Habitat and habitat management requirements of the shrill carder bee (*Bombus sylvarum*) and the brown-banded carder bee (*Bombus humilis*) in South Essex

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A thesis submitted in partial fulfilment of the requirements of the University of East London for the degree of Doctor of Philosophy

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DECLARATION

I certify that this work has not been accepted in substance for any degree, and is not concurrently being submitted for any degree other than that of the degree of Doctor of Philosophy being studied at the University of East London. I also declare that the work is the result of my own investigations except where otherwise identified by references and that I have not plagiarised another's work.

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ABSTRACT

A study of the habitat and habitat management requirements of two UK Biodiversity Action Plan Priority bumblebee species, *Bombus sylvarum* and *Bombus humilis*. Maps were produced of the South Essex distributions of the bees and a combination of field surveys, pollen sampling, habitat manipulation and microsatellite DNA analysis were used to investigate the forage requirements, nesting preferences and spatial dynamics of the bees.

Bombus humilis and *B. sylvarum* queens were recorded as the latest emerging of the *Bombus* species observed, not appearing until late May. Fabaceae, Scrophulariaceae and Lamiaceae were recorded as being the floral families most frequently and consistently visited by the bees with pollen being most commonly collected from Fabaceae and Scrophulariaceae. Rarefaction analysis of dietary preference data revealed that the mean number of plant species *B. sylvarum* workers would be expected to visit during 20 flower visits was significantly fewer than for *B. humilis* and *B. pascuorum* ($p=0.009$ and $p=0.004$ respectively). The mean number of plant species *B. humilis* would be expected to visit during 20 flower visits was similar to that of the nationally ubiquitous species *B. pascuorum* and *B. lapidarius* but was consistently lower than another ubiquitous species *B. terrestris/lucorum*, although not significantly so ($p=0.182$).

Forage patches sown with favoured forage species of the bees (*Lotus glaber*, *Odontites verna*, *Ballota nigra*, *Lotus corniculatus* and *Trifolium pratense*) successfully attracted *B. humilis* and *B. sylvarum* workers. No technique was found for reliably locating nests, and only three nests were located in the study. Those that were found were situated in relatively undisturbed tall rough grassland with a sunny exposure.

For the first time, microsatellite DNA analysis was used to assess foraging distances of rare bumblebees. Mean foraging distances estimated from distances separating sister bees were calculated as 756m for *B. humilis* and 232m for *B. sylvarum*. These distances were considered to be appropriate spatial scales for nesting and foraging habitat management. Results from this study were fed into a 10-year habitat management program for the bees at Hadleigh Castle Country Park, South Benfleet, Essex.

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Bees.....why would you want to conserve bees? (Anon. 2003, Anon. 2004, Anon. 2005, Anon. 2006, Anon. 2007)

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ACRONYMS

ASS: Arable Stewardship Scheme

BAP: Biodiversity Action Plan

BBCT: Bumblebee Conservation Trust

BDMS: Bumblebee Distribution Maps Scheme

bp: Base pairs

BST: British Summer Time

BWARS: Bee Wasp and Ant Recording Society

BWG: Bumblebee Working Group

CSS: Countryside Stewardship Scheme

DEFRA: Department for Environment, Food and Rural Affairs

DI: Deionised

DNA: Deoxyribonucleic acid

EEDA: East of England Development Agency

ES: Environmental Stewardship

EWT: Essex Wildlife Trust

GPS: Global Positioning System

HCCP: Hadleigh Castle Country Park

JNCC: Joint Nature Conservation Committee

MAFF: Ministry of Agriculture, Farming and Fisheries

MSA: Microsatellite Analyser

N.A.: No data available

OCCL: Old County Council Landfill

PCR: Polymerase chain reaction

S.E.: Standard error of the mean

SPTA: Salisbury Plain Training Area

SSSI: Site of Special Scientific Interest

TBE: Tris-Borate-EDTA

Chapter 1

Introduction

1.1 Background

Pollinators are keystone species in terrestrial ecosystems. They are essential for the maintenance of diversity in wildflowers and therefore also indirectly responsible for the persistence of the herbivores which feed from the wildflowers and in turn numerous groups of interdependent organisms along trophic pathways. Humans are one such group, dependent on bee, bird and bat pollination. The services of these organisms are estimated to affect 35 percent of the world's crop production, increasing the output of 87 of the leading food crops worldwide (Klein *et al.*, 2007).

Bees are the world's dominant pollinators. The 17,000 known bee species collectively interact with most of the planet's quarter of a million angiosperm species (Buchmann and Ascher, 2005). Bee pollination is essential for seed production in many crop and wildflower species because of the foraging behaviour and constancy of the bees. The majority of insects forage for their own individual needs, but bees collect pollen and nectar to supply their next generation, this necessitates more flower visits to be made than by other flower-visiting insects. This frequency of visits combined with unique morphological adaptations (long tongue length, coat of pollen-collecting hairs and ability to warm themselves enabling them to work at lower temperatures) makes them generally much more efficient pollinators than most other insects on all but small open flowers (Corbet *et al.*, 1991).

Historically honeybees (*Apis mellifera*) have been the principal managed pollinators across the globe (Paxton *et al.*, 2004). In the US, economic gains from honeybees are estimated to range between $1.6 and $5.7 billion (Potts *et al.*, 2006) and it is estimated that 7 out of the 60 agricultural crops that are crucial to the North American economy (e.g. *Medicago sativa*) would be lost if the insects that pollinate them became extinct (Ghazoul, 2005). In Europe, pollination by honeybees is worth approximately €4.25 billion and pollination by other taxa €0.75 billion (Potts *et al.*, 2006). In fact, the seed set of many of the major EC crops are dependent or enhanced by bee pollination (Corbet *et al.*, 1991).

Unfortunately, bee keeping with *A. mellifera* has suffered numerous setbacks over the last 30 years (Doebler, 2000; Paxton *et al.*, 2004; Blake, 2007) to such an extent that seeking alternatives to managed pollinators has become a necessity. This has driven research towards native pollinators and over the past few years has highlighted the vital role of wild, unmanaged bees, solitary and social, in the pollination of numerous fruit, nut and seed crops (Kremen *et al.*, 2002). In fact, wild pollination is now considered to provide 'insurance' for continued crop pollination and the pollination of wild flowers in the absence of honeybee pollination (Potts *et al.*, 2006).

Studies of native bee populations, both solitary and social, have also revealed disturbing trends of declines in European populations over the last 40-50 years (Biesmeijer *et al.*, 2006). This has occurred to such an extent that a potential 'Global Pollinator Crisis' has been debated (Ghazoul, 2005; Steffan-Dewenter *et al.*, 2005). Although evidence of pollinator declines can be demonstrated, the effects of such declines are poorly understood. Currently little is known about the loss of native pollinators and with sizeable gaps in our knowledge of pollination ecology and the specific interactions between individual pollinator species and the plants they pollinate (Williams, 1995), designing conservation strategies will prove impossible. What is certain is that if the trend of decline in pollinators continues the wild plant species they pollinate will eventually disappear and seed crops may become uneconomical to grow (Osborne and Corbet, 1994).

Recently Europe-wide programmes such as the European Pollinator Initiative (European Pollinator Initiative, 2007) have highlighted a need for increased knowledge of both wild and managed individual pollinator species ecology. The group of wild bees which has received the most attention are bumblebees (*Bombus* species). Bumblebees are the main wild-population pollinator group and are thus targeted by such research initiatives for their value as crop and wildflower pollinators. Their sociality, abundance, long flying season and broad flower choice (Corbet *et al.*, 1991) make them ideal pollinators filling pollination niches which cannot efficiently be filled by other bees. However, there is little information on the specific value of bumblebees as pollinators of wildflowers (Dramstad and Fry, 1995). If pollinators and wildflowers are to be conserved, knowledge of individual

bumblebee species ecology is vital. This study investigated the habitat requirements of two UK Biodiversity Action Plan species of bumblebee, the shrill carder bee (*Bombus sylvarum*) and the 'brown-banded' carder bee (*Bombus humilis*).

1.2 A historical perspective on British bumblebees

Historically Britain had a bumblebee fauna of 25 species, comprising of 19 'true bumblebees' (all subgenera excluding *Psithyrus*) and 6 'cuckoo bee' species (subgenus *Psithyrus)*, the inquilines in the nests of the true bumblebees (Williams, 1982).

Prior to a 1980 bumblebee mapping scheme developed by the International Bee Research Association (Williams, 1982) there was limited scientific information on the abundance and distribution of British bumblebees. The majority of our knowledge of their status came through anecdotal evidence of distributions in works such as Sladen's The Humble-bee (1912) and naturalist's notes such as Hallet's (1928) in which bumblebee species recorded were observed as having a plentiful distribution throughout the British Isles.

The 1980 bumblebee mapping scheme revealed that, although bumblebees were found throughout mainland Britain, their distributions were not as broad as previously believed. In fact, by comparing each species' post 1960 distribution with records made before 1960, it was possible to identify marked reductions in distribution in a number of species (Figure 1.1). These changes included drastic reductions in the distributional ranges of several species (Williams, 1982).

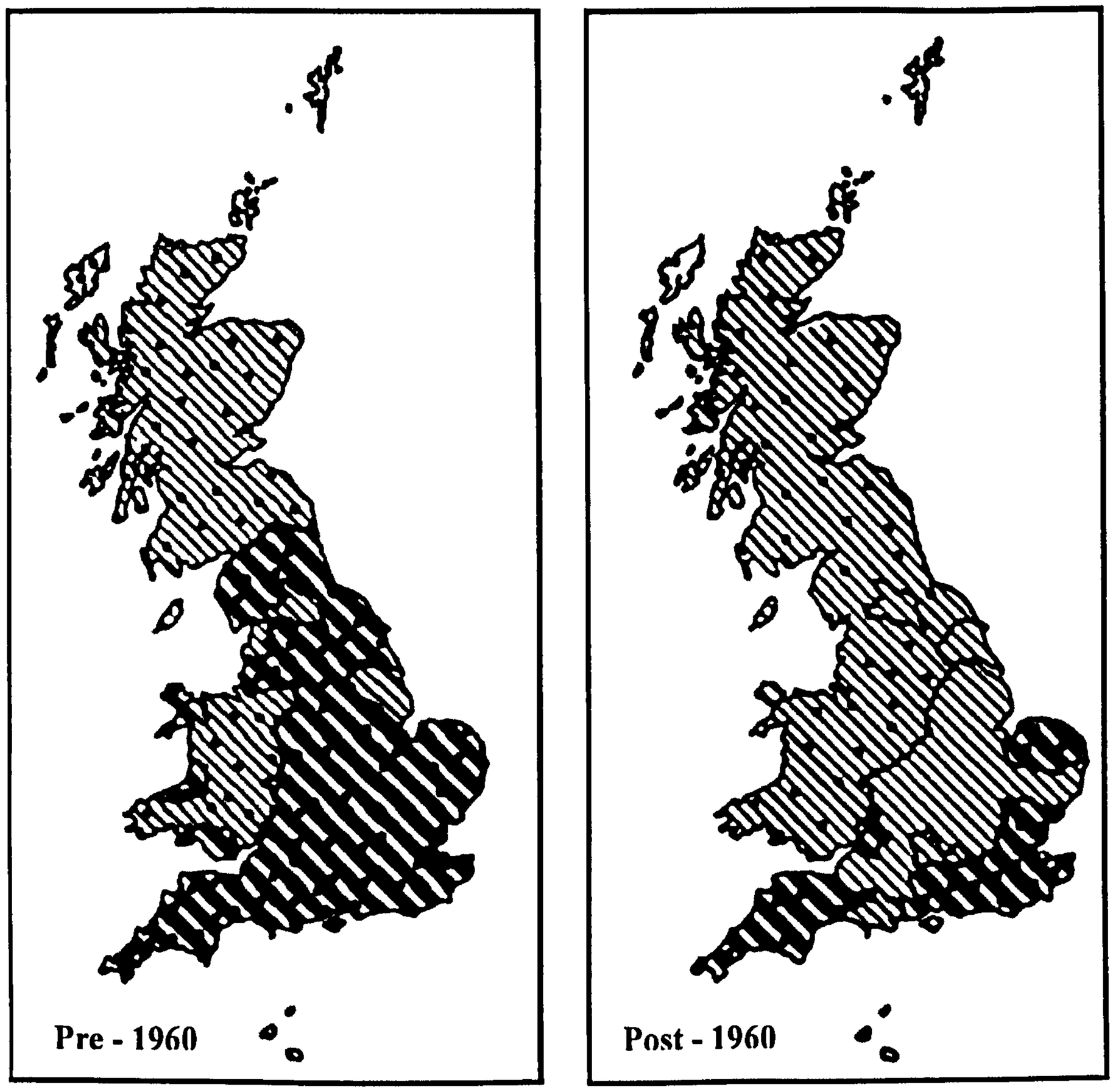


Figure 1.1. Pre-1960 and post-1960 distributions of *Bombus* species [excluding *Psithyrus*] in England, Wales and Scotland. From a classification of all BDMS vice-county records (Williams, 1982):

- Diagonal lines correspond to the Mainland Ubiquitous Species (all regions except the Outer Scottish Isles) Species include: *B. hortorum*, *B. pascuorum*, *B. pratorum*, *B. lucorum* (and *magnus*), *B. terrestris*, *B. lapidarius* and *B. ruderarius*.

- Dots correspond to the 'Widespread' Local Species (all regions except the Central Impoverished Region), after 1960 more northern and western in distribution. Species include: *B. monticola*, *B. jonellus*, *B. soroeensis* and *B. muscorum*.

- Broad stripes correspond to the Southern Local Species (just the Southern Region) Species include: *B. subterraneus*, *B. ruderatus*, *B. sylvarum* and *B. humilis*.

Concerns over changes in bumblebee distributions and numbers grew and in the mid 1990s five bumblebee species were put forward as potential UK Biodiversity Action Plan Species (UKBAP, 2007): *B. distinguendus*, *B. humilis*, *B. sylvarum*, *B. subterraneus* and *B. ruderatus*. In 1997 a Bumblebee Working Group (BWG) was set up with the purpose of further investigating the status of the proposed UK BAP species (Edwards and Jenner, 2006). Evidence from the work of this group indicated that the five species in question were seriously threatened in the UK and that five other species showed cause for concern: *B. jonellus*, *B. muscorum*, *B. soroeensis*, *B. ruderarius* and *B. monitcola* (Edwards, 1997; 1998; 1999; 2000; 2001; 2002; 2004).

As well as recognising the rare and threatened status of many of the UK's bumblebees, the BWG concluded that three of 19 true bumblebee species are now extinct in the British Isles:

i) *Bombus cullumanus* (Kirby) which has not been recorded in the UK since 1926 (Alford, 1975)
ii) *Bombus pomorum* (Panzer) last recorded in Kent in 1864 (Alford, 1975).
iii) *Bombus subterraneus* (Linneaus) last recorded at Dungeness, Kent in 1988 (Williams 2005). Now a UK Biodiversity Action Plan species considered to be extinct in the UK (Edwards, 2004).

Two *Bombus* species investigated by the BWG were the shrill carder bee (*Bombus sylvarum*) and the brown-banded carder bee (*Bombus humilis*). Both species have undergone dramatic decreases in their distributional ranges and are now found at only a handful of sites across southern Britain (Figures 1.2 & 1.3). Both are in the subgenus Thoracobombus, the carder bees (Sladen, 1912), and are amongst the longer-tongued bumblebees and both species are now recognised as priority National Biodiversity Action Plan Species in the UK (UKBAP, 1995; 1999).

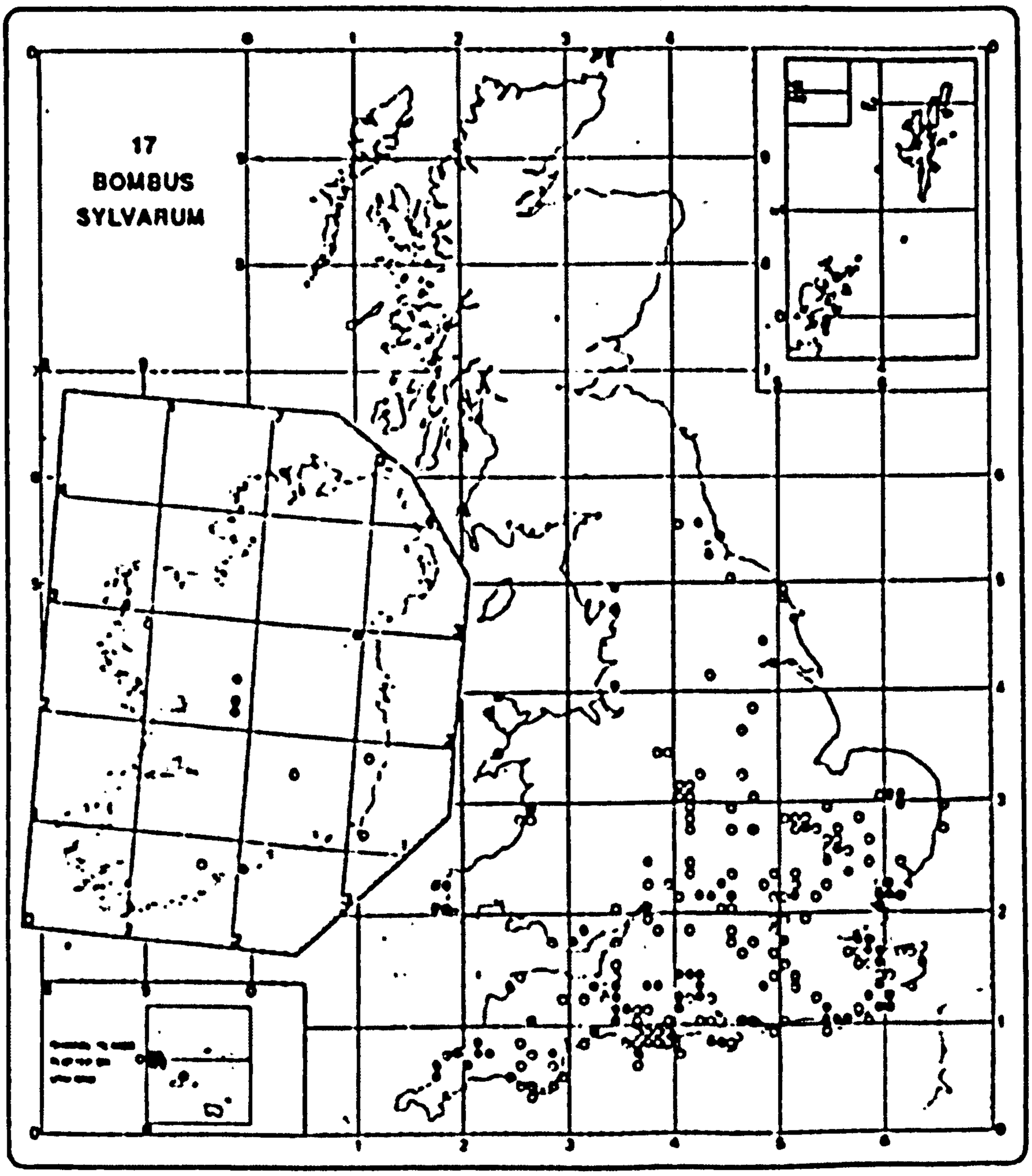


Figure 1.2. The distribution of *B. sylvarum* in the UK and Ireland. Open circles represent records from before 1960 but not since; Black dots represent records from 1960 onwards (Prŷs-Jones and Corbet, 1987).

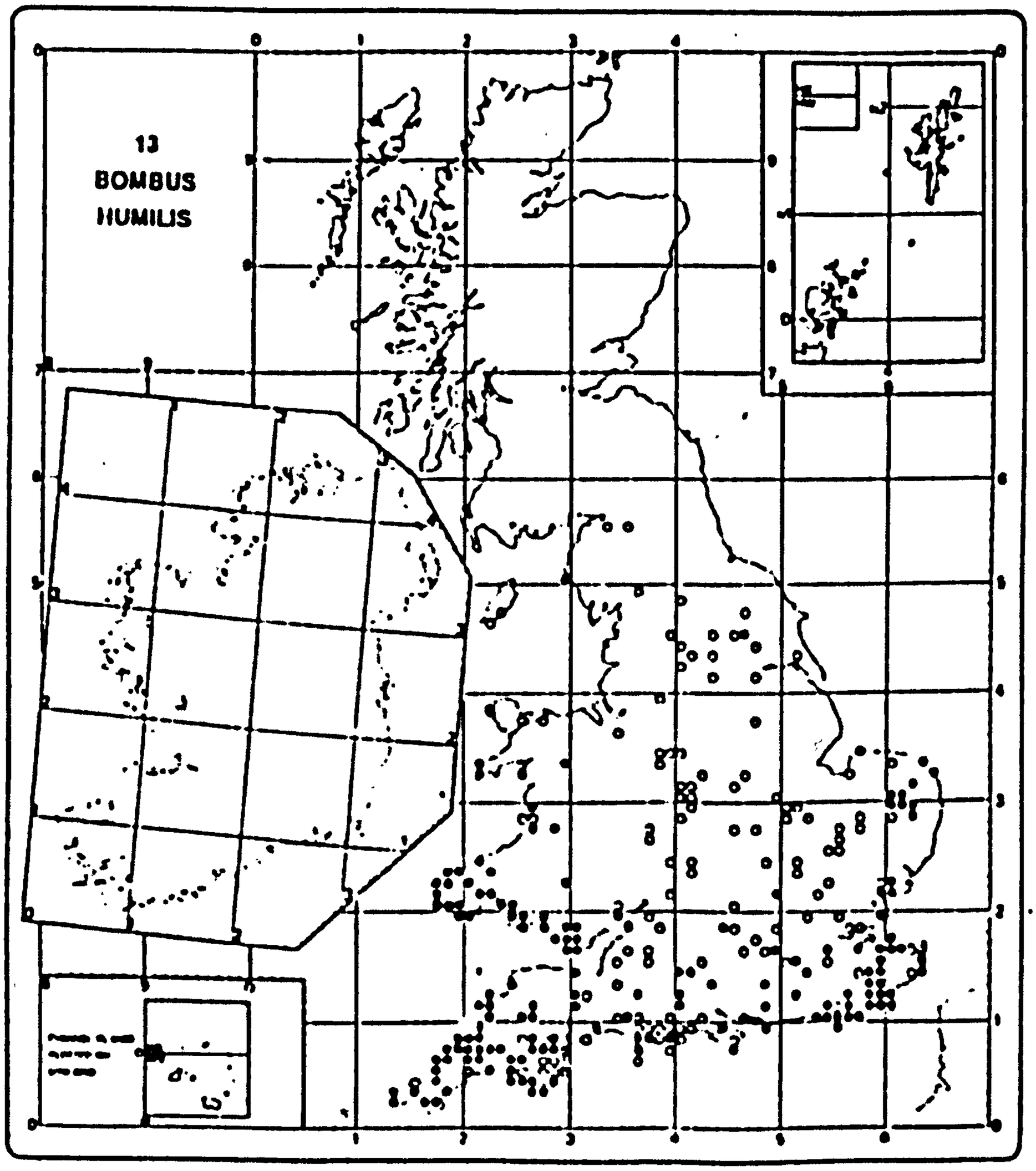


Figure 1.3. The distribution of *B. humilis* in the UK and Ireland. Open circles represent records from before 1960 but not since; Black dots represent records from 1960 onwards (Prŷs-Jones and Corbet, 1987).

1.3 *Bombus humilis* and *Bombus sylvarum* UK distributions

In the early 20th century *B. sylvarum* was widespread and common, especially in southern England (Sladen, 1912). Surveys in the 1970s showed that it had experienced a significant decline, with only one third of its previous range still being occupied (Williams, 1982). This decline continued and in 1997, despite great efforts in the field by members of the Bumblebee BAP Group, it was recorded in only two localities in south-east England and one in south Wales (Edwards, 1997). There are great concerns that the species may become extinct in the British Isles (Philp, 1998) with currently only five discrete populations confirmed across southern England and Wales (Edwards and Jenner, 2006).

At the beginning of the 20th century *B. humilis* was also widespread, reaching Yorkshire and Lancashire in the north, but was considered uncommon in southern England. By the 1970s it too had disappeared from much of its previous range (Williams, 1982) and has continued to decline since. Although *B. humilis* are more widely distributed than *B. sylvarum*, strong populations can now only be found in coastal areas of south Wales, south-western England, Salisbury Plain and the Thames Estuary (Prŷs-Jones and Corbet, 1987).

These two species often occur in proximity (Edwards, 1998) and both appear in substantial numbers across a number of sites throughout southern Essex. This study was based at these sites.

1.4 Reasons for decline

Loss of suitable habitat to agricultural intensification is widely accepted as being the main cause of the decline in British bumblebees (Williams, 1986; Osborne *et al.*, 1991; Osborne and Corbet, 1994; Goulson, 2003; Goulson *et al.*, 2005). During the 20th century, grasslands on flatter soils have been improved for intensive agriculture and the remaining semi-natural areas left largely unmanaged

(Bourn and Thomas, 2002). This spread of agriculture has resulted in the fragmentation, degradation and loss of semi-natural habitats greatly reducing the local biodiversity of temperate regions (Steffan-Dewenter and Tscharntke, 2002; Robinson and Sutherland, 2002; Weibull *et al.*, 2003).

Bumblebees are believed to be impacted by these changes in agroecosystems (Pywell *et al.*, 2005, Carvell *et al.*, 2006), specifically by:

i) the loss of habitat variability e.g. loss of microhabitats and architecture,
ii) the loss of flower-rich headlands and hedgerows,
iii) the loss of flower-rich hay meadows (cut once a year) caused by a switch to silage production (cut several times a year),
iv) greater fertiliser and pesticide use,
v) shorter periods of fallow or loss of fallow fields sown with Fabaceae such as red clover (*Trifolium pratense*).

These changes have resulted in the loss and fragmentation of essential bumblebee foraging and nesting habitat. Consequently there has been a decline in the abundance of a number of highly preferred bumblebee forage plants, especially members of Fabaceae and Lamiaceae. It is the loss of these plant species which is believed to have had the greatest impact on British bumblebees (Rich and Woodruff, 1996, Pywell *et al.*, 2005, Carvell *et al.*, 2006).

Evidence for the link between agricultural intensification and declines in British bumblebees comes from numerous investigations and experiments carried out indicating the value of bumblebee forage on arable land (Dramstad and Fry, 1995; Croxton *et al.*, 2002; Pywell *et al.*, 2004). It has been shown that by providing suitable forage along arable field margins it is possible to significantly increase the number of bumblebees foraging on agricultural land (Kells *et al.*, 2001; Carvell *et al.*, 2004; Pywell *et al.*, 2005); see Figure 1.4. Results from these experiments have been so successful that the UK's Countryside Stewardship Scheme (CSS), the Arable Stewardship Scheme (ASS) and more recently Environmental Stewardship (ES) have all recognised the potential importance of field margins as habitats for pollinators in agro-ecosystem conservation (MAFF, 1998; 1999; DEFRA, 2005a).

A nectar and pollen seed mix designed for bumblebees and other pollinators has even been included as a scheme option (DEFRA, 2005b). It is believed that by improving the quality of agricultural land as a resource for bumblebees, it might be possible to create a network of forage and nesting sites to effectively conserve bumblebees on a national scale (Kells *et al.*, 2001).

However, the answer to the problem of how to conserve all of the UK's bumblebee species does not appear to be so simple. Whilst a number of the UK's bumblebees are declining, suffering decreases in their distributions, a subset of 6 UK species (*B. lucorum*, *B. terrestris*, *B. lapidarius*, *B. pascuorum*, *B. hortorum* and *B. pratorum*) do not seem to have been similarly effected by the fragmentation and loss of suitable habitat caused by agricultural intensification. In fact, all six species remain abundant and current evidence suggests that there have not been declines in their ranges. In some cases their ranges appear to have increased as other *Bombus* species have disappeared (Goulson, 2003).

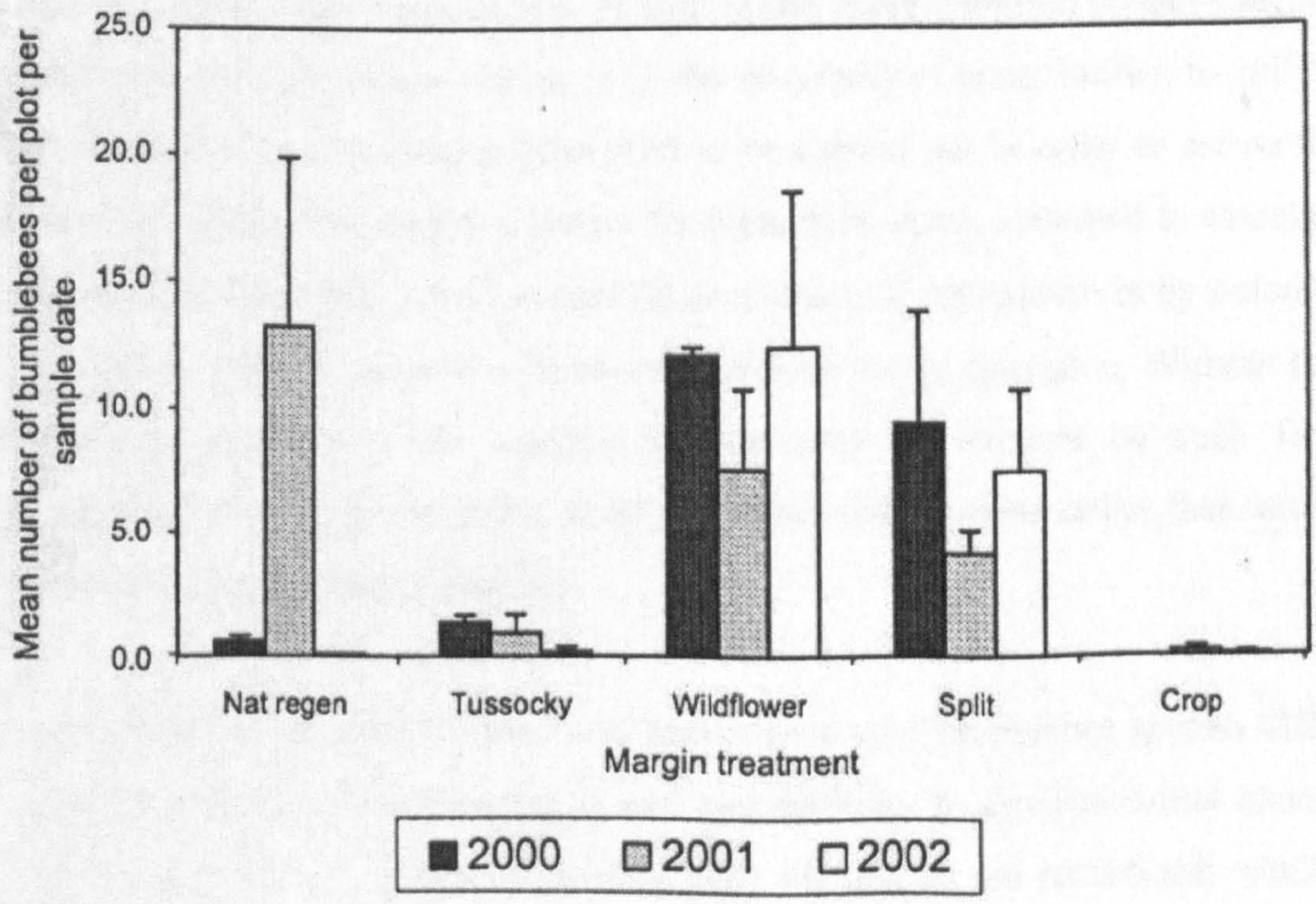


Figure 1.4. Change in total bumblebee abundance on different margin treatments of arable land (Carvell *et al.*, 2004).

The general trend in the UK is that bumblebee species with restricted geographic ranges also tend to be less abundant on sites they share with the 6 species which remain widely distributed (Williams, 1986). Whilst UK agricultural schemes are undeniably vital for the long-term survival of Britain's bumblebees, they might consolidate populations of more ubiquitous bumblebee species whilst doing little for the conservation of rarer species.

Thus far, the results of arable field margin experiments aimed at conserving bees have echoed the trends that are being witnessed in bumblebee declines. A disturbing lack of rarer bumblebee species have been recorded in these studies (Kells *et al.*, 2001; Carvell *et al.*, 2004) and thus their ability to conserve all British species has yet to be proved. This could be in part due to the floral matrix created in field margins being representative of shallower-flowered annuals favouring disturbance, rather than the deeper flowered perennials favoured by bumblebees (Kells *et al.*, 2001). This has to some extent been demonstrated by the more recent successful orientation of seed mixes towards longer-tongued bumblebees than general nectar mixes (Pywell *et* al., 2005), but even in these studies, trial areas have only really been successful in attracting the more common longer-tongued bumblebees. Further studies within or in the proximity of areas known to still be supporting populations of rarer bees need to be carried out in order to assess the benefits of agri-environmental schemes for these bees. Also, a method to calculate increase in colony number and successful production of reproductives by colonies is required to effectively assess the benefits of such forage provision. Without this information, monitoring the number and diversity of workers on such field margins may merely be recording shifts in worker distributions rather than actual increases in bumblebee populations.

What is clear is that there is very little known as to why bumblebee species differ so widely in their range, abundance, and susceptibility to environmental change (Goulson and Darvill, 2004). Morphologically all species are remarkably similar (Goulson *et al.*, 2005) with the only obvious differences being variation in body size and tongue length (Goulson *et al.*, 2005), characteristics which not only vary between species but also between individuals, castes and nests of the same species. Most British bumblebees have similar annual life cycles (although a few species

are partially bivoltine), and all depend exclusively on nectar and pollen for food (Goulson *et al.*, 2005). Beyond this, very little is known about what differentiates species.

Several theories have been put forward to explain differential declines between bumblebee species; tongue length (Goulson and Darvill, 2004), distributional range (Williams, 2005), colony size/timing (Bowers, 1985a), foraging range (Walther-Hellwig and Frankl, 2000a; Darvill *et al.*, 2004), pesticide use (Thompson, 2001). However, without an in-depth knowledge of individual species ecology and habitat requirements, it is impossible to understand what is driving these changes. In particular, the question of whether species have precise habitat and life cycle requirements has yet to be answered. For conservation in the UK to be effective therefore, it is vital for us to develop a better understanding of individual species ecology, the requirements for each stage of their life cycle: nesting, foraging, mating and hibernating (Figure 1.5), and the spatial dynamics which populations operate over. This aim has been recognised as a priority of the Bumblebee Working Group (Edwards, 1998), and it was also the focus of this study.

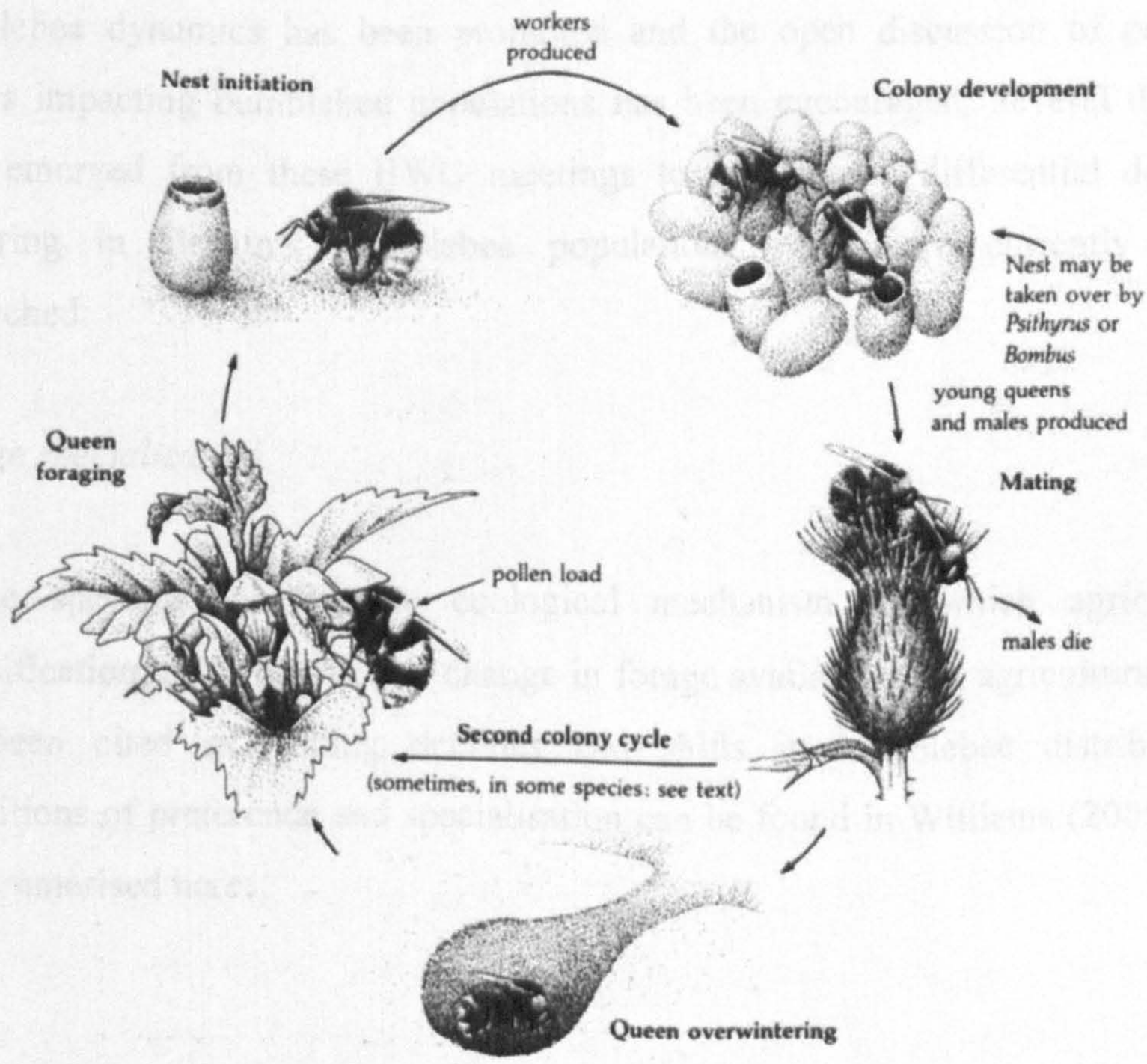


Figure 1.5. British bumblebee lifecycle (Prŷs-Jones and Corbet, 1987).

1.5 Research thus far

1.5.1 Differential bumblebee declines

Due to their bright distinctive appearance and unusual sociality amongst insects, naturalists have shown a keen interest in bumblebee behaviour throughout history. The most famous perhaps being Darwin's observations at Down House, Bromley (Darwin, 1859). It was not until the publishing of the Bumblebee Distribution Maps Scheme in Alford's bumblebee atlas (1980) that the plight of many of Britain's bumblebees was first recognised. Alford's mapping scheme and subsequent bumblebee surveys (Williams, 1982; 1985) led to a Bumblebee Working Group (BWG) being created in 1997 to investigate the status of Britain's bumblebee species and the driving forces behind their UK declines.

Research into bumblebee behaviour and ecology has increased greatly both nationally and globally since the formation of the BWG and annual BWG meetings have created a forum by which the need for further understanding of bumblebee dynamics has been promoted and the open discussion of possible factors impacting bumblebee populations has been encouraged. Several theories have emerged from these BWG meetings to explain the differential declines occurring in Britain's bumblebee populations which are currently being researched:

Forage specialisation

Forage specialisation is one ecological mechanism by which agricultural intensification, and in particular change in forage availability on agricultural land, has been cited as driving declines and shifts in bumblebee distributions. Definitions of preference and specialisation can be found in Williams (2005), and are summarised here:

Dietary preference – is defined as the choice of one forage species over another when both are available and abundant but, if one or the other forage species is absent, the other species is able to be used.

Dietary specialisation – is defined as a greater degree of narrowness in food preference or the situation where a bumblebee species is limited to a particular food plant species or group.

These definitions mean that when a bee is visiting plant species more frequently merely because they are more abundant in a habitat, this cannot be classified as preference or specialisation.

In balanced ecosystems numerous bumblebee species and colonies forage in the same geographic locations. For this to be occurring, some degree of intra- and inter-specific resource partitioning to avoid competition must be occurring (Heinrich, 1976). A study by Dafni and Shimda (1996) on *B. terrestris* introductions demonstrated this by showing that when bee reproduction is limited by floral resources, competition between bees can result in changed population abundance and distributions. Lytzau Forup and Memmott's (2005) study of the relationship between bumblebee and honeybee abundances has more recently provided further evidence for the effects of floral abundance on bee diversity and partitioning between bees.

Forage availability is vital for the successful development of bumblebee colonies and therefore also their populations. In a study of food supplementation and reproductive success, Pelletier and McNeil (2003) demonstrated the importance of adequate nectar and pollen resources throughout a season. They showed that colonies whose nectar and pollen supplies were increased regularly throughout a season reached larger sizes (number of workers) and had a higher reproductive success than a control group, by 51% and 86% respectively. In particular, food supplementation increased the number of males produced and the probability of producing gynes (young queens). They also suggested that shortfalls in food supply may have a detrimental effect on parameters leading to lower colony success including: drops in brood temperature, an increase in immature

development time, production of fewer or smaller individuals, as well as the production of fewer sexuals. These factors may also contribute to increased vulnerability of colonies to predation and parasitism.

With colony development dependent upon on adequate nectar and pollen supplies, competition for foraging resources are paramount to reproductive success. Therefore, if resources have become limited due to changes in agricultural practice (Rich and Woodruff, 1996; Pywell *et al.*, 2005; Carvell *et al.*, 2006) it would be expected that bumblebee populations would be impacted and, if resource partitioning between species is occurring, that some species might be more significantly impacted than others. Thus, it has been hypothesised that resource partitioning in terms of forage preference is at least partly responsible for the differential declines recorded between bumblebee species (Goulson and Darvill, 2004).

Numerous studies have demonstrated correlations between bumblebee tongue lengths, corolla tube lengths and flower handling times (Dennis and Haas, 1967; Inouye, 1980; Ranta and Lundberg, 1980; Pyke, 1982) indicating that bumblebees are most efficient at collecting nectar from flowers with corolla tube lengths most closely correlated with a bumblebee's tongue length. As there is much inter-species variability with regards to bumblebee tongue lengths (Goulson and Darvill, 2004), it has been hypothesised that foraging on flowers with corolla lengths corresponding to *Bombus* species tongue lengths is one way that forage resource partitioning between bumblebee species occurs (Prŷs-Jones and Corbet, 1987; Goulson and Darvill, 2004; Goulson *et al.*, 2005).

The bumblebee species that have remained abundant are mostly short-tongued species that emerge early in the year. These species are commonly found in a variety of habitats from urban gardens to agricultural monoculture crops and are considered to be able to exploit a broad range of floral resources (Goulson *et al.*, 2005). The longer-tongued late emerging species such as *B. humilis*, *B. ruderatus* and *B. subterraneus* are the bees which have suffered the greatest declines in range and abundance. They are considered to be more dependent on a narrower dietary breadth than more common bumblebee species. Studies have shown that the

longer-tongued bees tend to forage preferentially on Fabaceae flowers as a pollen resource (Goulson and Darvill, 2004) and it is this group of plants that has been lost particularly through agricultural change (Carvell *et al.*, 2006). It has also been hypothesised that this preference occurs because species emerging later in the season are limited in their choice of available forage resources (Goulson *et al.*, 2005) and they need to raise their brood quickly, so specialize on flowers with more protein-rich pollen (Ellis, 2006).

As yet however providing reliable evidence that bumblebees have varying dietary breadths and dietary specializations which correspond to the declines being observed has proved difficult. Paul Williams of the Natural History Museum has been studying UK bumblebee populations since the 1980s, particularly at a site in Dungeness, Kent, where he has recorded an uncommonly broad diversity compared to other sites nationally (Williams, 1982; 1989). Results from his studies at Dungeness have contradicted theories on dietary specialisation (Williams, 2005).

Using data from Dungeness, he was unable to demonstrate a relationship between relative rarities of bumblebee species and their dietary preference or proboscis length, and concluded that bumblebee declines may not be explained by narrow food-plant specialization. He argued that past studies of dietary breadth and specialisation (Goulson *et al.*, 2005) had been unreliable due to the varied sample sizes of rare compared to more common bumblebee species, and that they had utilized data from different geographical locations with different forage availabilities which were not comparable (Williams, 2005). He did however comment that Dungeness data may be unrepresentative of dietary breadth and specialization patterns among British bumblebees in general, and stressed the need for another study providing the same comparability (Williams, 2005). Prior to this South Essex study however, no such dataset has been made available.

Categorising bumblebee species into tongue length groupings also comes with difficulties as there is much variation in tongue length between castes and individuals even within a bee species (Goulson *et al.*, 2005). Also, two of the more ubiquitous species (*B. hortorum* and *B. pascuorum*) are credited as possessing

some of the longest mean tongue lengths of the British bumblebees (Williams, 1989), so providing definitive proof of the role of forage resource partitioning in UK bumblebee declines has thus far proved elusive.

Edge of range

Instead of variations in bumblebee dietary breadths, Williams has proposed a relationship between rarity and decline of bumblebees and each species' European range as the driving force behind changes in UK bumblebee distribution. He argues that range represents a species' overall niche breadth and perhaps climatic and habitat specialization, and that it may represent a better indication of a species' risk of decline with diminishing resources. The theory is based on a marginal mosaic model of bumblebee populations which he first proposed in his thesis (Williams, 1985). The model stated that regionally rarer species tend to be found at lower local abundances where they are present (Williams, 1988). He suggested that this would occur at the edges of bumblebee's European ranges and that under these conditions if resources become limited and abundances decreased further across all bumblebee species it is likely to be the least abundant species that would be extirpated first.

He concluded that declines being seen in bumblebee populations are a combination of an as yet unidentified aspect of niche specialization affecting regional range size and land use changes affecting forage resources (Williams, 2005). Whilst this hypothesis itself may be useful for understanding the theoretical side of bumblebee declines and predicting where management efforts are most required, it does not explain the mechanics of localised declines, leaving many unanswered questions before conservation strategies can be implemented.

Time of emergence and habitat specialization

Part of the remit of the BWG was to carry out field surveys and investigations to identify the extent of British bumblebee distributions and aspects of individual species ecology. The coordinator of the working group, Mike Edwards, compiled the results of these observational studies and research projects (Edwards, 1999;

2000; 2001; 2002) from which he proposed a theory that time of emergence and habitat specialization are the driving forces behind differential bumblebee declines.

Whilst not based on experimental data, Edwards proposed that British bumblebees can be roughly divided into two groups, early emerging woodland species and later emerging grassland species, and theorised that the grassland group have been impacted to a much greater extent by agricultural intensification causing the loss of wildflower-rich grasslands over much of the UK. Foraging queens from the early emerging woodland group are less affected due to their reliance on woodland, park and garden plants in the spring. Emerging earlier in the season also means that woodland groups have longer to raise their brood and are therefore possibly less reliant on the quality of forage available and would therefore have a greater probability of colony success.

Edwards concluded that greater knowledge of individual species ecology would be required to further support this theory and this was recognised as an aim for further research by the Bumblebee Working Group. Currently such knowledge is unavailable and the need for studies of individual species ecology is still required for Edwards' theory to be substantiated. What is apparent however from current knowledge is that, similarly to the previous forage specialisation theory, whilst many of the UK's declining bumblebee species appear to follow the suggested trends (e.g. *B. humilis*), there are other closely related species which do not (e.g. *B. pascuorum).*

Other theories which may in part explain the observed patterns of decline are discussed below.

Pesticides

Historically testing of pesticide toxicity to pollinators has been carried out on honeybees. More recently studies have suggested that bumblebees may be more susceptible to pesticide toxicity than honeybees due to specific behavioural characteristics. Applications of pesticides (e.g. pyrethroids) to flowering

crops/weeds are generally carried out early in the morning or in the evening. This is done to avoid periods when honeybees are most active and, in the case of pyrethroids specifically when it is cooler as they are more toxic at lower temperatures. Thompson (2001) proposed that because mornings and evenings tend to be the peak foraging time for bumblebees, whose numbers are thought to drop during the middle of the day (Alford, 1975; Plowright and Laverty, 1984), it might put them at greater risk from pesticide exposure than honeybees. Thompson also suggested that, because of the smaller colony sizes of bumblebees and because wild populations are not monitored as closely, lethal and sub-lethal effects on bumblebees may go unnoticed.

Since Thompson's paper, several studies on pesticide toxicity to bumblebees have been carried out. Tests on a broad spectrum of pesticides have revealed severe impacts on colony vitality (Gels *et al.*, 2002) and the foraging activities and survival of bumblebees (Incerti *et al.*, 2003). It has also been shown that foraging workers do not avoid insecticide treated areas (Gels *et al.*, 2002). More recently, a study by Ali (2005) in Canada revealed that exposure to the pesticide spinosad (at levels similar to those which they could encounter in the environment during larval development) impaired the foraging ability of adult bumblebees. From these results there seems little doubt that increased pesticide use could be impacting bumblebees as a whole. However, little evidence has been produced to indicate that impacts could be driving differential declines in bumblebees.

Whilst there is some evidence to suggest that susceptibility to pesticides can vary even between bumblebee families (Marletto *et al.*, 2003), differential pesticide susceptibility is unlikely to be an explanation for bumblebee declines. An alternative theory could be that foraging behaviour could put certain bumblebee groups at greater risk to pesticide exposure. The quantity of nectar a bee drinks may affect pesticide exposure (Thompson, 2001) and there is evidence to suggest that long-tongued bumblebees drink faster than shorter-tongued bees of similar size (Harder, 1983). Thompson (2001) also proposed that bodyweight can have a bearing on pesticide exposure in bumblebees with lighter bees being able to take up proportionally larger nectar loads than heavier bees. Many of the declining bee

species are also the smaller bee species (Peat *et al.*, 2005) and therefore may be more susceptible.

These theories however are hypothetical and if pesticide use is causing the differential declines being observed in British bumblebees, perhaps a more likely explanation could be increased exposure of the grassland species proposed by Edwards (1999; 2000; 2001; 2002) compared to those of woodlands and parks. If bumblebees are divided into woodland versus grassland species, it would be expected that species foraging in woodlands, gardens and parks might have less pesticide contact than those on grasslands and field margins. Unfortunately, as yet no research has been carried out to look at pesticide concentration in larval food of foraging bees in pesticide treated and untreated areas. These data would also have to be combined with data from further research on habitat use by declining bumblebee species. What is certain however is that combined with other impacts such as declines in forage availability, pesticide use has the potential to significantly impact bumblebee populations and increasingly the need to make bumblebee conservation areas pesticide free is being recognised (Osborne *et al.*, 1991; Dramstad and Fry, 1995).

Climate change

Another theory which has been suggested as impacting UK bumblebee populations is global climate change. Current climate models indicate that a global climate change has been occurring over the last 100 years (Houghton *et al.*, 2001). Recent decreases in biodiversity in Europe have been linked to this climate change (Schmitt and Hewitt, 2004) and shifts in bumblebee distributions are one phenomenon that has been suggested as being attributable to these changes (Plowright *et al.*, 1997). Britain is at the edge of many species' climatic range (Williams, 2005), so it would be expected that effects of changes in climate would be observed here. Indeed, an example of a change in bumblebee behaviour has been recorded that is considered to be climate-driven. Historically in the UK, *B. terrestris* has been reported as producing one colony per year, with queens produced by the colony hibernating through the winter (Sladen, 1912; Alford, 1975,) but in recent years *B. terrestris* workers have increasingly been recorded

foraging throughout winter (Goulson 2003). These observations have been largely confined to southern England and are believed to be a result of the milder winters associated with climate change. However, definitively linking such potentially climate-driven behaviour with changes in bumblebee distribution has yet to be done.

The seeming replacement of *B. muscorum* throughout much of its range by *B. pascuorum* has been suggested as evidence for the effects of climate change (Plowright *et al.*, 1997). *Bombus muscorum* is considered to be physiologically suited to cooler damper conditions (Edwards and Broad, 2005) whereas *B. pascuorum* is thought to favour warmer conditions (Ellis, 2006), so the patterns of change being observed are those that would be expected due to climate change. It is difficult however to prove that this is the driving force behind the distributional changes. Following the Plowright *et al.* (1997) study, Macdonald (2001) investigated the range expansion of *B. terrestris* and *B. lapidarius* from 1976 onwards, but was unable to identify any link between the spread and climate change. Whilst it is certain that any climate change will impact both bumblebee distributions and food plant distributions, it is difficult to tie this with the trends currently being witnessed in bumblebee distributions.

Loss of nest sites and hibernation sites

Research carried out across Europe both prior to the formation of the BWG and directly resulting from BWG discussion has also investigated the nesting behaviour and requirements of bumblebees and identified the habitats bumblebees favour for nesting (Wojtowski, 1963; Sakagami and Katayama, 1977; Harder, 1986; Fussell and Corbet, 1992a; Svensson *et al.*, 2000, Carvell, 2002; Svensson, 2002; Kells and Goulson, 2003; Osborne *et al.*, 2007). The nesting habitats identified included many of those which have been disappearing due to agricultural intensification: hedgerows, established grassland, field margins and boundaries. Bumblebees often nest in or near the disused nests of small mammals, birds or previous generations of bumblebees, so disturbance of the soil surface by ploughing or herbicide treatment may destroy their nesting places (Osborne and Corbet, 1994). Agricultural intensification has increased the use of herbicides and

a shift from hay to silage production has lead to areas traditionally supporting bumblebee nests being cut and disturbed more frequently. This, combined with habitat fragmentation of landscapes into habitat islands, has eliminated nesting sites and isolated suitable nesting patches from available forage patches (Buchmann and Ascher, 2005).

It has been proposed that declines in certain bumblebees can be partly explained by differential nesting habitat requirements between species. Many of the declining species are carder bees which are known to nest at or below the soil surface in dense grassy tussocks (Fussell and Corbet, 1992a; Carvell, 2002; Kells and Goulson, 2003) whereas many of the more common species prefer to nest in underground cavities (Fussell and Corbet, 1992a; Kells and Goulson, 2003). Common species in general are far more adapted to nesting in a variety of habitats such as gardens and parks (Osborne *et al.*, 2007), whereas the carder bees seem to be more reliant on the undisturbed tall grassland which is being lost with agricultural intensification. In fact, one of the key habitats identified for UK BAP species *B. humilis* and *B. sylvarum* is brownfield sites in part due to their unmanaged nature allowing the development of mature grass tussocks (Harvey, 2004). It would therefore be expected that species dependent upon the grasslands being lost through agricultural intensification would be more impacted than those with broader nesting requirements. This is in fact the general trend that is being observed. However, it is not the case for all species. *Bombus pascuorum* is a carder bee considered to nest preferentially in dense grassy tussocks (Fussell and Corbet, 1992a) but its distribution appears to be increasing in the UK rather than decreasing (Plowright *et al.*, 1997). From this it should be concluded that whilst loss of nesting habitat may be impacting bumblebee populations, it may be only one of several factors driving the differential declines in bumblebees that are being recorded.

In addition to nest sites, bumblebees also require mating and overwintering sites. There has been little research on particular species requirements and preferred habitats for these stages of the bumblebee life cycle (Alford, 1969). What evidence there is suggests that the habitat features they prefer, hedgerows and undisturbed

grasslands, could be similarly impacted by changes to the agricultural landscape (Alford, 1975; Carvell, 2002; Macdonald, 2006).

Predation, parasitism and pathogens

Predators, parasites and pathogens are also known to impact upon colony development in bumblebees. Beyond general descriptions of such species (Alford, 1975; Schmid-Hempel, 1998; Goulson, 2003) however, very little is known about their prevalence and impacts on bumblebee populations. Whilst there has been some suggestion that agricultural intensification may have shifted populations of bumblebees and their natural enemies into more concentrated areas of the natural environment and therefore increased the probability of them coming into contact (Juliet Osborne, personal communication), no experimental investigation has thus far been carried out.

1.5.2 *Bombus humilis* and *Bombus sylvarum* ecology

Prior to the publication of the 1980 bumblebee mapping scheme (Alford, 1980) and subsequent journal articles highlighting the plight of bumblebees (Williams, 1982) much of the knowledge of individual species ecology came from naturalist observations and records (Sladen, 1912; Hallet, 1928; Alford, 1975). Whilst these records provided some information on *B. humilis* and *B. sylvarum* ecology, they were recorded as observations rather than scientific studies, so provided little information on inter-species variation and interaction. To conserve all of the UK's bumblebee species an understanding of how species ecology differs, how species interact and what is driving the differential declines is required.

Due to the rare nature of *B. humilis* and *B. sylvarum* populations in the UK, large scale studies of their habitat requirements and life cycle ecology have not been possible. This has meant that the majority of research has been carried out on the more ubiquitous species in the UK. Results from much of this research was summarised in section 1.5.1. This summary demonstrated that many factors may

be influencing bumblebee declines in the UK and with such complex interactions occurring definitively identifying the cause may be impossible. Without this knowledge, conservation efforts must be targeted towards the reversal of all factors considered to be influencing bee declines. This includes the recreation of lost habitats on a national scale, with suitable forage and nesting habitats and pesticide free zones. To achieve this, an in depth knowledge of individual species ecology for all British bumblebees has been recognised as a priority by the BWG (Edwards, 1998).

Prior to this South Essex study, several investigations of the ecology and habitat requirements of the two UK BAP species *B. humilis* (Figure 1.6) and *B. sylvarum* (Figure 1.7) had been carried out. They are summarised below:

Figure 1.6. *Bombus humilis* queen foraging on *Lathyrus latifolius*.

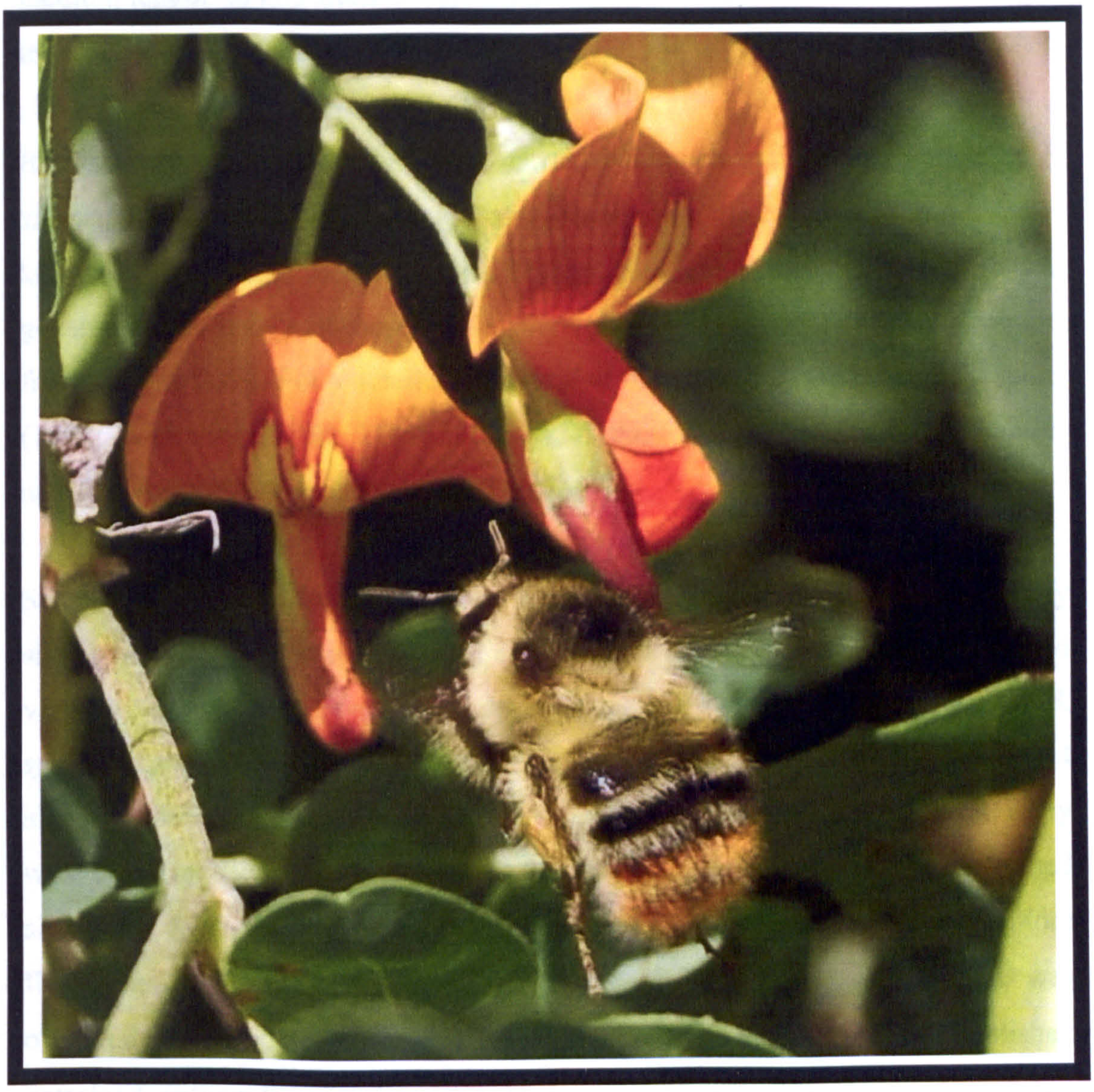


Figure 1.7. *Bombus sylvarum* queen foraging on *Colutea arborescens*.

The first studies to investigate aspects of *B. sylvarum* and *B. humilis* ecology and competition between these and other species appear to have been a study by Pekkarinen (1979) and one by Teras (1985). In the study by Pekkarinen (1979) *B. pascuorum*, *B. muscorum*, *B. terrestis*, *B. sylvarum* and *B. humilis* were all recorded as possessing similar tongue lengths, but little competition for food amongst the bees was identified. The Teras (1985) study revealed *B. sylvarum* as an extremely late emerging species which visited fewer plant species than other bumblebees. *Bombus sylvarum* was recorded making more than half of their visits to fabaceous plants and workers of the species were reported as having tongues of

medium length. In this study *B. sylvarum* was also found to be a forest edge rather than forest or open terrain preferring species.

It was not however until the BWG carried out much of its investigation of *B. sylvarum* and *B. humilis* ecology and distribution that much of our current knowledge of range declines, distributions and ecological requirements was generated. The results of these studies were published in a series of annual reports from 1997 to 2004. For a detailed overview of their research see Edwards' reports (1997; 1998; 1999; 2000; 2001; 2002; 2004), their findings in terms of *B. humilis* and *B. sylvarum* ecology are summarised here.

Searches for *B. humilis* and *B. sylvarum* were initiated in an attempt to identify sites which still supported the species (Edwards, 1997). Initial surveys revealed very few sites to be supporting the bees. Where they were observed however, *B. sylvarum* and *B. humilis* were often found in proximity, although they were not always observed sharing exactly the same components of their joint habitat. They were consistently associated with well-established open grasslands in well-drained situations. Such grasslands were often not particularly flower-dense with more floriferous areas nearby tending to be ignored by foraging workers. It was observed that the workers seemed to prefer to remain in typical established grassland nesting habitat. An example being *B. humilis* foraging workers being found on ungrazed areas of Portsdown Hill, Hampshire, but were completely absent on adjacent tightly grazed areas.

Further surveys revealed that *B. sylvarum* distribution was extremely limited and on sites which supported the bees, workers were rare. *B. humilis* were more widely distributed and tended to be found in greater numbers on the search sites where both species were recorded (Edwards, 1998). Both species were found to be late emerging, with workers not appearing till late June or July, confirming an observation made by Alford (1975) prior to the publication of the 1980 bumblebee mapping scheme.

Following the initial surveys to identify current distributions of these two *Bombus* species, the focus of the surveys was switched towards identifying their foraging

requirements. Both *B. sylvarum* and *B. humilis* were found to be generalist bumblebee foragers although foraging resources were found to be predominantly from a few families (Edwards, 1999). This was found to be particularly the case for pollen resources, which were analysed by a small scale pollen basket collection study. Families Fabaceae, Lamiaceae and Scrophulariaceae were found to be of particular importance although the need for further research of pollen gathering behaviour was highlighted.

Following these initial forage surveys, the BWG supported an MSc project student (Claire Carvell) surveying vegetation structure and changes in the availability of floral resources, observing foraging bumblebees, searching for nests and collecting pollen samples (Edwards, 1999). The study recorded bumblebees visiting 20 out of 57 available flower species and showing a distinct preference for areas grazed by cattle during the previous year. Analysis of forage preferences of *B. humilis* and *B. sylvarum* suggested there may be a separation but it was concluded that there was insufficient data to reach a definitive conclusion. The study recognised the need for a high density of bumblebee forage but also identified a need for longer term forage assessment.

Overall, observational surveys and pollen analyses revealed many of the favoured forage plants of the two bee species. The need for large quantities of this forage throughout the colony lifetime through a succession of flowering by different species and by a planned cutting/grazing regime was identified as a key target for conservation. *Odontites verna* in particular was recorded as being an important late summer forage plant for *B. sylvarum*. The importance of grazing management was recognized at Castlemartin, Pembrokeshire, with the best populations being recorded in the winter-grazed areas. Grazing by sheep however, was considered unfavourable between the months of April and September (Edwards, 1999).

Only one population of *B. sylvarum* was found during the BWG surveys occupying less than $2km^2$ of continuous good quality habitat and even there the area was known to be at least twice as big ten years previously. It appeared that *B. humilis* was able to survive in smaller individual areas, but still required a fairly extensive matrix of such areas. From their observational studies, the BWG

concluded that *B. sylvarum* seemed to require the restoring of large areas to maintain viable populations in their entirety, highlighting the need for areas of at least $10km^2$ of suitable habitat matrix to support populations of both bee species (Edwards, 2002). The practicalities of restoring habitats of this size in the UK's current fragmented landscape are impossible so the BWG concluded that the way forward is through more precise bumblebee habitat-informed, landscape orientated modifications to modern farming practice which can restore a viable matrix of the necessary partial habitats within a modern farming system (Edwards, 1998).

As well as forage and distribution surveys, the BWG investigated other aspects of the bees' life cycle requirements, but very little was discovered about hibernation and few nests were located (Edwards, 1999). Only a few *B. humilis* nests were found and even fewer of *B. sylvarum.* Those of *B. humilis* were slightly underground at the base of grass swards amongst the layer of dead litter in grasslands. From queen nest searching behaviour it was considered likely that *B. sylvarum* also nests in grasslands having a layer of dead litter, but where grassland is denser than that for *B. humilis* possibly using old vole runs (Edwards, 1998).

The lack of nests located in these surveys led to attempts to encourage *B. sylvarum* and *B. humilis* queens to utilise nest boxes (Edwards, 2000; 2002). All attempts proved unsuccessful preventing accurate measures of nest density. Nest densities for individual species were therefore theorised as being the result of interaction between nest site resource, forage resources and maximum nest size for that species, but the need for further research was highlighted.

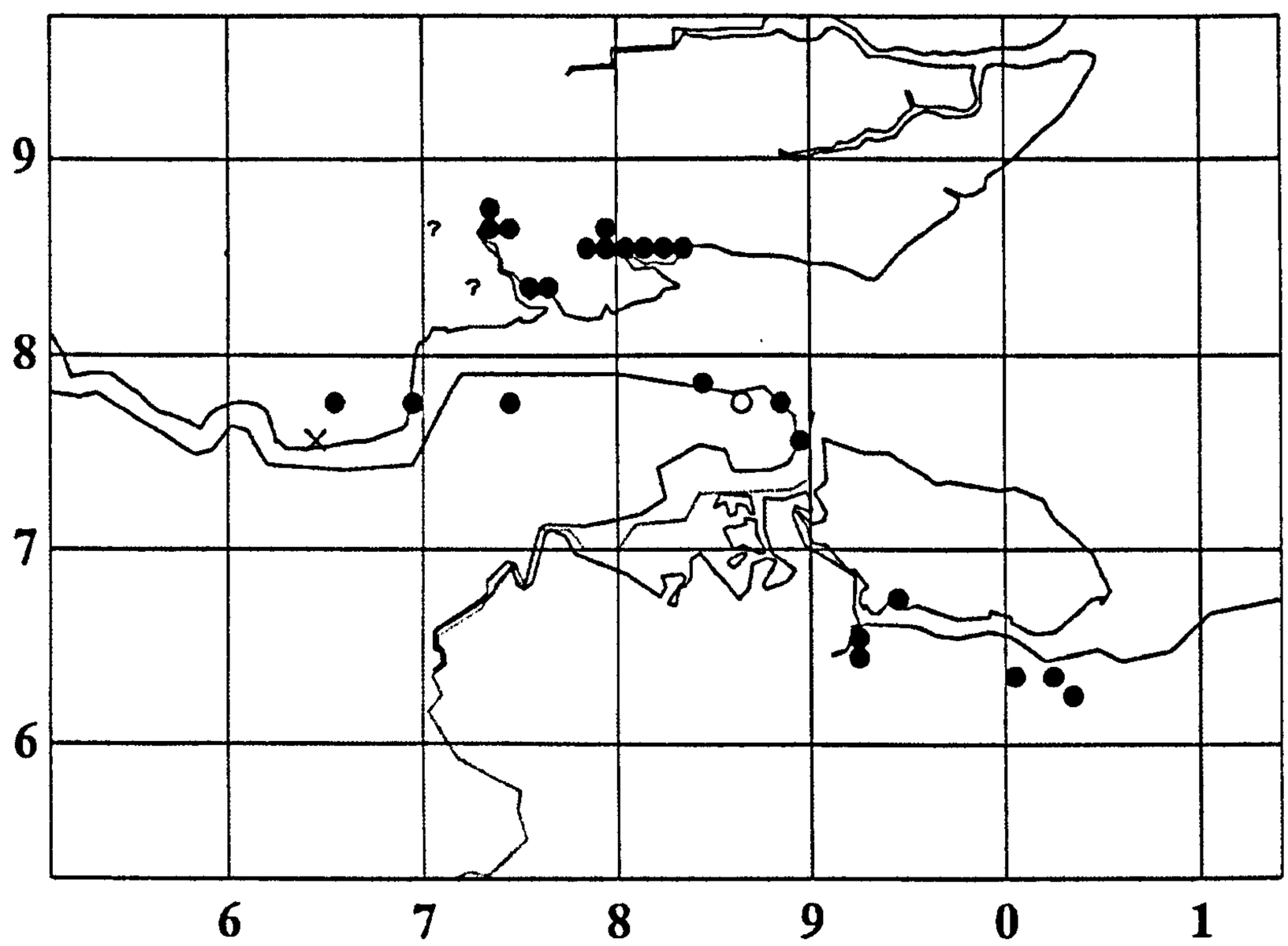
Mark-recapture surveys carried out concurrently indicated that individual workers re-visited the same forage areas over and over again, explaining the patchy distribution witnessed in uniformly good quality habitat (Edwards, 2000). This confirmed theories that bumblebees are forage patch constant (Free and Butler, 1959). Unfortunately due to the patch constancy of the bees, the investigation was unable to reveal the spatial dynamics of foraging bumblebees in terms of how far they travel from nest to forage, and therefore did not provide information on the spatial requirements of conservation habitat management. The BWG (Edwards, 2000) did report that work at Rothamsted using harmonic radar revealed that the

potential foraging range of bumblebees is much larger than is often assumed. The Rothamsted research showed that bumblebees did not necessarily forage on the closest resources to the nest. Distances of over five hundred metres between nest and forage points were frequent in studies of *B. terrestris*, indicating nest ranges of greater than $1km^2$ (Osborne *et al.*, 1999).

At the same time as the BWG national bumblebee surveys were being carried out Peter Harvey was investigating the status of *B. humilis* and *B. sylvarum* in Essex (Harvey, 1999). Harvey surveyed a range of sites across South Essex to identify the presence of *B. humilis* and *B. sylvarum* (Figure 1.8) and the importance of habitats and floral forage species. His surveys revealed that the most important habitat feature common to all the areas the bumblebee were seen was tall very flower-rich herbage. Characteristics of this habitat included abundant *Odontites verna* and evidence of previous abundance of clover and other species such as *Lotus* species together with the vicinity of scrub. He identified *Odontites verna* as a very important component during August and into September when other sources such as *Trifolium pratense* were virtually over. He also highlighted the importance of a continuity of forage sources throughout the season.

In a document for English Nature (Harvey, 2000a), Harvey confirmed the presence of a considerable population of *B. sylvarum* at the Canvey Northwick site, Essex, and continued to survey sites across South Essex for the presence of both *B. sylvarum* and *B. humilis* populations. Habitat surveys of the sites were carried out and plants most likely to be important to the bees were observed. Mark-recapture work confirmed the occurrence of forage patch constancy in *B. sylvarum* workers, and therefore the futility of marking bumblebees whilst foraging to assess their foraging distances (Harvey, 2000a).

Bombus sylvarum



X = Ferry Fields site, now being developed
? = possible sightings

Bombus humilis

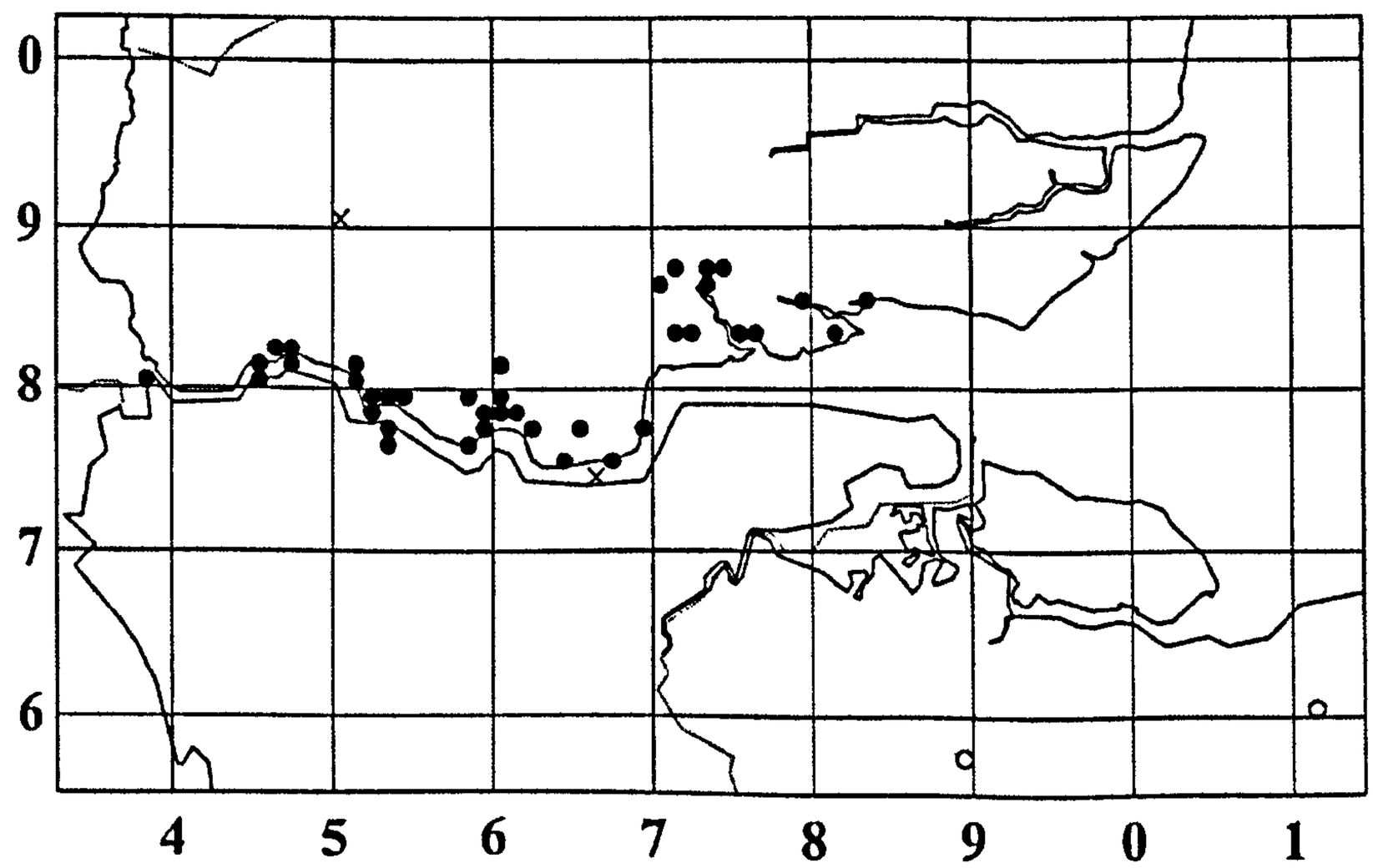


Figure 1.8. Distribution of *B. sylvarum* and *B. humilis* in the East Thames Corridor (Harvey, 2000a).

Harvey also carried out investigations of nesting requirements, but no *B. sylvarum* or *B. humilis* nests were located during the surveys and a nest box trial carried out at one of the sites was unsuccessful with several of the boxes being damaged or destroyed and no bumblebee nests being found in the other boxes (Harvey, 2000a). Recommendations for habitat management were made in the report, but it was concluded that further research into the foraging behaviour, nesting requirements and the spatial dynamics of the bees was required if effective habitat management plans are to be initiated in the area (personal correspondence).

From Harvey's surveys Canvey Island populations of *B. humilis* and *B. sylvarum* were recognised by the BWG as being of national importance. The fragility of the site was also recognised and the need for a wider, more dispersed population as well as this single local highly productive population was underlined (Edwards, 2002). This necessitated research into surrounding populations and the promotion of habitat restoration in the area to consolidate existing populations. The priority for research in the East Thames Corridor identified in the Edwards study (2002) was a driving force behind this study.

Further research on the value of the Thames corridor for bumblebees was carried out in a study of bumblebees in urban environments by Roselle Chapman and reported in her PhD thesis (Chapman, 2004). Chapman confirmed the presence of a network of sites supporting *B. humilis* populations along the Thames Corridor within London, specifically along the River Lea and on eastern derelict industrial sites. Sites varied from public gardens and parks to wasteland and were relatively fragmented within the urban environment. The phenology of the bees was recorded and workers foraging visits were recorded predominantly on flowers in the Lamiaceae family with many floral species visited being non-native to the UK. A competition experiment revealed that when other *Bombus* species were removed from forage patches, *B. humilis* workers spent significantly longer foraging at patches of flowers. However, due to problems with experimental methodology it was not possible to demonstrate that such competition was species specific.

Following Carvell's MSc project work for the BWG surveying *B. sylvarum* at Castlemartin Range, Pembrokeshire and Kenfig National Nature Reserve,

Glamorgan (Edwards, 1999) she carried out a study on habitat use and the conservation of bumblebees on Salisbury Plain Training Area (SPTA), Wiltshire (Carvell, 2002). SPTA is the largest remaining area of unimproved chalk grassland in northwest Europe and is known to support a broad diversity of bumblebee species including *B. humilis* and *B. sylvarum*. The study identified relationships between numbers of *B. humilis* and the structure and height of vegetation and also the total flower abundance. Relationships between bees and numbers of flowers varied between bumblebee species potentially indicating resource partitioning between the bees. *Bombus humilis* demonstrated a preference towards *Lotus corniculatus*, *Odontites verna* and *Trifolium pratense*. Unlike the four ubiquitous *Bombus* species, higher numbers of *B. humilis* were recorded from the track edges, reverting arable land and sheep-grazed grasslands than the recently cattle-grazed habitat suggesting that *B. humilis* may differ in its habitat requirements compared to other commonly recorded species. Correlations indicated that it preferred taller, more grass-dominated swards and was the only species to be positively correlated with the depth of moss or grass litter.

Bombus humilis abundance on the site was recorded as being higher than that of four mainland ubiquitous species (although one of the four, *B. ruderarius*, has since been classified as rare and threatened). Only 10 individuals of *B. sylvarum* were recorded in the study, previously much higher numbers had been recorded on the site (Edwards, 1998). This confirmed the urgent need for conservation efforts in the UK for this species and again highlighted the need for further research into the specific habitat requirements of the species (Carvell, 2002).

The study also highlighted the importance of small-scale disturbances from military vehicles in creating locally abundant patches of flowering plants, and hence forage resources for bumblebees, a factor that had previously been highlighted as important on Canvey Wick and other brownfield sites in South Essex (Harvey, 2000b).

Another research group to have developed from the BWG is that of Dave Goulson, originally based at the University of Southampton but currently at the University of Stirling where his research team have founded the Bumblebee Conservation

Trust (BBCT) (BBCT, 2007). Following on from the BWG floral preference surveys, Goulson and Darvill (2003) carried out a study on Salisbury Plains. This study investigated the floral preferences of *B. humilis* on the site and compared them with the other bumblebee species present. The study surveyed a series of sites across Salisbury Plains and recorded the foraging habits of all *Bombus* species observed. *Bombus humilis* was most frequently recorded in the central part of the plain but was rarely abundant. Results of foraging surveys corresponded with other studies in finding Fabaceae the preferred source of nectar and pollen for *B. humilis*. No *B. sylvarum* were recorded during the surveys.

Perhaps the most important work on *B. humilis* and *B. sylvarum* ecology from this research group however was that carried out by Jon Ellis. With the BWG investigating foraging requirements and national studies on the effectiveness of providing bumblebee forage on arable land (Carvell *et al.*, 2004)). The focus of Goulson's research group shifted towards the comparison of forage preferences between species and the population 'health' of remaining populations. Ellis' work has included two papers covering their foraging behaviour and population dynamics, particularly in terms of the effects of habitat fragmentation (Goulson *et al.*, 2005; Ellis *et al.*, 2007).

His first work on the bees was published in Goulson *et al.* (2005) but was covered in greater detail in his PhD thesis (Ellis, 2006). He investigated the causes of rarity in bumblebees, focusing on *B. humilis*, *B. muscorum sladeni* and *B. sylvarum*. The study observed differences in foraging behaviour between the three species. *Bombus humilis* was shown to exhibit the most general foraging behaviour visiting the greatest number of plant families. Fabaceae flowers comprised 72% of *B. humilis* visits. *B. sylvarum* were observed making 76% of their visits to Fabaceae and Scrophulariaceae. All species were observed to have a narrower diet breadth when collecting pollen.

The Goulson *et al.* study (2005) was carried out nationally and used observational surveys to record flowers visited for nectar and pollen. It was however unable to explain differences in rarity between these three species in terms of their foraging behaviour. The study was criticised by Williams (2005) because of its comparison

of foraging behaviour across a variety of sites with differing floral assemblages, and Ellis (2006) concluded that the study was more of an analysis of favoured forage species than a dietary comparison. He also concluded that other factors such as foraging range, competition and edge of range effects could be contributing to bumblebee declines and stated the need for further research into bumblebee biology, particularly nesting requirements and the advantages that early emergence might confer if nest sites are limiting (Goulson *et al.*, 2005; Ellis, 2006).

Ellis collected DNA samples from the bees he observed during his first study which he used for a second study investigating the effects of habitat fragmentation on the genetic structure of *B. humilis* and *B. sylvarum* (Ellis *et al.*, 2007). This second study found estimated effective population sizes to be low and found evidence of genetic structuring between populations indicating that the populations were isolated. Evidence for limited gene-flow between populations was provided by the apparent absence of any trend of genetic isolation by distance in *B. sylvarum* and *B. humilis.* It was suggested that this signified that current populations were so far apart that gene-flow between them was unlikely. Prior to this study, the only estimate of gene-flow in fragmented populations of bumblebees was a maximum distance of ~10km, see Figure 1.9 (Darvill *et al.*, 2006). This estimate was calculated from island populations of *B. muscorum* however and may not have been an accurate measure of genetic isolation distances on the mainland and across all bumblebee species.

During the study, a small proportion of diploid males were detected. It was speculated that production of diploid males could be driven by the low genetic diversity in fragmented populations and that diploid male production would reduce colony survivorship (Cook and Crozier, 1995; Duchateau and Mariën, 1995). It was also predicted that diploid male production could be occurring more frequently than detected in this study due to surveying time and colony survivorship following male diploid production (Ellis *et al.*, 2007).

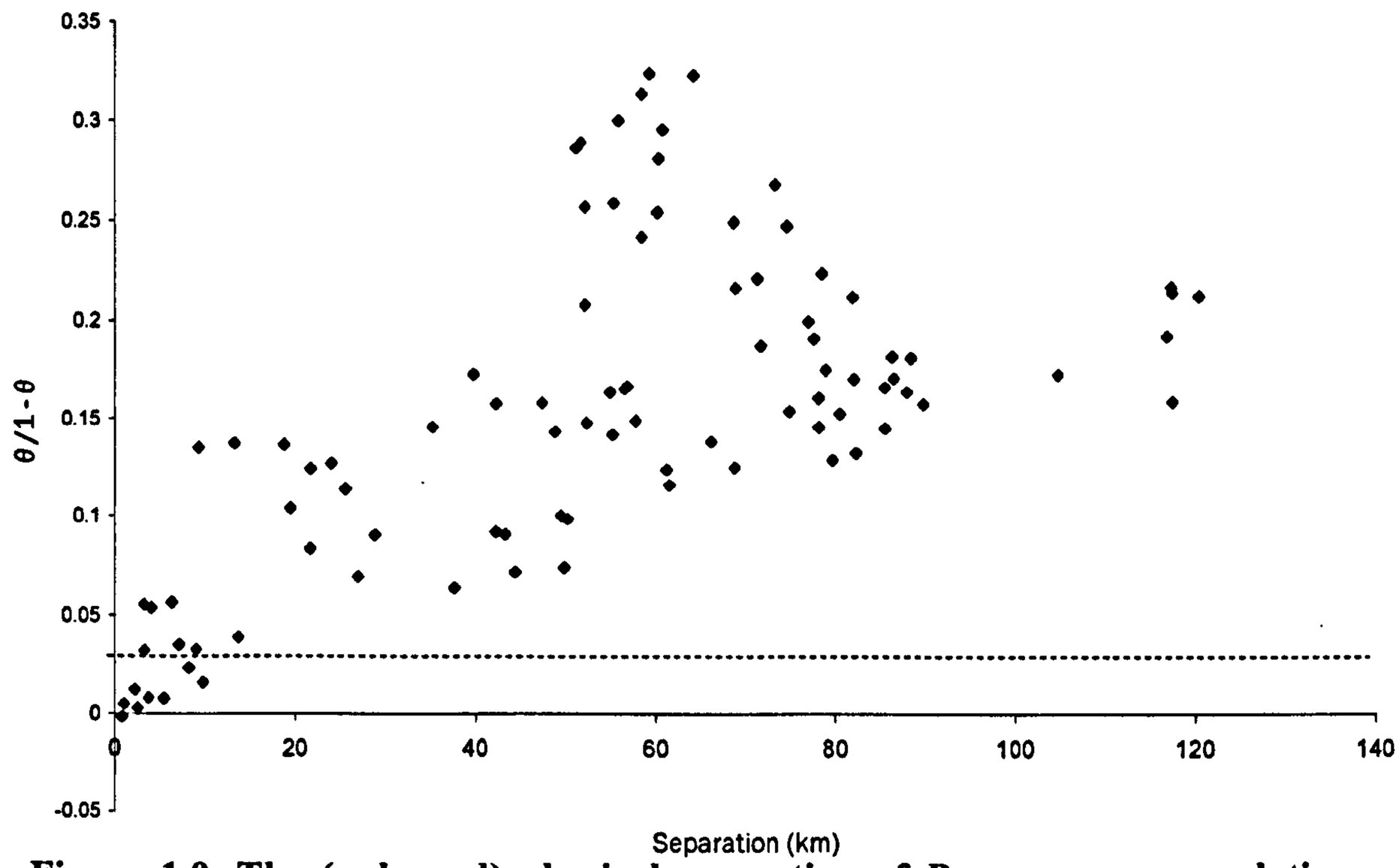


Figure 1.9. The (unlogged) physical separation of *B. muscorum* populations and the genetic differentiation between them. Mantel test, $p < 0.0001$ (Darvill *et al.*, 2006). Population pairs above the dotted line are significantly genetically differentiated from one another ($p < 0.05$).

Nest density estimates for both species were also calculated. Estimates were similar to those made for the ubiquitous carder bee species *B. pascuorum* (Darvill *et al.*, 2004). However, the methods employed to calculate nesting density only used the sample area and did not incorporate flight distances allowing for the fact that the bees sampled might be attracted to forage from sites outside the sample areas. This means that calculations were perhaps a more accurate estimation of population size rather than nesting densities.

Other than these main studies, very few journals articles have been published on *B. sylvarum* and *B. humilis* ecology. One study in Sweden investigated *B. sylvarum* ecology and identified boundary habitats as being of importance for worker foraging and open areas with withered grass and tussocks being where the queens were observed nest seeking (Svensson *et al.*, 2000). They also reported that *B. sylvarum* were not found in forest habitat. These findings were backed up by a further Svensson study (2002).

Two other studies have looked at a separate aspect of *B. humilis* and *B. sylvarum* ecology, investigating the effects of honeybee (*Apis mellifera*) foraging on bumblebee behaviour. A study by Lytzau Forup and Memmott (2005) surveyed 19 heaths across southern England and found *B. humilis* to be rare across all the sites. The investigation found a negative relationship between honeybee and bumblebee abundance, but no relationship between honeybee abundance and bumblebee diversity. A separate Walther-Hellwig *et al.* (2006) study found that *B. sylvarum*, along with *B. lapidarius*, *B. muscorum* and *B. pascuorum*, responded to competition from honeybees (*Apis mellifera*) by shifting the plant species they favoured for foraging. The studies concluded that honeybees might impact bumblebee populations and that competition can affect foraging behaviour, but it appeared likely that national bumblebee declines are in response to other factors. The studies did however highlight that increased use of *Apis mellifera* should not be given as a answer to the decline in pollinators as rather than treating the symptoms of the decline, it could be exacerbating the pollinator crisis.

Data generated from the BWG and associated research has stressed the importance of two main areas of investigation for bumblebee conservation in the UK. Firstly, the potential for the development of a program implementing a national network of habitat improvement primarily through creating bumblebee foraging and nesting habitat on arable land (Kells *et al.*, 2001; Carvell *et al.*, 2004; Pywell *et al.*, 2005). Secondly, to ensure that any habitat improvement program targets all declining bumblebee species rather than merely supporting populations of the more ubiquitous bee species. For this to be achieved the BWG has highlighted the need for more comprehensive research into the exact habitat requirements of individual bumblebee species, particularly the UK BAP species.

Whilst these studies have provided important information on the ecology of both *B. humilis* and *B. sylvarum* they have not been able to provide a complete overview of their life cycle requirements. A common theme in the conclusions to many of these studies was the need for further research into the habitat requirements of both these and other *Bombus* species, particularly with respect to whether species partition resources and the spatial dynamics over which populations and colonies operate. This furthering of knowledge is required if

management prescriptions are to be effective in conserving all bumblebee species in the UK. As such, the investigation of the habitat requirements of *B. humilis* and *B. sylvarum*, with a specific focus on the nationally important East Thames Corridor populations, was identified as the main aim of this study.

1.6 Aims of the investigation

Human alteration of natural landscapes is considered to be the primary cause of global biodiversity loss (Reid *et al.*, 2005). Human-driven habitat loss and fragmentation impacts the distribution, persistence and metapopulation dynamics of wildlife species, and is a major concern in conservation biology and landscape ecology (Gilpin, 1987; Opdam, 1990; Hanski and Gilpin, 1991; Reed, 2004). Prescribed management of semi-natural habitats for wildlife by man is now generally accepted as being necessary to promote the long-term survival and persistence of many species (Samways, 1993; With, 2004). However, without a comprehensive understanding of the ecological requirements of target species such management prescriptions are unlikely to succeed.

As highlighted in previous discussions, bumblebees are a conservation priority group in the UK. The shrill carder bumblebee (*Bombus sylvarum*) and the brown carder bumblebee (*Bombus humilis*) have been recognised as conservation priority species and both are now subjects of national Biodiversity Action Plans. The basis of these plans is to safeguard extensive areas of remaining habitat where these bees still occur and to promote informed management of these habitat areas. The Biodiversity Action Plans recognise that there is insufficient knowledge of these species and their habitat requirements in order to provide informed management prescriptions and emphasise the need for research to address this (UKBAP, 1995; 1999).

If the trend of decline seen in *B. sylvarum* and *B. humilis* populations over the last fifty years is to be reversed, increasing our understand of the foraging, nesting, breeding and hibernating requirements of these rare bees is necessary. Once we

have this knowledge, for it to be utilized effectively for conservation initiatives through land management, it is important that we understand the metapopulation dynamics of the bees and provide habitat management over the appropriate landscape spatial scales. The general aims of this project were therefore two fold:

i) To improve knowledge of *B. sylvarum* and *B. humilis* ecology in terms of specific resource requirements, inter- and intra-specific resource partitioning, and the spatial dynamics of the species.

ii) To provide bumblebee conservation guidance for land mangers in a format which can be incorporated into habitat management plans and is compatible with prevailing land management strategies.

Both *B. humilis* and *B. sylvarum* occur across a series of country parks and brownfield sites in the Basildon and Castle Point areas of South Essex, one of the few remaining strongholds still supporting both of these *Bombus* species in the UK. It was decided that this would be an ideal location to carry out the first study of *B. humilis* and *B. sylvarum* ecology and habitat management requirements in a fragmented landscape comprised of semi-natural grasslands, urban, arable and brownfield sites.

These aims were achieved through several research methods:

i) Site surveys – identification and mapping of sites within the South Essex area which were supporting populations of *B. humilis* and *B. sylvarum*. Timed counts of *B. humilis* and *B. sylvarum* workers to produce indices of relative numbers allowing comparison both between and within sites during a single field season and annually.

ii) Floral surveys – identification of which floral species *B. humilis* and *B. sylvarum* are visiting whilst foraging.

iii) Dietary specialisation – Comparison of *B. humilis* and *B. sylvarum* floral choices with those made by the more nationally ubiquitous *Bombus* species recorded on the same sites.

iv) Pollen analysis – analysis of pollen samples from foraging workers to assess which flowers are being visited for nectar collection and which for pollen collection. Comparison of pollen collecting behaviour of rare versus more ubiquitous species foraging on the same sites.

v) Forage patch creation – investigation of whether *B. humilis* and *B. sylvarum* can be attracted to experimental forage patches in areas previously not supporting bee populations.

vi) Habitat manipulation – habitat manipulation program at Hadleigh Castle Country Park (SSSI) in South Essex to establish effective techniques for bumblebee habitat creation.

vii) Further species ecology – Artificial forage, emergence time, time of first worker and nest surveying. Creation of artificial forage sources which can be manipulated for known tongue lengths to control which *Bombus* species are able to forage on them. Surveys of the first foraging queens of each *Bombus* species to determine emergence times for habitat management forage provision and to investigate any regional variability compared to national timings. Surveys of first workers to appear at a forage patch in the mornings to assess whether *B. sylvarum* and/or *B. humilis* are at a competitive disadvantage being unable to forage at cooler temperatures than the more nationally ubiquitous species. Surveys of the South Essex sites for *B. humilis* and *B. sylvarum* nests to assess nesting requirements. Investigation of a novel method to reliably locate rare bumblebee nests.

viii) Population estimates and flight distances – using molecular techniques to estimate the effective population size and heterozygosity of the South Essex *B. humilis* and *B. sylvarum* populations, the number of colonies utilising each site for foraging and a comparison of maximum foraging distances of *B. humilis* and *B. sylvarum* workers.

ix) Management guidelines – production of best practice management guidelines for *B. humilis* and *B. sylvarum* habitat improvement using results from the investigation and research summary.

Results of the study were incorporated into a 10-year program of habitat management for *B. humilis* and *B. sylvarum* at Hadleigh Castle Country Park, South Benfleet, Essex. It was also intended that the results of the study will be used in the design of management plans for sites within and surrounding the South Essex metapopulation and also for the promotion and conservation of both of these species nationally. Results from the project will be used as guidance to aid the protection of existing bumblebee habitats and the restoration of bumblebee habitat within the East Thames Corridor to consolidate populations of both *Bombus* species.

Chapter 2

Site surveys

2.1 Introduction

Prior to this study, the Canvey Wick site, Canvey Island, Essex had been recognised by the Bumblebee Working Group (BWG) (Edwards, 1999) and Peter Harvey (Harvey, 2000a) as being of national importance to *B. sylvarum* populations and was considered to be the key site for *B. humilis* and *B. sylvarum* populations in South Essex. The site has been targeted by the BWG as a priority for protection (Edwards, 2000) and despite the East of England Development Agency (EEDA) obtaining outline planning permission for the site, it was designated a SSSI in February 2005 with the majority of the site being preserved as a nature reserve (English Nature, 2005).

With the conservation value of this site recognised it is important that populations of *B. humilis* and *B. sylvarum* here are consolidated by the protection and enhancement of this and surrounding sites. Current understanding of metapopulation theory highlights the importance of several interconnected populations existing in a fragmented landscape (Hanski and Gilpin, 1991). Populations in fragmented habitats are sustained by immigration, local extinction and colonisation, and regional stochasticity (Opdam, 1990). It is believed that the presence of such a network of interconnected subpopulations greatly increases the probability of a species' persistence within a fragmented landscape (Bourn and Thomas, 2002) and preserves genetic heterozygosity (Usher, 1997).

Therefore, identifying sites surrounding the Canvey Wick site which also support *B. humilis* and *B. sylvarum* populations and mapping the extent of their distributions could be beneficial to the conservation of the Canvey Wick populations. By understanding the spatial distributions of current populations, conservation efforts can be targeted towards areas within and surrounding the current populations. By doing this, populations can be better protected, consolidated and potentially populations can be expanded to rebuild links between remaining UK populations. The potential for this rebuilding of links comes in the form of agri-environmental schemes (DEFRA, 2005a) developed through research into the benefits of improving bumblebee forage on agricultural land (Kells *et al.*, 2001; Carvell *et al.*, 2004; Pywell *et al.*, 2005). It is hoped that eventually

informed habitat restoration can reintroduce important bumblebee forage plants to much of the farmed landscape reconnecting nationally fragmented bumblebee populations (Carvell *et al.*, 2006).

The first stage of this project therefore was surveying sites to identify which supported populations of *B. humilis* and *B. sylvarum*. Once surveyed, the current South Essex populations were mapped to generate a tool for informing local landowners and planners of the current range of these populations. Also, by identifying sites currently being utilised by the bees and their relative values to foraging bees, it was possible to plan further stages of the research into individual species' ecological requirements by identifying the most appropriate sites for foraging surveys, nest surveys, habitat creation and molecular ecology studies.

2.2 Methods

Site surveys

Surveys investigating the presence or absence of *B. sylvarum* and *B. humilis* on sites across South Essex were carried out during the summer of 2003, 2004 and 2005. These involved slow 'bee walks' adapted from standard methodology (Banaszak, 1980; Saville *et al.*, 1997). Rather than walking a set line transect, walks started at the entrance of a site and systematically covered the whole site paying particular attention to patches supporting flowers known to be favoured for forage by the bees (Harvey, 1999). Whilst walking, bumblebee counts were carried out. Observations were made approximately 2m either side of the observer and speed of walking was about 10m per minute. The walks were replicated for each visit to the same site both in terms of distance and approximate duration.

Bee walks were carried out during July, August and September, the months reported in literature as being the main foraging period of *B. humilis* and *B. sylvarum* workers (Edwards and Jenner, 2006). All searches were conducted between 9:30 and 17:00 BST and during warm weather favourable to bumblebee

activity (temperatures greater than 15°C). Identification of the bees followed Prŷs-Jones and Corbet's Naturalist Handbook key (1987). *Bombus sylvarum* could be identified by observation whilst foraging, but to differentiate *B. humilis* from the similar colour groupings of *B. muscorum* and *B. pascuorum* it was necessary to capture individuals using Thorne Supplies (Thorne, 2007) queen bee marking plunger cages (Kwak, 1987) and identify species morphology using a field lens. If workers were old and worn, using abdomen hair colouration to differentiate between *B. humilis* and *B. pascuorum* was occasionally impossible. Under these circumstances the individuals were not included in the recordings.

Harvey's previous studies in the region demonstrated a divergent distribution between *B. humilis* and *B. muscorum* with *B. humilis* being restricted to the East Thames Corridor and *B. muscorum* being confined to the northeast and east of Essex (Harvey, 1998). Thus, it was not expected that *B. muscorum* would be observed during this study. Differentiating *B. humilis* from *B. muscorum* is generally done by the presence of dark hairs at the wing bases on the thorax. This method however is not considered entirely reliable (personal correspondence with Ben Darvill and the BWG). Occasionally during this study, individual bees thought to be *B. humilis* but without dark hairs on the abdomen were found. This was particularly the case for male bumblebees. When this occurred the individuals were collected and examined under a microscope to assess identity. This scenario was rare however, and the vast majority of bees were released after 5-10 seconds of observation.

Initial sites surveyed in 2003 were selected through consultation with Peter Harvey and from sites previously identified in his reports as supporting the bees (Harvey, 1998; 1999; 2000a). These included Wat Tyler Country Park (TQ737863), Hadleigh Castle Country Park (TQ800869), the Canvey Wick site (TQ768835), Vange Marsh North (TQ734874), the Old County Council Landfill site (TQ736872), the Untidy Industry site (TQ738874), Vange Hill (TQ721876), Creekside (TQ771862) and Two Tree Island (TQ825853).

Further sites were added to the surveys in 2004 and 2005: Vange Marsh South (TQ728863), Fobbing Marsh (TQ720836), the Hadleigh Castle Country Park side

of Belton Hills (TQ823860), the Southend District Council side of Belton Hills (TQ827860), Thames Barrier Park (TQ412799), the Cleanaway Pitsea Landfill site (TQ740857), Burnt Mills (TQ740906), Langdon Hills Country Park (TQ705867), One Tree Hill (TQ695859), Bells Hill Meadow (TQ707868), All Saints Church Yard (TQ715867), Pitsea Mount (TQ738877) and Swanscombe Peninsula, Kent (TQ605759).

GPS

Following the identification of sites utilized by *B. humilis* and *B. sylvarum* the sites were mapped using a Trimble differential mapping GPS system (Trimble, 2007). Known forage patches and site boundaries were walked and recorded. The spatially referenced data was then transferred to ArcGIS 9.0 (ESRI, 2007) mapping software to produce a map of sites supporting both *Bombus* species.

Timed indices

In July, August and September of 2003/4/5 timed surveys of foraging *B. humilis* and *B. sylvarum* individuals were carried out in order to assess the relative values of each site for the bees. By creating relative indices of bumblebee numbers it was possible to determine the value of sites to the bees and to assess whether the importance of individual sites varied within and between years. The surveys were carried out using modified 'bee walks' (Banaszak, 1980; Saville *et al.*, 1997) under the same climatic conditions as those for site surveys and at approximately the same time of day. These bee walks comprised of a thirty minute non-linear walking survey in a forage-rich area of approximately 300m^2 on each site. The number of individuals of *B. humilis* and *B. sylvarum* observed during the thirty minutes was recorded. The number of timed counts at each site is recorded in Table 2.2.

Unless observed bees are collected or marked during surveys it is impossible to know whether the same bees have been counted twice, but marking or collecting was considered an impractical technique for these surveys due to the time it would take impacting surveying time. For this reason the bee walks were carried out in a

slow and methodical manner in an attempt to avoid counting the same bee twice. It was considered that this combination of avoidance of counting the same individual and repeated methodology both within and between sites would generate comparative counts rather than actual counts. The counts therefore created a comparison of the relative value of the sites seasonally and annually in terms of the relative number of bees they supported.

The timed bee walks used the same identification techniques as the site surveys. Several counts were carried out on each forage patch and a mean bee count per hour was calculated for each site. The number of repeated surveys at each patch was dependent upon the number of visits to the particular site by the observer and the availability and duration of forage at each site. This meant that the number of repeated counts were not standardised across the sites, but as counts were only made when climatic conditions were favourable and forage was available to the bees, timed counts can be considered to be an accurate representation of bee numbers during times of maximum forage availability. Using this method therefore, it was possible to generate an index of the relative carrying capacity in terms of foraging workers of each site when forage was at its most abundant.

2.3 Results

Site surveys

Surveys identifying the presence or absence of *B. humilis* and *B. sylvarum* on sites in South Essex revealed a network of sites supporting both species. However, *B. sylvarum* and *B. humilis* were not recorded on several sites which were considered to be suitable for both bumblebee species based on habitat suitability described by Harvey (1998; 1999; 2000a) (results are displayed in Table 2.1).

Sites supporting populations of both *Bombus* species were plotted to produce a map of the South Essex *B. humilis* and *B. sylvarum* metapopulations (Figure 2.1). From this map it could be seen that the populations were centred on the Canvey

Wick site, Wat Tyler Country Park and Hadleigh Castle Country Park. The Old County Council site and Two Tree Island were also recorded supporting high numbers of the two species. A network of smaller sites supporting lower numbers of the bees was also recorded surrounding these sites. The maximum distance separating one of the identified sites from the others was recorded as being 2.8km (Burnt Mills to Pitsea Mount), although it is possible that smaller unrecorded 'stepping-stone' sites may have existed between the two. Sites further away than this were not recorded as supporting the bees even though habitat on the sites appeared to be suitable.

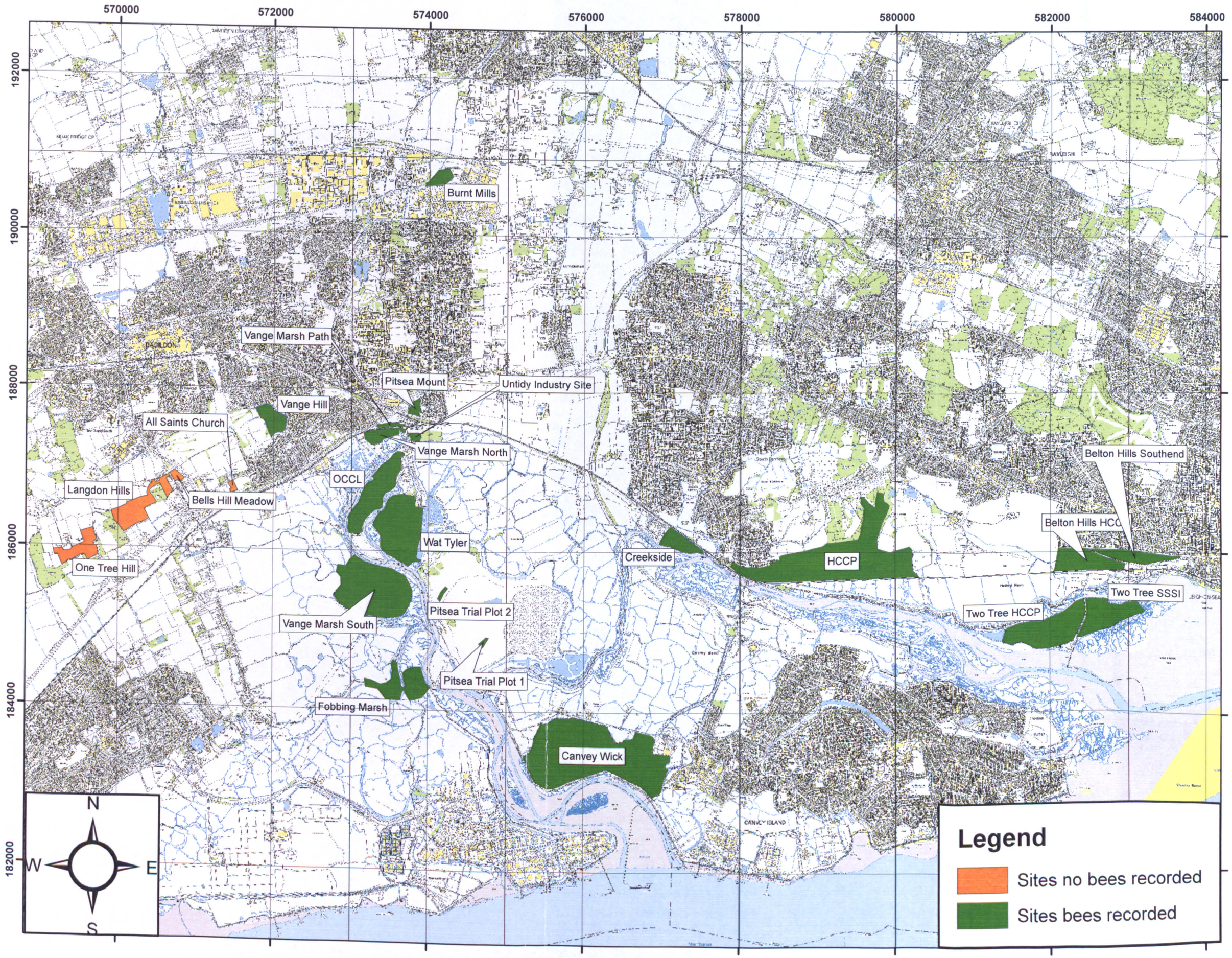
Table 2.1. Sites surveyed for the presence of *B. humilis* and *B. sylvarum* individuals in the East Thames Corridor (2003-2005). 'High numbers' = >50 workers observed, 'numbers' = between 10 and 50 workers, 'several workers' = from 3 to 9 workers (All sites surveyed were considered to contain suitable habitat to support both *Bombus* species)

Site Name	Grid Reference	*B. sylvarum* observed	*B. humilis* observed
Wat Tyler Country Park	TQ 737863	Yes, in high numbers	Yes, in high numbers
Hadleigh Castle Country Park	TQ 800869	Yes, in high numbers	Yes, in high numbers
Canvey Wick	TQ768835	Yes, in high numbers	Yes, in high numbers
Vange Marsh North *	TQ734874	Yes, in numbers	Yes, in numbers
Old County Council Landfill Site	TQ736872	Yes, in high numbers	Yes, in high numbers
Untidy Industry Site	TQ738874	No	Yes, several workers
Vange Hill *	TQ721876	Yes, in numbers	Yes, in high numbers
Two Tree Island (HCCP)	TQ823851	Yes, in numbers	Yes, in numbers
Two Tree Island (Essex Wildlife Trust)	TQ825852	Yes, in high numbers	Yes, in high numbers
Vange Marsh South	TQ728863	No	Yes, two workers
Fobbing Marsh	TQ720836	No	Yes, two workers
Creekside	TQ771862	Yes, several workers	Yes, in numbers
Belton Hills (HCCP)	TQ823860	Yes, several workers	Yes, several workers
Belton Hills (Southend Council)	TQ827860	Yes, several workers	Yes, several workers
Thames Barrier Park	TQ412799	No	Yes, in numbers
Burnt Mills	TQ740906	No	Yes one worker
Cleanaway Pitsea Landfill Site	TQ740857	Yes, in numbers	Yes, in high numbers
Langdon Hills Country Park	TQ705867	No	No
One Tree Hill Country Park	TQ695859	No	No
Bells Hill Meadow	TQ707868	No	No
All Saints Church Yard	TQ715867	No	No
Pitsea Mount	TQ738877	Yes, several workers	Yes, in numbers
Swanscombe Peninsula	TQ605759	No	Yes, in numbers

* - Bees were seen in these numbers in the first year of the study but changes in land management meant that similar numbers were not observed in later years.

Figure 2.1. Map of sites supporting the South Essex populations of *B. humilis* and *B. sylvarum*. (overleaf) Sites are those which were recorded as supporting populations of *B. humilis* and/or *B. sylvarum* during site surveys.

Bombus humilis and B. sylvarum forage sites, South Essex



Site indices

The number of timed surveys carried out on each site is recorded in Table 2.2.

Table 2.2. Number of timed bee counts at each site, 2003-2005. Record of the number of timed bee counts that mean timed *B. humilis* and *B. sylvarum* sightings were calculated from in Figures 2.2-2.5.

	Number of times each site was surveyed (n)				
Site	2003	2004	2005	Aug 04	Sept 04
Canvey Northwick	12	9	8	5	3
Canvey Northwick scrapes	3	4	3	2	2
Creekside	2	4	2	2	2
HCCP	7	6	10	4	2
OCCL	2	5	4	2	3
Two Tree HCCP	3	6	3	3	3
Two Tree EWT	3	6	4	3	3
Vange Hill	4	4	2	2	2
Vange Marsh North	3	4	2	2	2
Wat Tyler Country Park	7	5	7	3	2

Results of indices counts revealed that mean counts of *B. humilis* and *B. sylvarum* varied both annually between sites (Figures 2.2 and 2.3) and seasonally within the same site (Figures 2.4 and 2.5).

Mean counts represented in Figures 2.2 and 2.3 demonstrated the value of the Canvey Wick site for supporting foraging *B. humilis* and *B. sylvarum*. They also demonstrated similar consistently high counts for both species at Hadleigh Castle Country Park (HCCP), Two Tree Island (EWT) and Wat Tyler Country Park. *Bombus humilis* counts were also high at the Old County Council Landfill site (Figure 2.2) but the site was only recorded as having large numbers of *B. sylvarum* in 2005 (Figure 2.3).

Results also demonstrated that the value of sites to the bees varied considerably throughout colony cycles and between years. This was particularly evident when

trends in annual bumblebee counts for sites were compared. Figure 2.2 shows that mean counts of *B. humilis* at the Canvey Wick site decreased gradually each year from 2003 to 2005, but at the Old County Council Landfill site and HCCP the numbers increased during the same period. These patterns were also witnessed for *B. sylvarum* (Figure 2.3) with mean counts decreasing gradually at the Canvey Wick site and increasing at the Old County Council Landfill site. Also of interest was the pattern of the highest annual mean numbers of the species at each site. The lowest mean numbers of both *B. humilis* and *B. sylvarum* at the Two Tree Island EWT site were recorded in 2004, but at Wat Tyler Country Park the highest mean numbers for both species were recorded in the same year.

Seasonal variations in the mean number of bees supported by each site are shown in Figures 2.4 and 2.5. From August to September 2004 highest mean numbers of foraging bees appeared to shift from the main sites of Canvey Wick, Wat Tyler Country Park and Hadleigh Castle Country Park, to the Old Country Council Landfill site, Creekside and Two Tree Island. Observational studies indicated that these changes appeared to coincide with forage availability on the sites.

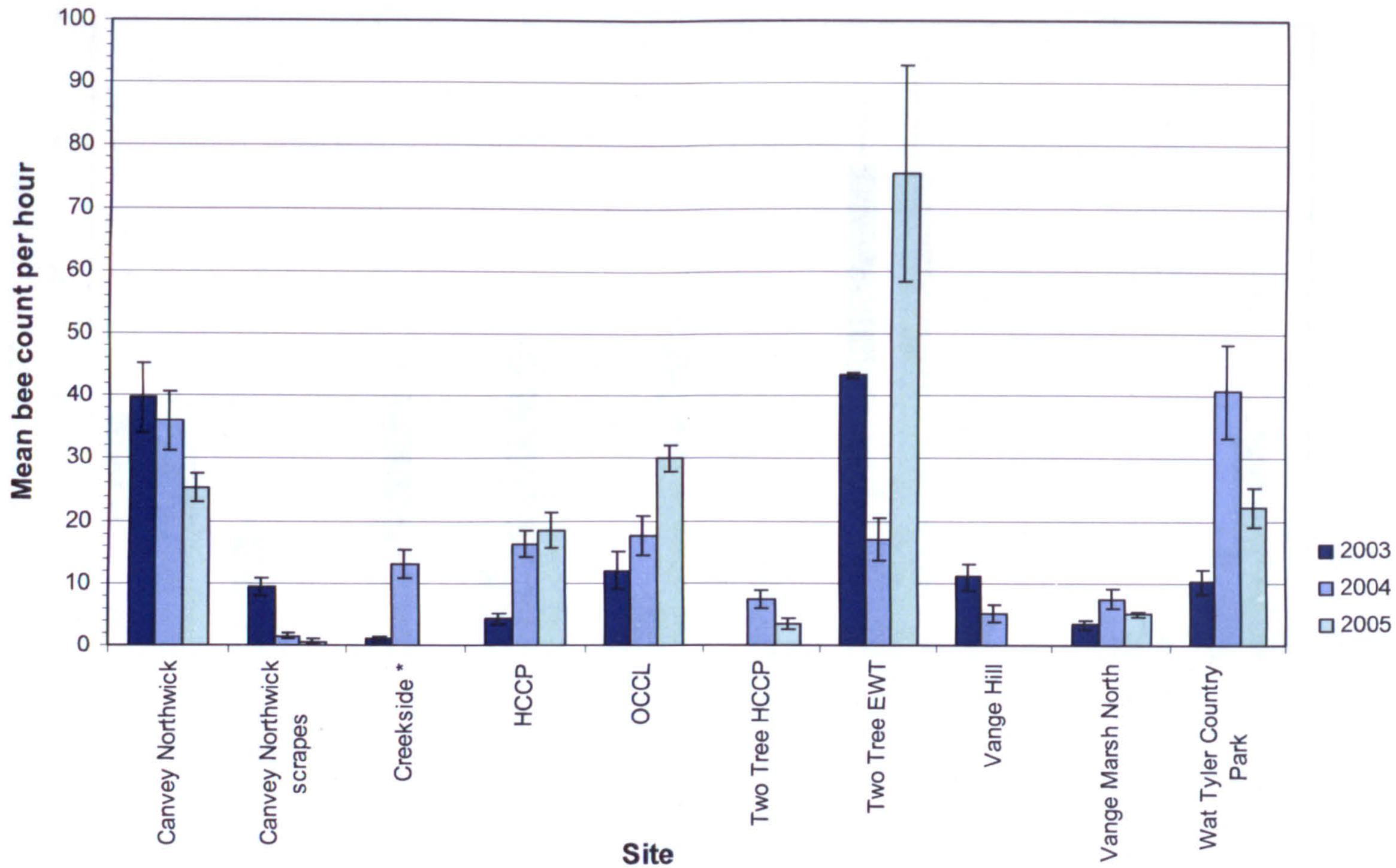


Figure 2.2. Mean timed *B. humilis* sightings 2003-2005. Values represent mean annual number of bees recorded at each site during timed walks over approximately equal sized forage rich areas. Values were recorded as number of bees observed per hour. Sampling effort varied at each site dependent upon frequency of visits and availability of forage. Sampling effort (n) is recorded in the Table 2.2. [Error bars represent standard error of the mean] (* A fire at Creekside in 2005 meant that there was no available forage).

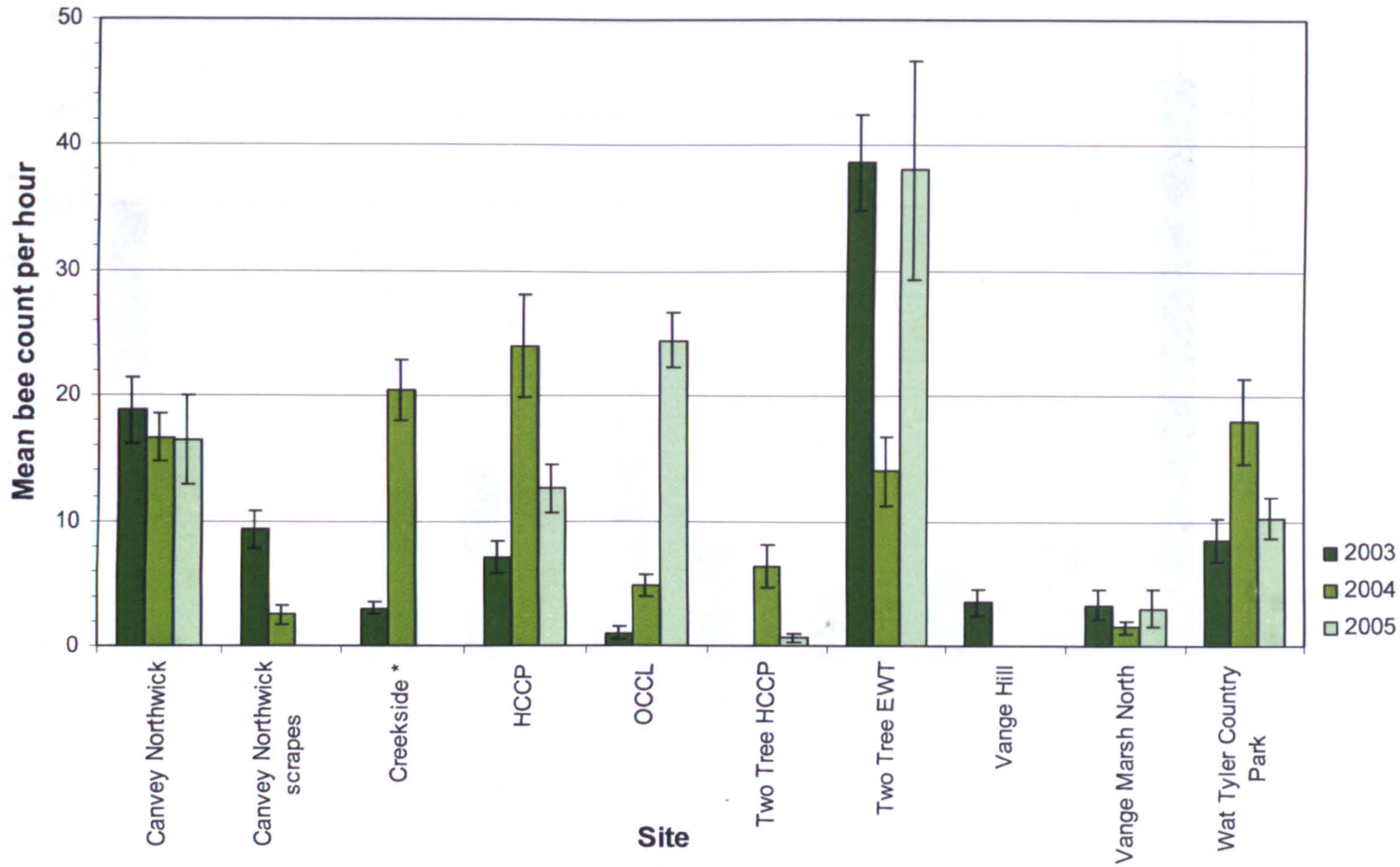


Figure 2.3. Mean timed *B. sylvarum* sightings 2003-2005. Values represent mean annual number of bees recorded at each site during timed walks over approximately equal sized forage rich areas. Values were recorded as number of bees observed per hour. Sampling effort varied at each site dependent upon frequency of visits and availability of forage. Sampling effort (n) is recorded in the Table 2.2. [Error bars represent standard error of the mean](* A fire at Creekside in 2005 meant that there was no available forage)

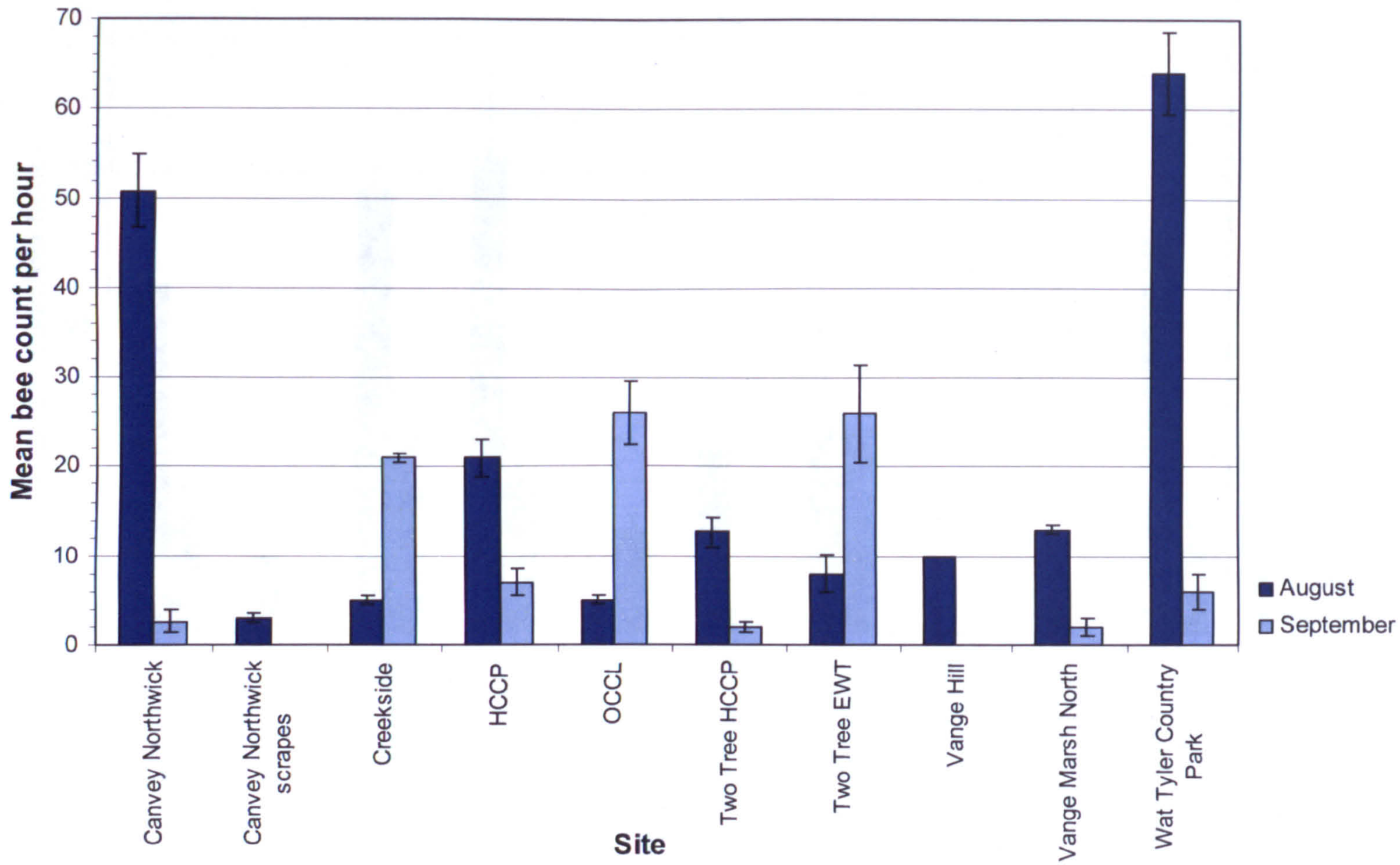


Figure 2.4. Mean timed *B. humilis* sightings, August and September 2004. Values represent maximum number of bees recorded at each site during timed walks over approximately equal sized forage rich areas throughout one forage season. Values were recorded as number of bees observed per hour. Sampling effort varied at each site dependent upon frequency of visits and availability of forage. Sampling effort (n) is recorded in the Table 2.2. [Error bars represent standard error of the mean]

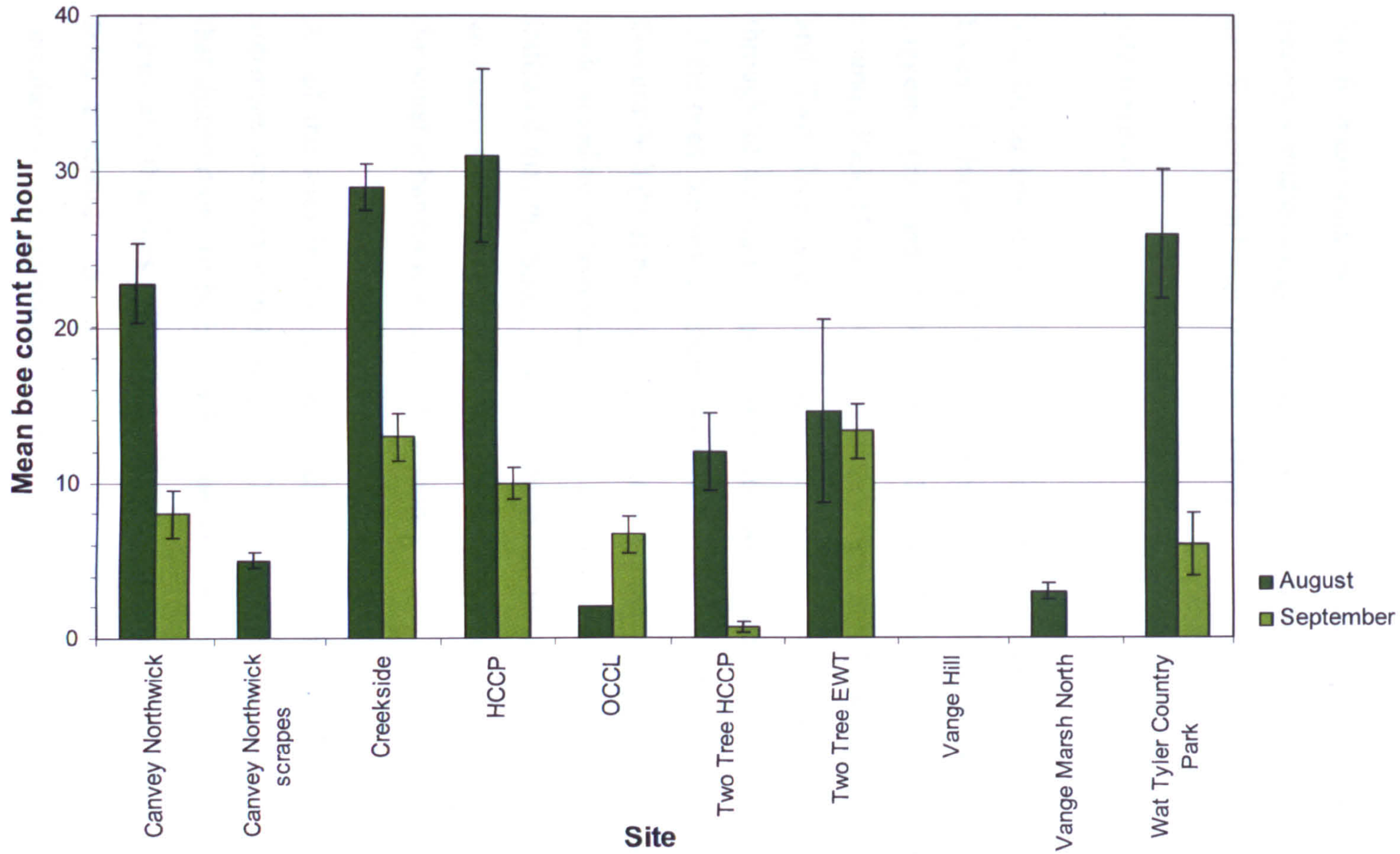


Figure 2.5. Mean timed *B. sylvarum* sightings, August and September 2004. Values represent maximum number of bees recorded at each site during timed walks over approximately equal sized forage rich areas throughout one forage season. Values were recorded as number of bees observed per hour. Sampling effort varied at each site dependent upon frequency of visits and availability of forage. Sampling effort (n) is recorded in the Table 2.2. [Error bars represent standard error of the mean]

2.4 Discussion

Bombus muscorum

No *B. muscorum* were observed in this study. This corresponded with Peter Harvey's studies suggesting that there is a differential distribution of *B. muscorum* and *B. humilis* in Essex (Harvey, 1998).

Site surveys

The initial site survey identified the Canvey Wick site as central to the South Essex *B. humilis* and *B. sylvarum* metapopulations as had been previously suggested (Edwards, 1999; Harvey, 2000a). The surveys also identified Wat Tyler Country Park, Hadleigh Castle Country Park, the Old County Council Landfill site and Two Tree Island as supporting strong populations of both bee species throughout the study years. Other sites were identified supporting lower numbers of the bees, but several sites surveyed surrounding this region were found to have favourable habitat but were not found to support these bees (Table 2.1). If current understanding of favoured habitat by *B. humilis* and *B. sylvarum* was accurate, this indicated that the South Essex metapopulations were already fragmented to such an extent that inter-patch distances were preventing the bees from utilising all of the suitable habitat patches in the region.

All of the sites in the region which were found to support *B. humilis* and *B. sylvarum* are shown in Figure 2.1. The extent of sites on the map demonstrated that distributions of both *Bombus* species were restricted in this region of South Essex and that sites supporting them were fragmented within the landscape. Data from a separate *B. sylvarum* study has demonstrated that remaining UK populations are genetically isolated (Ellis *et al.*, 2006) and evidence from a *B. muscorum* study has estimated that 10km might be sufficient distance to isolate bumblebee populations (Darvill *et al.*, 2006). With remnant UK populations of both *B. humilis* and *B. sylvarum* spaced at much greater distances than this (Prŷs-Jones and Corbet, 1987), it seems likely that should this population disappear, the

distances required for natural recolonisation of the region from other UK populations would represent an impossible barrier.

Without population estimates and an accurate knowledge of the spatial dynamics of the bees, particularly with respect to migration and foraging distances, it is impossible to know whether existing sites are sufficiently close to each other to indefinitely support *B. humilis* and *B. sylvarum* metapopulations in the region. If not, this population may also be in similar decline to those seen nationally. To understand the dynamics of the populations, monitoring and investigation of the spatial dynamics of the bees within the fragmented landscape is required. With such knowledge, more informed conservation management could be implemented in the region. Monitoring and spatial dynamics are discussed further in subsequent chapters.

With or without this knowledge of spatial dynamics however, what is apparent from this initial study is that greater fragmentation, potentially removing the numerous smaller stepping-stone sites connecting the main sites, could impact populations in the region. Protecting and enhancing all of the sites identified in the study as well as creating new habitat should be a priority to ensure the future of the bees in the region.

Further evidence for the need to conserve all of the sites came from the indices counts. The initial method of presence/absence surveying could not be used to identify the relative importance of each site in terms of the number of bumblebees they supported. Timed counts were therefore used to produce relative indices values for each site to give a more accurate impression of how they varied within and between years (Figures 2.2 – 2.5). As expected, the results confirmed the value of the Canvey Wick site to *B. humilis* and *B. sylvarum* populations (Edwards, 1999; Harvey, 2000a), but equally the value of a range of other sites surrounding Canvey Wick; Wat Tyler Country Park, Hadleigh Castle Country Park, the Old County Council Landfill and Two Tree Island.

Whilst the importance of the Canvey Wick site to the area cannot be denied due to its size and abundance of forage, index counts recorded equally high or higher

numbers of *B. humilis* and *B. sylvarum* on surrounding sites. The value of a range of sites was particularly evident when indices counts were used to compare annual and seasonal trends in bee numbers. Annual trends (Figures 2.2 and 2.3) demonstrated that while mean bee numbers were decreasing annually on some sites (including the Canvey Wick site) they were increasing on others. Also, years with the highest number of bees recorded on a site were not consistent across all sites. Whilst some sites had poor years in terms of mean numbers of both *B. humilis* and *B. sylvarum*, other sites recorded their highest numbers for the species during the same year. The importance of a range of sites was also demonstrated when comparing sites seasonally to show changes in the value of sites throughout the main worker forage periods of August and September (Figures 2.4 and 2.5). Results indicated that whilst the main three sites (Canvey Wick, HCCP and Wat Tyler) were the most important for foraging workers in August, during September the OCCL site and Two Tree Island became much more important for foraging bees as forage availability declined at the other sites.

These findings appeared to demonstrate that, rather than protecting one key site, conserving all of the sites currently supporting the *B. humilis* and *B. sylvarum* metapopulations in the East Thames Corridor would be the most effective method to protect the range of habitats required by the bees. These results mirrored those reported in other studies (Sawchick *et al.*, 2002; Diekötter *et al.*, 2006), showing that the relative value of sites fluctuates both within a season and annually and that individuals move within the landscape to occupy the most suitable forage patches. If we are to provide continuous forage for both *Bombus* species therefore, it is necessary to maintain a range of sites with varying ability to provide forage under a range of climatic and seasonal conditions. In this way it is possible to provide insurance that if forage fails on one site there are numerous other sites within the range of the metapopulation from which the bees can forage.

The risk of dependence on a single site was highlighted by the Creekside populations of both *Bombus* species in 2005. A fire at this site removed all of the available forage in the early summer and no individuals of either species were recorded here during the 2005 surveys. A visit to the site in 2006 revealed that forage was again available and numbers on the site were at least as high as

previously recorded. Presumably recolonisation had occurred through individuals from a nearby site which had produced suitable forage in 2005.

Another observation from this initial study was with regards to the geographical distribution of the population. Remaining national populations of *B. humilis* and *B. sylvarum* have been observed to be predominantly coastal in distribution (Harvey, 2000a; Edwards, 2004). This had also been found to be the case for populations in the East Thames Corridor, with no sites being recorded further than 10km from the River Thames in previous studies (Harvey, 2000a). Results from the initial site surveys support these previous findings. The furthest site from the Thames that was surveyed was Burnt Mills. Burnt Mills is situated approximately 8.5km away from the River Thames. Even though surveys revealed this site had abundant forage resources for both *B. humilis* and *B. sylvarum*, only one *B. humilis* worker was recorded on the site in 2003. No *B. sylvarum* were recorded on the site and no further *B. humilis* workers were recorded in subsequent years. The availability of suitable forage but lack of the bees indicated that Burnt Mills might be too distant from the main metapopulation and that this is the edge of both *Bombus* species' distribution in the region.

Most recent national records of *B. humilis* and *B. sylvarum* have been coastal and it has been suggested that this may not entirely be due to the survival of suitable habitat in such regions (Harvey, 2000a). Other factors such as climate might be influencing the trends. This certainly seems to be the case in South Essex where the bees are distributed on the warmer drier coastal sites (DEFRA, 2007). Such a distribution would be expected if, as suggested by Williams (2005), *B. humilis* and *B. sylvarum* are operating in the UK at the edge of their climatic range.

Another explanation for this phenomenon of coastal distributions might be due to coasts and large bodies of water acting as leading lines for migrating bumblebees. Evidence for migration in bumblebees has been fairly anecdotal. However, the recent colonisation of the UK by *Bombus hypnorum*, presumably from France, has been suggested as evidence of bumblebee migrations (Goulson and Williams, 2001). Whether or not *B. hypnorum* naturally colonised from mainland France or was accidentally imported by man on horticultural products, the speed of its

apparent spread throughout southern UK (BWARS, 2007) has provided evidence for the ability of these bees to migrate and colonise suitable habitat. Similar patterns of colonisation have also been observed in New Zealand following bumblebee introductions in 1885 (Goulson, 2003). Numerous observational studies have suggested that coasts seem to act as leading lines for bees during migration (Luckham, 1906; Birket, 1956; Owen, 1956; Philp, 1957; Rudebeck, 1965; Mikkola, 1978), but have provided no evidence explaining such dispersal patterns. A more recent study by Davill *et al.* (2006) demonstrated that 3km was enough distance to prevent gene flow and therefore migration between Hebridean islands. This indicated that large bodies of water might act as barriers to the dispersal of at least some bumblebee species and might explain why coast lines act as leading lines for migration.

If bumblebees do migrate and large bodies of water act as barriers to this, the leading line effect of coasts would concentrate the migrating bumblebees in such areas. Coastal sites consisting of suitable foraging and nesting habitat would therefore be more likely to be located by an increased proportion of the migrating bees. Such action would therefore effectively decrease the distance between suitable habitat patches compared to a similar inland migration system by increasing the likelihood of a high proportion of migrating bees locating the same patch and therefore increasing the probability of effective immigration and colonisation of a site (Gilpin, 1987). This could be of particular importance to *Bombus* species with declining populations. Currently this 'leading lines' theory is hypothetical and further research into the dynamics and distances involved in bumblebee migrations, particularly those of rarer bumblebees, would be needed before any conclusions could be made. Whether it is the leading effect of water bodies or the warm dry climate however, the Thames and Essex coastline provide an ideal location for habitat creation and attempts to increase the distribution of the current populations.

What was certain from this initial study is that the South Essex area continues to support nationally important populations of *B. humilis* and *B. sylvarum* whilst they have disappeared elsewhere in the UK (Williams, 1982). The reason for this must in part be due to habitat conditions favoured by the bees being abundant in the

area. These habitat associations have been investigated in the subsequent chapters of this investigation. Without this knowledge of habitat requirements however, what can be summarised from this chapter's data is that it is likely that the fragmented habitat in South Essex mimics the minimum $10km^2$ threshold of continuous habitat that has been reported as a requirement for the remaining *B. sylvarum* populations across the UK (Edwards, 2000) and that further fragmentation of the region could threaten the future of the South Essex populations of both *Bombus* species.

Chapter 3

Forage preferences

3.1 Introduction

Bumblebees are entirely dependent upon nectar and pollen as food resources (Goulson and Darvill, 2004). These resources are obtained from flowers and studies have identified the availability of a succession of suitable flowers as a major factor contributing to colony success of bumblebees (Bowers, 1985b; Pelletier and McNeil, 2003). The diversity of bee species in an area has been linked to the floral diversity of the area (Banaszak, 1983; Bowers, 1985b; Kells *et al.*, 2001) and reductions in forage availability due to the loss of suitable habitat to agricultural intensification is widely accepted as being the main cause of the decline in British bumblebees (Williams, 1986; Osborne *et al.*, 1991; Osborne and Corbet, 1994; Goulson, 2003; Goulson *et al.*, 2005). These changes in agriculture are thought to have caused declines in a number of highly preferred bumblebee forage plants, especially members of Fabaceae and Lamiaceae (Rich and Woodruff, 1996; Pywell *et al.*, 2005; Carvell *et al.*, 2006). All of these studies have highlighted the importance of the availability of suitable, adequate and continuous forage resources for the successful development of bumblebee colonies, and thus provision of such resources are a priority for the conservation of rare bumblebees (further discussion on this topic can be found in the introduction, section 1.4).

Due to the practical limitations of studying rare bumblebees most studies of bumblebee ecology and foraging behaviour have focused on the more ubiquitous species. In Europe, this has included *B. terrestris*, *B. lucorum*, *B. lapidarius*, *B. pascuorum*, *B. hortorum* and *B. pratorum* (Croxton *et al.*, 2002; Dramstad and Fry, 1995; Hirsch *et al.*, 2003). The remaining species have received little attention. To conserve populations of declining bumblebees an understanding of their foraging preferences is required, specifically whether they differ from those of the more ubiquitous species. Although there have been some studies of *B. humilis* and *B. sylvarum* foraging behaviour (Williams, 1989; Carvell, 2002; Goulson and Darvill, 2003; Chapman, 2004), with the exception of Chapman's study of *B. humilis* ecology on urban sites, the studies have either been carried out over short time periods or on large continuous grassland sites where these species have been a rarity compared to the more ubiquitous species. The South Essex population

provided an ideal opportunity to study the foraging behaviour of these bees in a fragmented landscape on a variety of sites where the bees were still abundant. Initial observations in the region had been made in a provisional study by Peter Harvey (1999), but this study was restricted in duration and scope and concluded that further research in the area was needed to accurately assess the forage requirements of these bees.

The principle aim of this initial study was to identify the foraging preferences of *B. humilis* and *B. sylvarum* across the South Essex sites. *Dietary preference* is defined as the choice of one forage species over another when both are available and abundant but, if one or the other forage species is absent, the other species is able to be used (section 1.5.1). Analysis of dietary preference would therefore require experimental manipulation of forage resources. Numerous previous studies have demonstrated that bumblebees make active floral choices when foraging (Banaszak, 1983; Fussell and Corbet, 1991; 1992b; Carvell, 2002; Goulson *et al.*, 2005), it was therefore likely that bees observed foraging on flowers in this investigation were actively choosing where to forage and on which floral species. However, due to the field based nature of the study, the patchy dynamics of forage on the sites and the unknown spatial scales over which the bees operate, it was decided that a comparison of floral availability with forage choices would not have generated an accurate representation of the forage choices facing the bees. For this reason, no record of flower abundance on sites was made.

Therefore, in the absence of a more accurate technique, direct observational surveys were carried out to identify the floral species being most frequently visited by the bees. By carrying out such observational surveys of foraging bees, the flowers that bumblebee queens, workers and males were visiting could be assessed in terms of their proportion of all *B. humilis* and *B. sylvarum* floral visits. Based on these observations and the fact that both *B. humilis* and *B. sylvarum* persist in the South Essex area when they have disappeared from much of the rest of the UK, it was assumed that floral species visited by the bees were suitable for the individual *Bombus* species' persistence. Floral species identified were therefore considered to represent the foraging behaviour of *B. humilis* and *B. sylvarum* workers, queens

and males and could be included in conservation initiatives to provide suitable forage throughout colony development.

3.2 Methods

Once sites supporting populations of *B. humilis* and *B. sylvarum* were identified (map Figure 2.1) surveys were carried out on each site to record flower visits by *B. humilis*, *B. sylvarum* and any other *Bombus* species observed foraging on the sites. For the purpose of this study however, only sightings of *B. humilis* and *B. sylvarum* were utilized.

The surveys comprised of a modified version of the bee walk transects used by Banaszak (1980) and Saville *et al.* (1997). The bee walk methodology was modified because forage distribution on the sites was considered to be too patchy and discontinuous for linear walks to be used. Non-linear walks encompassing the main flowering patches within the sites were considered to be a more effective method for observing bees on the sites. The walks involved bumblebee counts whilst walking through areas of suitable forage. Observations were made approximately 2m either side of the observer and speed of walking was about 10m per minute. Walks were replicated for each visit to the same site both in terms of distance and approximate duration, so that within-site records were comparable.

Bee walks were carried out from July to October in 2003 and from April to October in 2004 and 2005 to identify floral use during all stages of *B. humilis* and *B. sylvarum* life cycles:

i) queen nest initiation and production of first workers,
ii) foraging workers and colony development,
iii) production of males and gynes (young queens) and the end of the colony.

The survey dates were based on the months reported in literature as being the main foraging period of *B. humilis* and *B. sylvarum* workers (Prŷs-Jones and Corbet, 1987), observations of regional trends by Peter Harvey (personal discussion) and on observed timings from the surveys in 2003. All searches were conducted between 9:30 and 17:00 BST and during warm weather favourable to bumblebee activity (temperatures greater than 15°C).

Identification of the bees followed Prŷs-Jones and Corbet's Naturalist Handbook key (1987). Bees which could not be identified whilst foraging were captured using Thorne Supplies (Thorne, 2007) queen bee marking plunger cages (Kwak, 1987) and were identify by species morphology using a field lens. Problems of differentiating between *B. humilis*, *B. pascuorum* and *B. muscorum* were solved as discussed in chapter 2 (section 2.2). Identification of flowers followed Stace (1997).

Initial sites surveyed in 2003 were selected through consultation with Peter Harvey and from sites previously identified in his reports as supporting the bees (Harvey, 1998; 1999; 2000a). Further sites were added to the surveys in 2004 following the 2003 site surveys. Sites are listed in Table 3.1.

During the bee walks the species, caste and number of bees observed were recorded. The species of flower each bee was seen foraging on and the position of the forage on the site were also recorded. The surveying technique was repeated at each site, all surveys were carried out within the South Essex metapopulation distributions and the floral species available were fairly consistent across all of the sites (appendix 12.1) so it was assumed that all of the bees recorded were feeding with the same foraging choices.

It was intended that data generated would be compared to national results (Williams, 1989; Carvell, 2002; Goulson and Darvill, 2003) and to studies done by Peter Harvey in the region (Harvey, 1999). The results would be used to identify which floral families and species are the preferred forage sources for *B. humilis* and *B. sylvarum* in the South Essex area.

Table 3.1 List of sites surveyed, 2003 - 2005. Sites on which bee walks were carried out to record flower visits by *B. humilis* and *B. sylvarum*.

Name of site	OS grid reference
Burnt Mills	TQ740906
Canvey Wick site	TQ768835
Cleanaway Pitsea Landfill site	TQ740857
Creekside	TQ771862
Fobbing Marsh	TQ720836
Hadleigh Castle Country Park	TQ800869
Hadleigh Castle Country Park side of Belton Hills	TQ823860
Old County Council Landfill site	TQ736872
Southend District Council side of Belton Hills	TQ827860
Two Tree Island	TQ825853
Untidy Industry site	TQ738874
Vange Hill	TQ721876
Vange Marsh North	TQ734874
Vange Marsh South	TQ728863
Wat Tyler Country Park	TQ737863

3.3 Results

Results of observed foraging visits are presented as relative number of total visits made to each flower species. Results for all *B. humilis* and *B. sylvarum* observations across all sites are summarised below:

For management guidance of South Essex sites, individual site records of 2003 and 2004 *B. humilis* and *B. sylvarum* floral foraging visits were included in the appendix (section 12.1).

Bombus humilis workers

Bombus humilis workers were observed foraging on a fairly broad variety of flowers. From Figure 3.1 the key floral species for the bees in terms of relative visits appeared to be *Ballota nigra, Lotus glaber* and *Odontites verna* with

Trifolium pratense also receiving a high proportion of visits. *Lathyrus latifolius*, a *Lavandula* species, *Medicago sativa* and *Centaurea nigra* were also fairly commonly visited by foraging *B. humilis* workers.

In terms of plant families, Figure 3.2 demonstrated that Fabaceae, Lamiaceae and Scrophulariaceae received almost all of the visits from *B. humilis* workers. Of these, Fabaceae appeared to be the most favoured. Asteraceae also received a small proportion of the visits.

In total, *B. humilis* workers were recorded foraging on 45 floral species from 15 plant families. The majority of these species and families were recorded as receiving very few visits from these bees and were thus not considered priority target species for conservation efforts.

Bombus sylvarum workers

Ballota nigra, Lotus glaber and *Odontites verna* appeared to be the favoured flower species of foraging *B. sylvarum* workers (Figure 3.3). *Melilotus officinalis* was also observed as receiving a proportionally high number of visits but only in the 2003 surveys. *Trifolium pratense* was not observed to be a preferred foraging resource for *B. sylvarum* workers.

Bombus sylvarum workers foraged relatively equally on the plant families Fabaceae, Scrophulariaceae and Lamiaceae (Figure 3.4), more so perhaps than *B. humilis* workers which seemed to favour Fabaceae flowers.

In total, *B. sylvarum* workers were recorded foraging on 25 floral species from 8 plant families. The majority of these species and families were recorded as receiving very few visits from these bees and were thus not considered priority target species for conservation efforts.

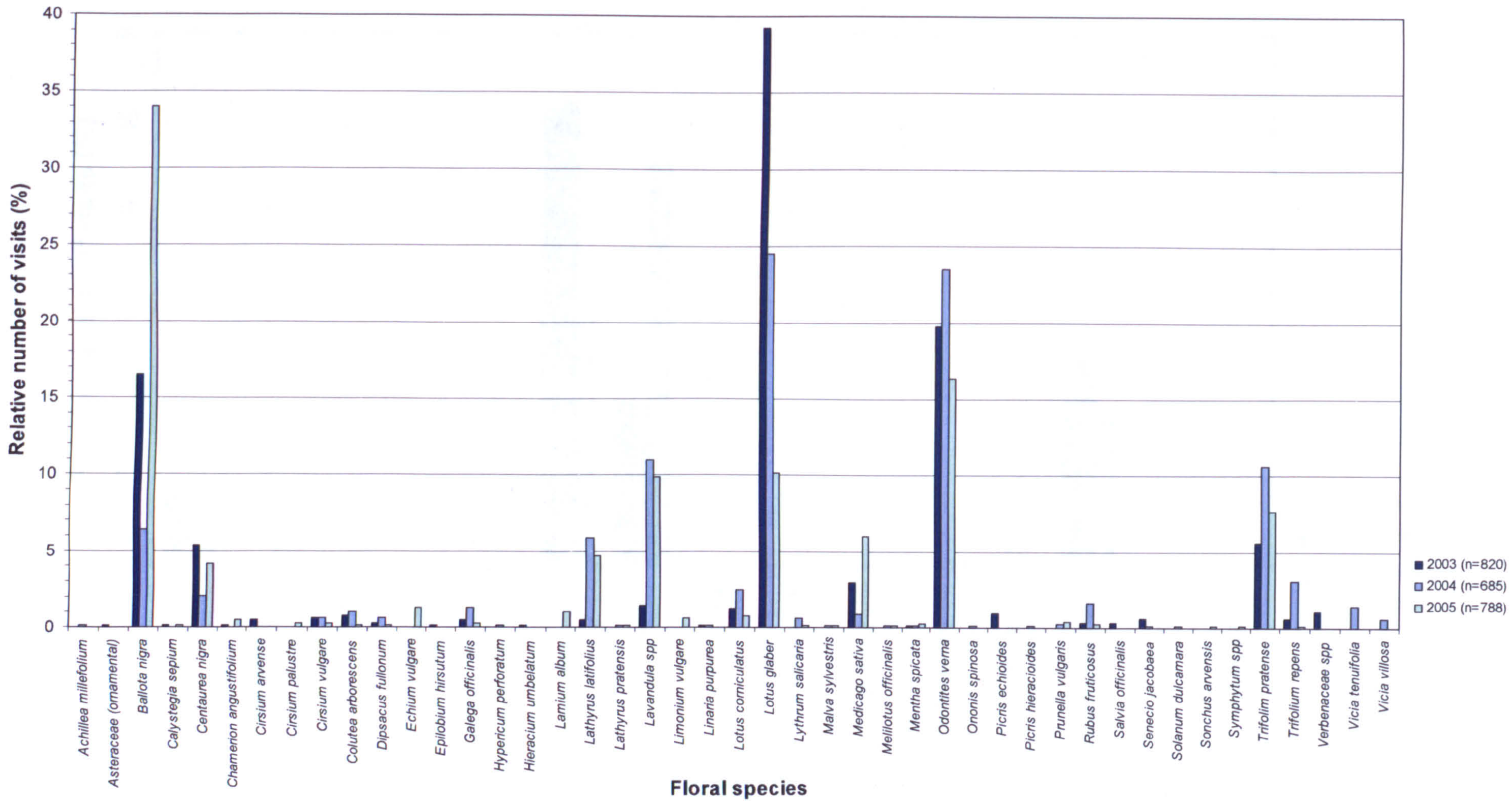


Figure 3.1. Relative plant species visits of *B. humilis* workers 2003-2005.
Values represent the proportion of *B. humilis* worker flower visits observed to each flower species across all sample sites.

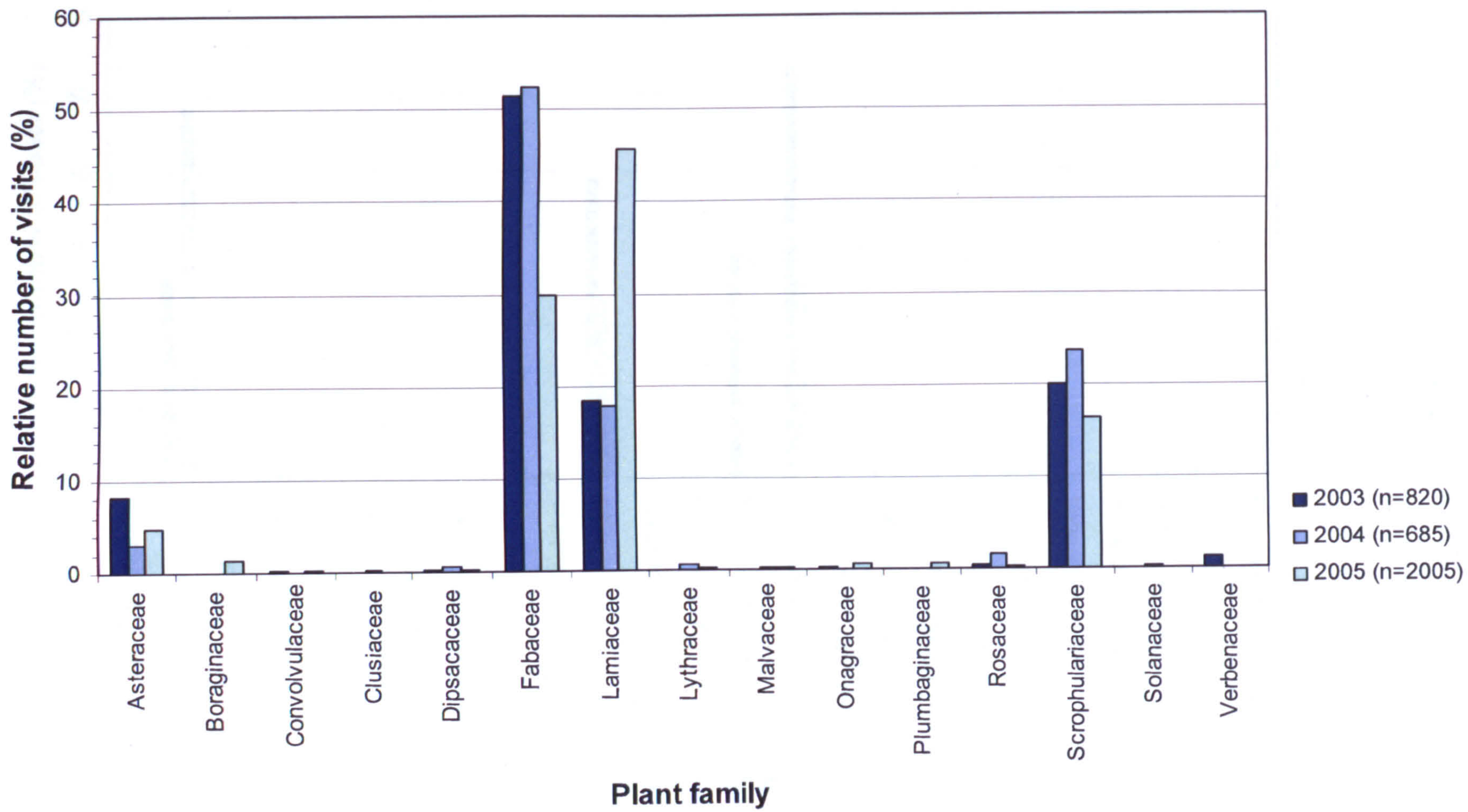


Figure 3.2. Relative plant family visits of *B. humilis* workers 2003-2005.
Values represent the proportion of *B. humilis* worker flower visits observed to each flower species across all sample sites.

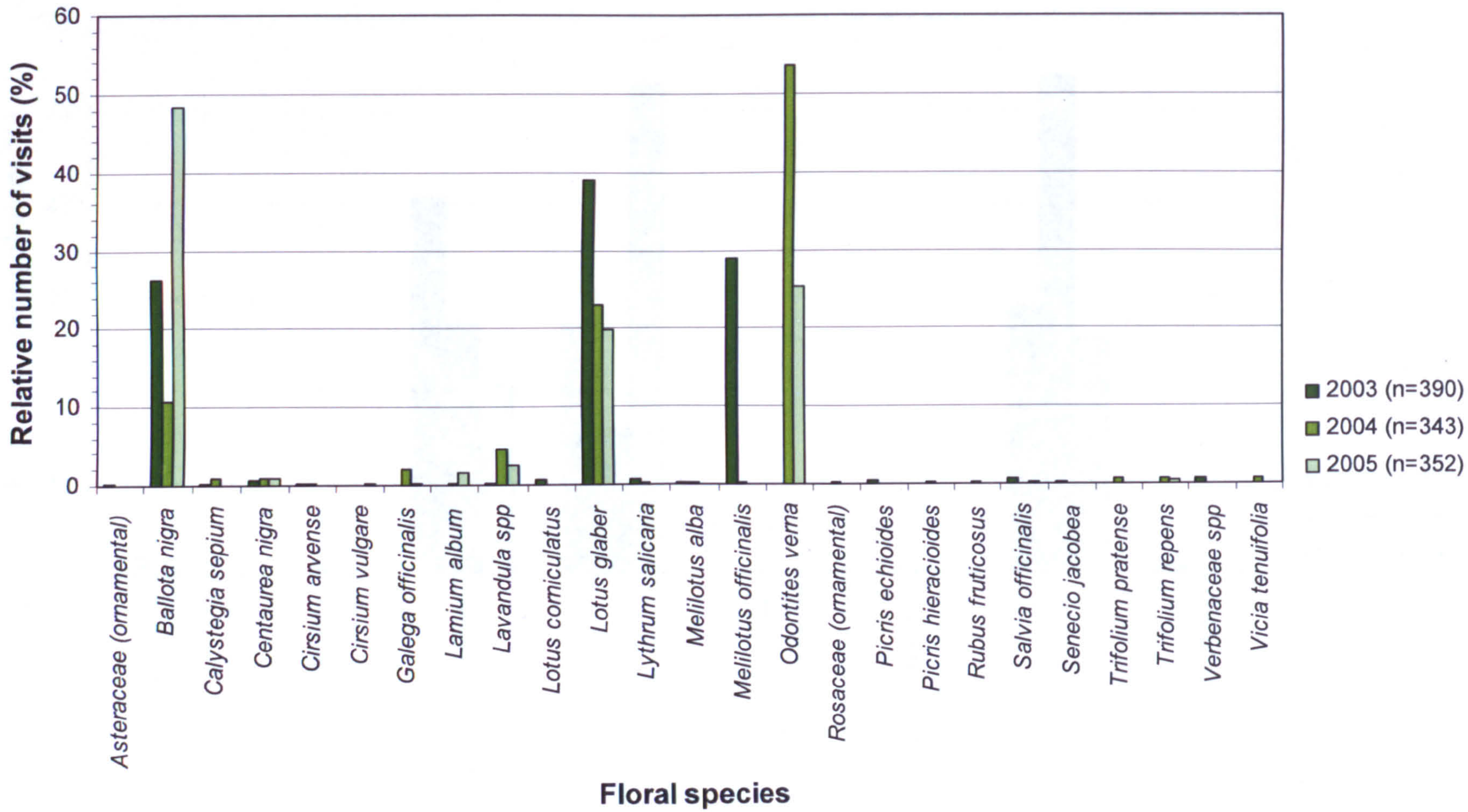


Figure 3.3. Relative plant species visits of *B. sylvarum* workers 2003-2005.
Values represent the proportion of *B. humilis* worker flower visits observed to each flower species across all sample sites.

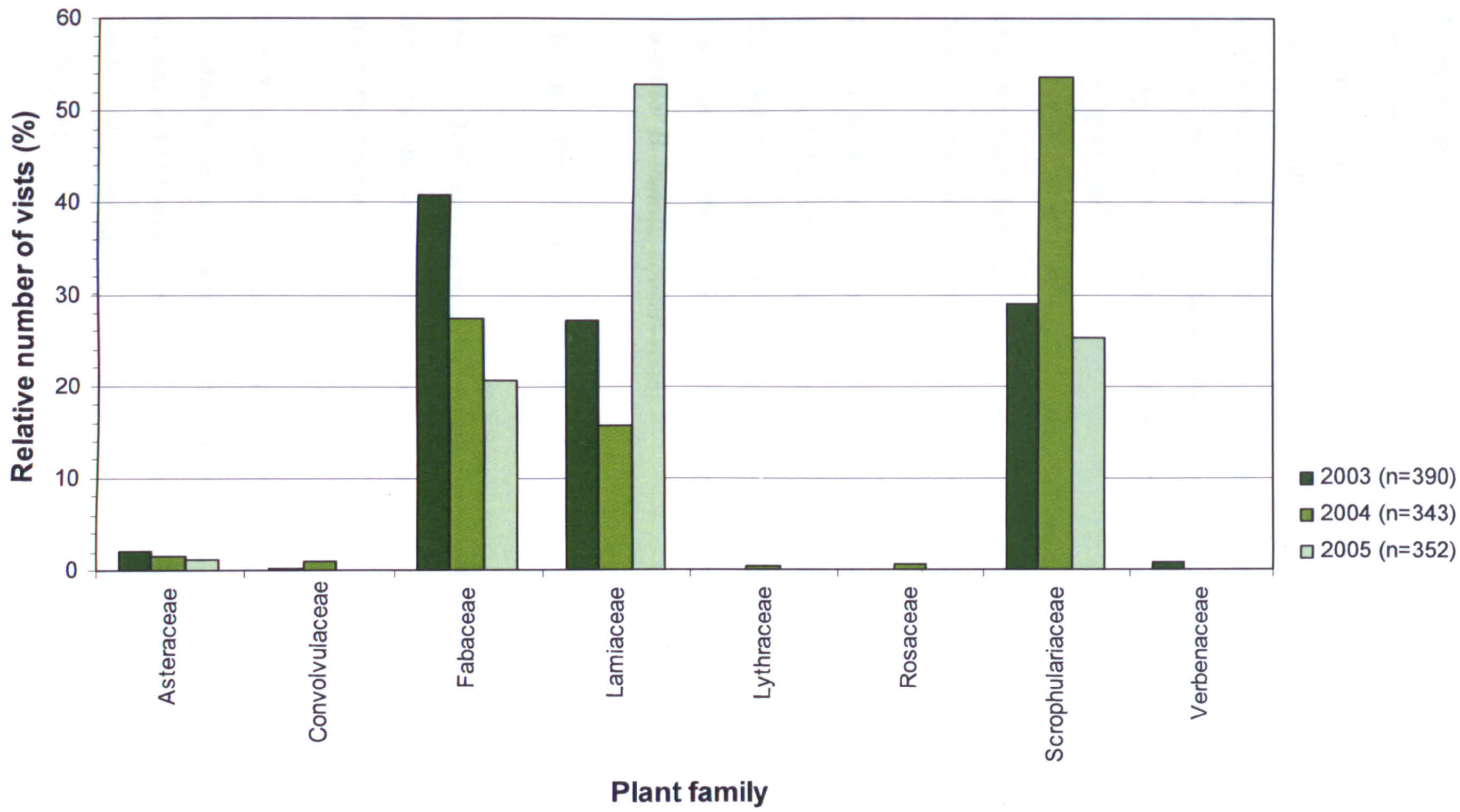


Figure 3.4. Relative plant family visits of *B. sylvarum* workers 2003-2005.
Values represent the proportion of *B. sylvarum* worker flower visits observed to each flower family across all sample sites.

Bombus humilis queens

From the data in Figure 3.5 it was possible to conclude that *Lathyrus latifolius*, *Trifolium pratense*, *Vicia sativa* and *Vicia villosa* were the most important forage sources for *B. humilis* queens. Almost all of the visits were recorded to these floral species. In 2003, *Lotus glaber* received a proportionally high number of visits. The total number of observed queens that year was only 5 though, and when combined with the results from 2004 and 2005 it was possible to conclude that the number of visits to *L. glaber* was small and was perhaps just a product of the late start to surveying in 2003.

Data for plant families (Figure 3.6) supported these findings with forage visits being recorded almost exclusively to Fabaceae flowers.

In total, *B. humilis* queens were recorded foraging on 17 floral species from 6 plant families. Fabaceae flowers appear to be by far the most important of these, with the other plant families receiving very few visits from these bees. A supply of Fabaceae flowers coinciding with queen activity would therefore appear to be a target for *B. humilis* conservation.

Bombus sylvarum queens

Data for 2003 was not included as only two queens were recorded. This was due to the surveying not beginning until July and this being late in the season for recording queens.

For *B. sylvarum* queens *Ballota nigra* and *Lathyrus latifolius* were the most frequently visited forage sources (Figure 3.7), with *Colutea arborescens*, *Vicia villosa* and *Odontites verna* also receiving a substantial number of visits. This was confirmed by results in Figure 3.8, with Fabaceae and Lamiaceae receiving most of the visits.

In total *B. sylvarum* queens were recorded visiting 12 floral species from 4 plant families.

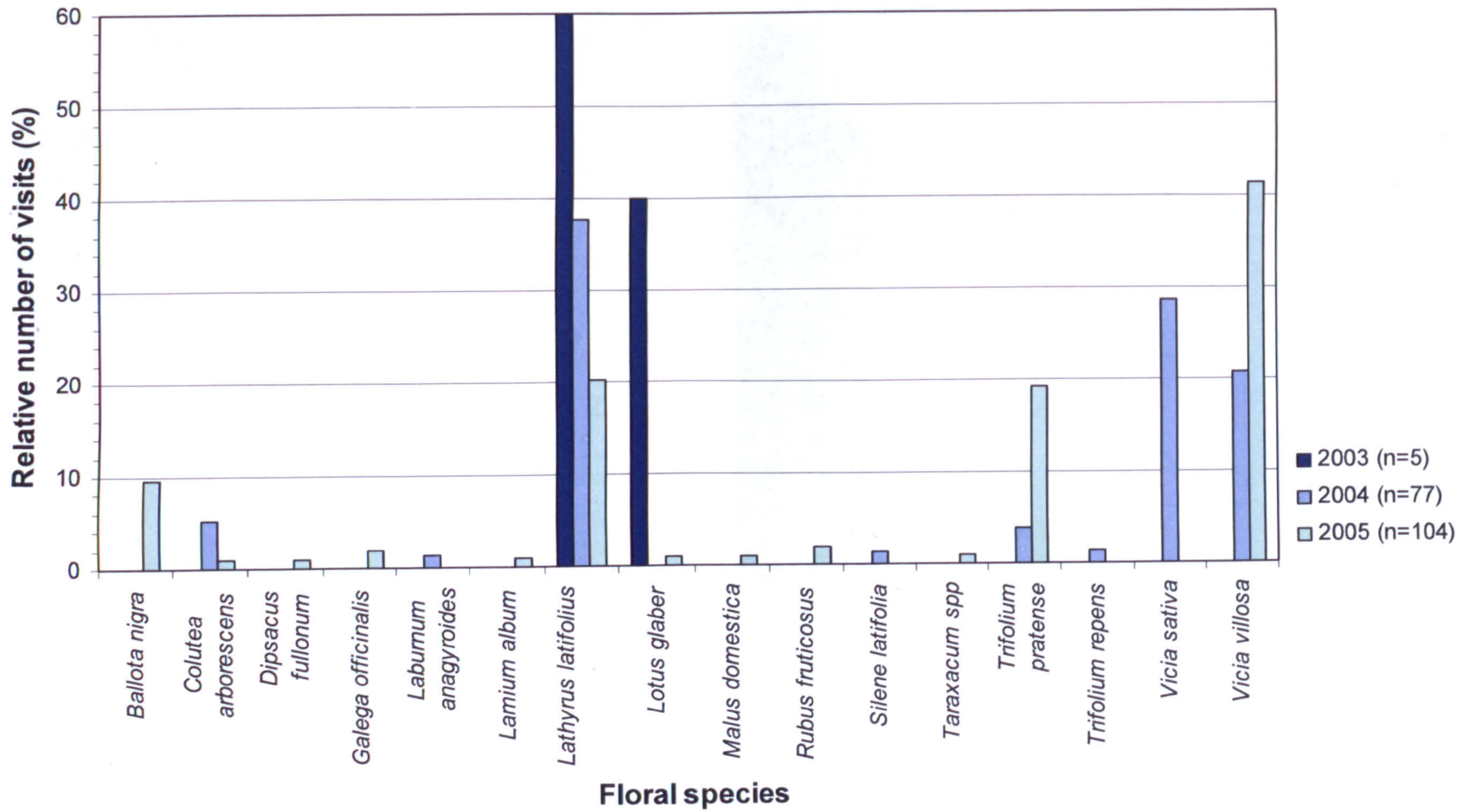


Figure 3.5. Relative plant species visits of *B. humilis* queens 2003-2005.
Values represent the proportion of *B. humilis* queen flower visits observed to each flower species across all sample sites.

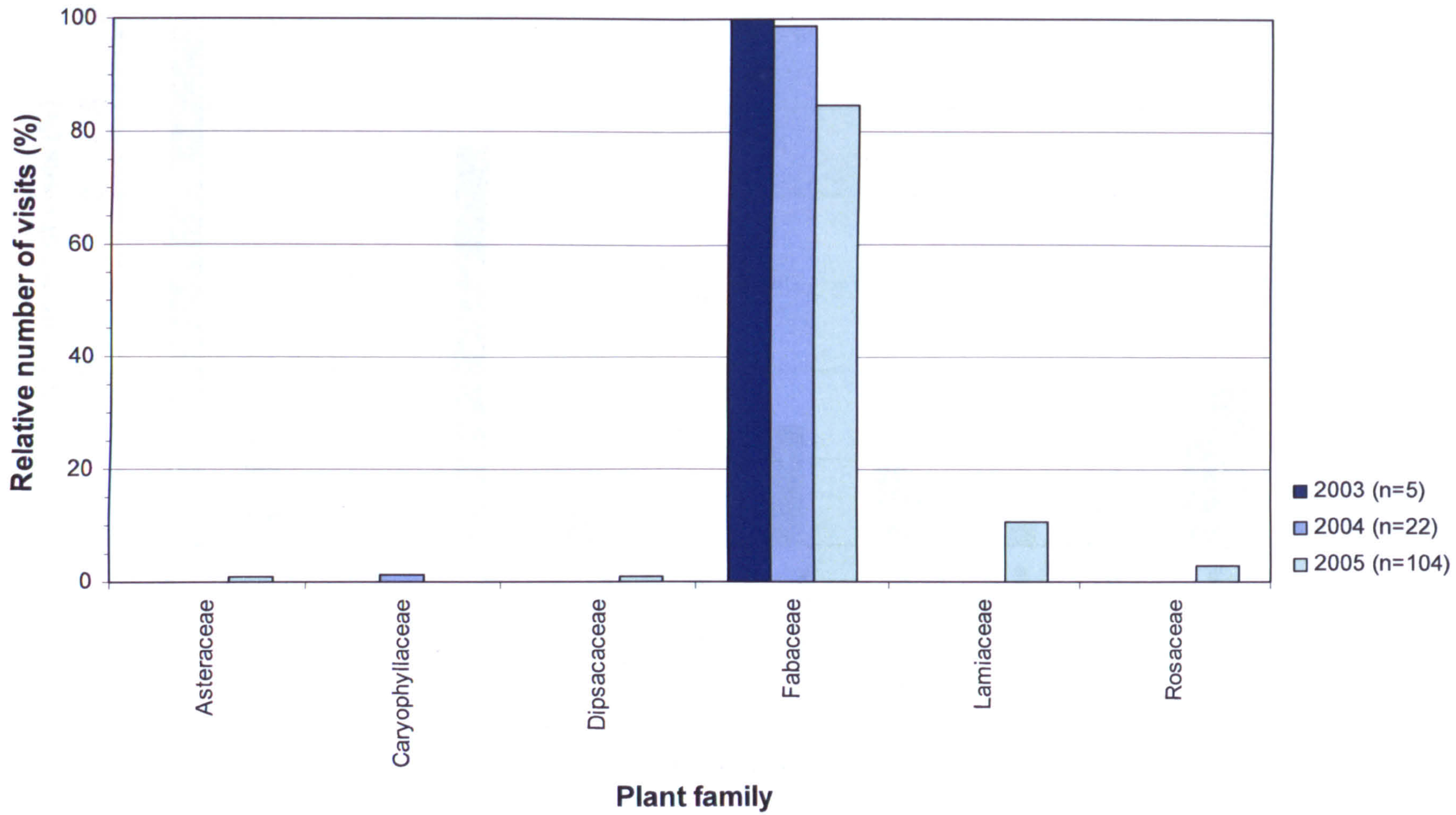


Figure 3.6. Relative plant family visits of *B. humilis* queens 2003-2005.
Values represent the proportion of *B. humilis* queen flower visits observed to each flower family across all sample sites.

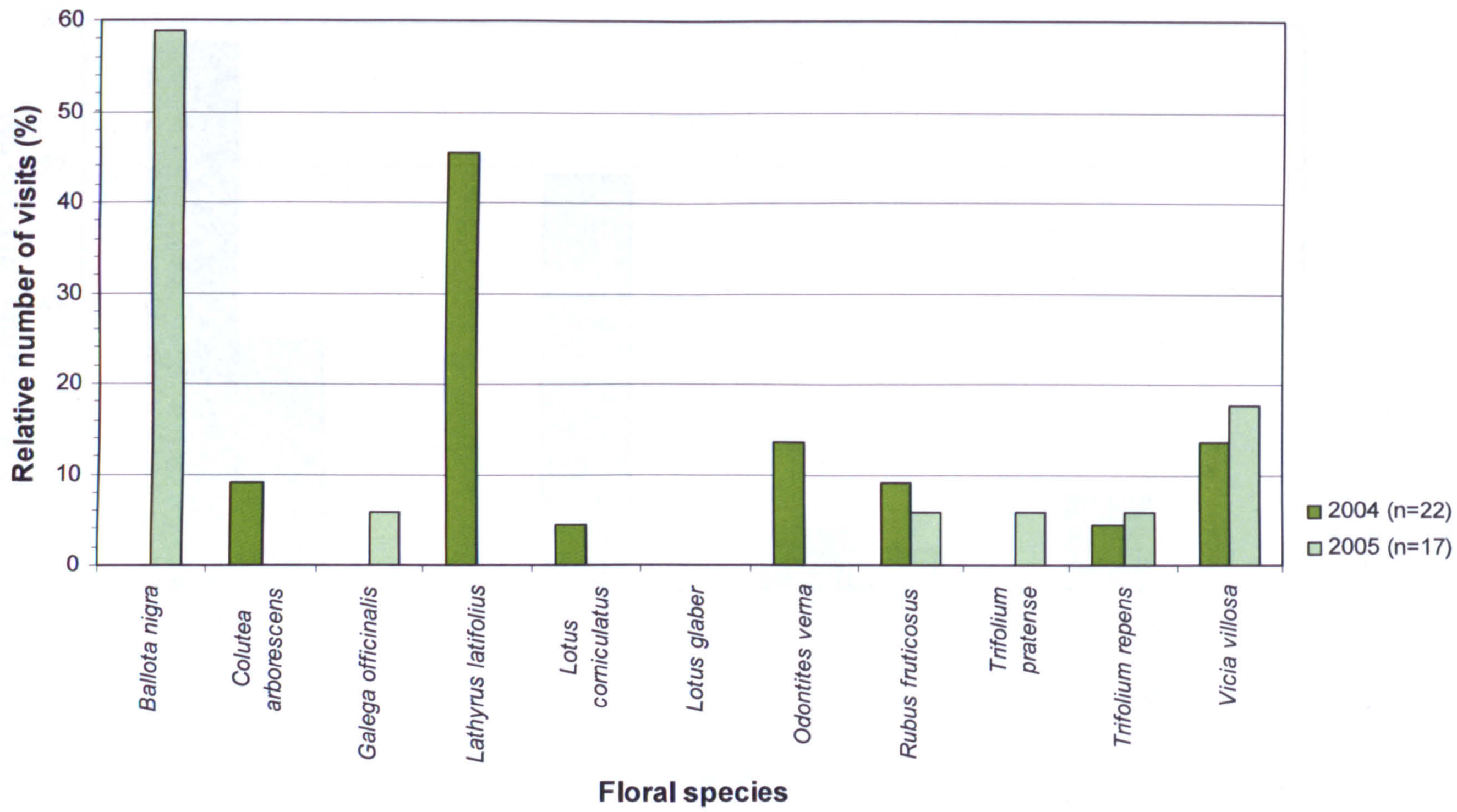


Figure 3.7. Relative plant species visits of *B. sylvarum* queens 2004-2005.
Values represent the proportion of *B. sylvarum* queen flower visits observed to each flower species across all sample sites. (2003 data not included as only 2 queens were recorded)

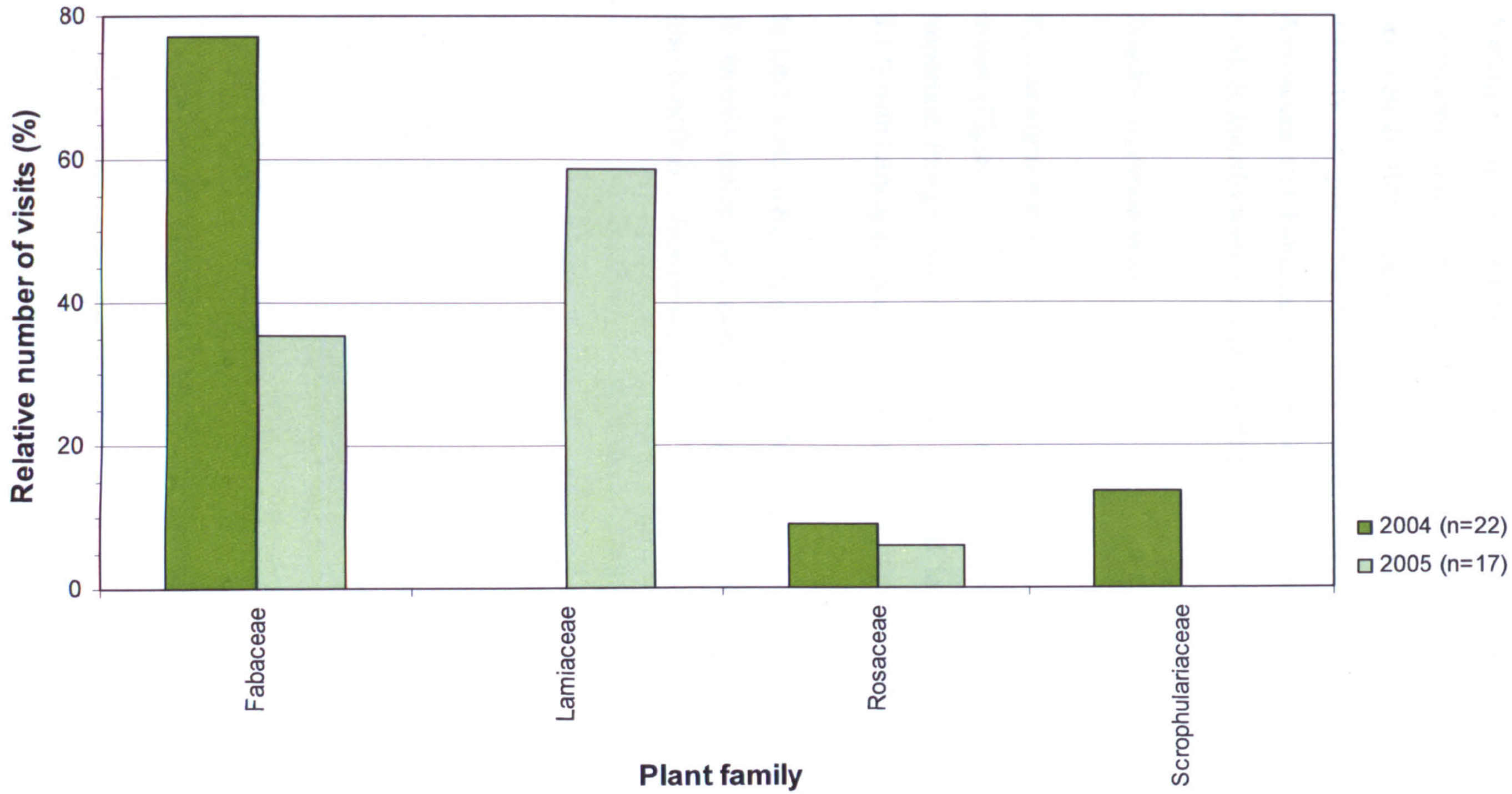


Figure 3.8. Relative plant family visits of *B. sylvarum* queens 2004-2005.
Values represent the proportion of *B. sylvarum* queen flower visits observed to each flower family across all sample sites. (2003 data not included as only 2 queens were recorded)

Bombus humilis males

Ballota nigra received the majority of visits by *B. humilis* males (Figure 3.9). *Centaurea nigra* also appeared to be important with the rest of the visits shared between 26 floral species. Due to this high proportion of visits to *B. nigra*, the majority of plant family visits were recorded to Lamiaceae (Figure 3.10). Asteraceae and Fabaceae also received substantial proportions of the visits. In total, *B. humilis* were recorded visiting 11 plant families to forage.

Bombus sylvarum males

Ballota nigra was found to be by far the most visited floral species by *B. sylvarum* males (Figure 3.11), although *Centaurea nigra* was also recorded as being an important forage source. This was reflected in the plant family survey (Figure 3.12) with Lamiaceae receiving the majority of the visits followed by Asteraceae.

In total, visits were recorded to 13 floral species from 5 plant families. Similarly to *B. humilis* males, provision of forage resources for *B. sylvarum* workers should also benefit *B. sylvarum* males.

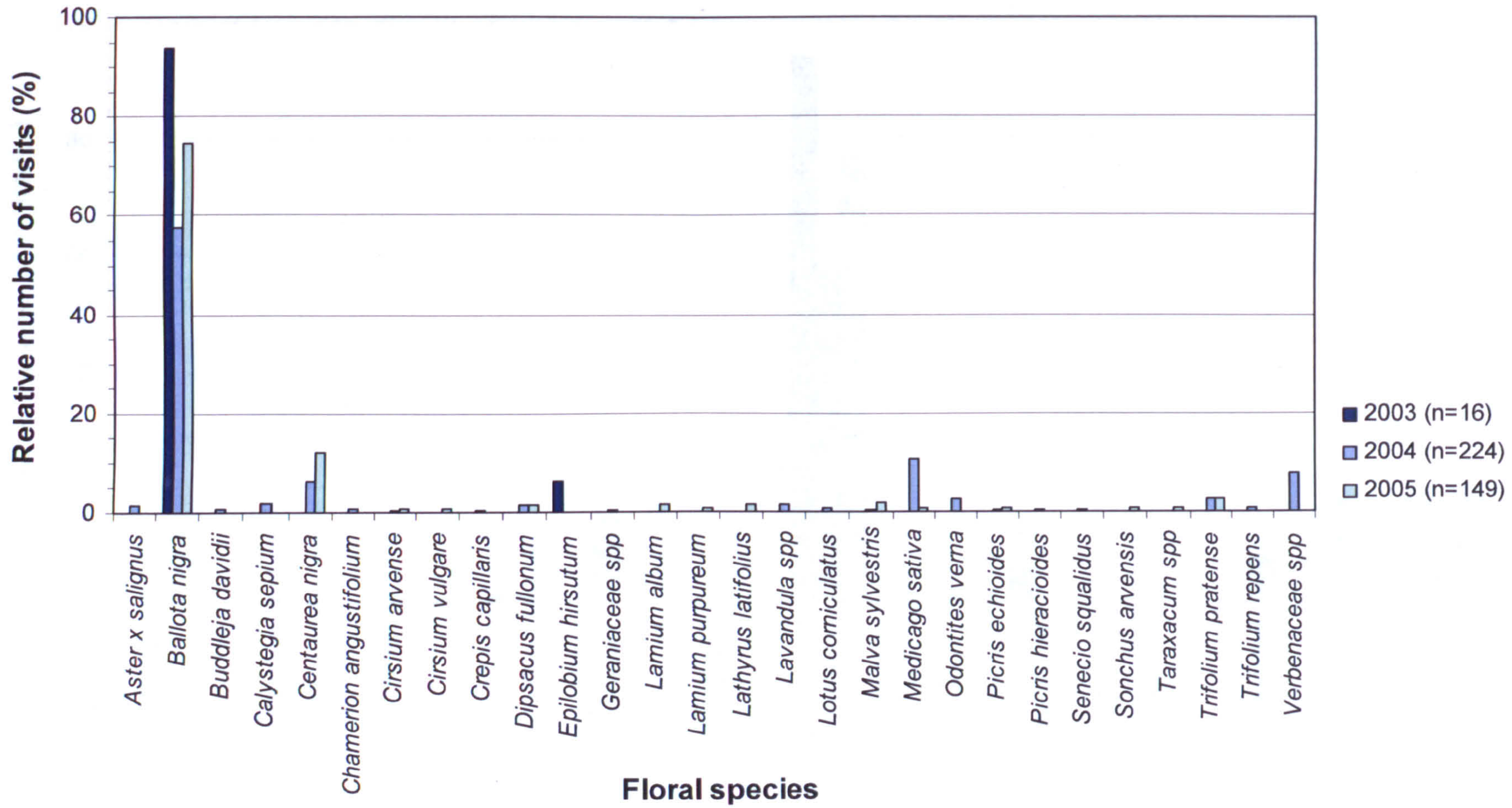


Figure 3.9. Relative plant species visits of *B. humilis* males 2003-2005.
Values represent the proportion of *B. humilis* males flower visits observed to each flower species across all sample sites.

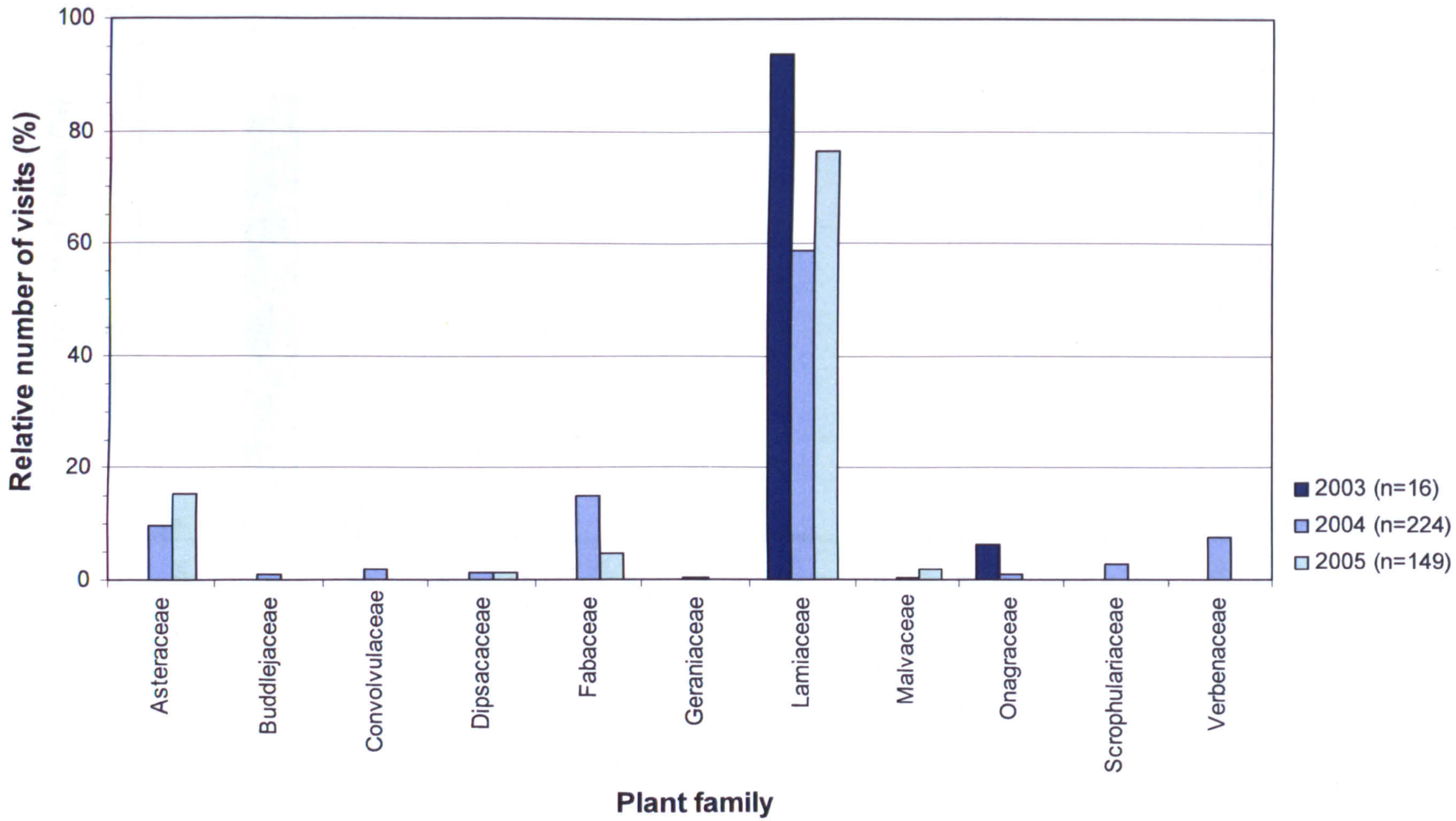


Figure 3.10. Relative plant family visits of *B. humilis* males 2003-2005.
Values represent the proportion of *B. humilis* male flower visits observed to each flower family across all sample sites.

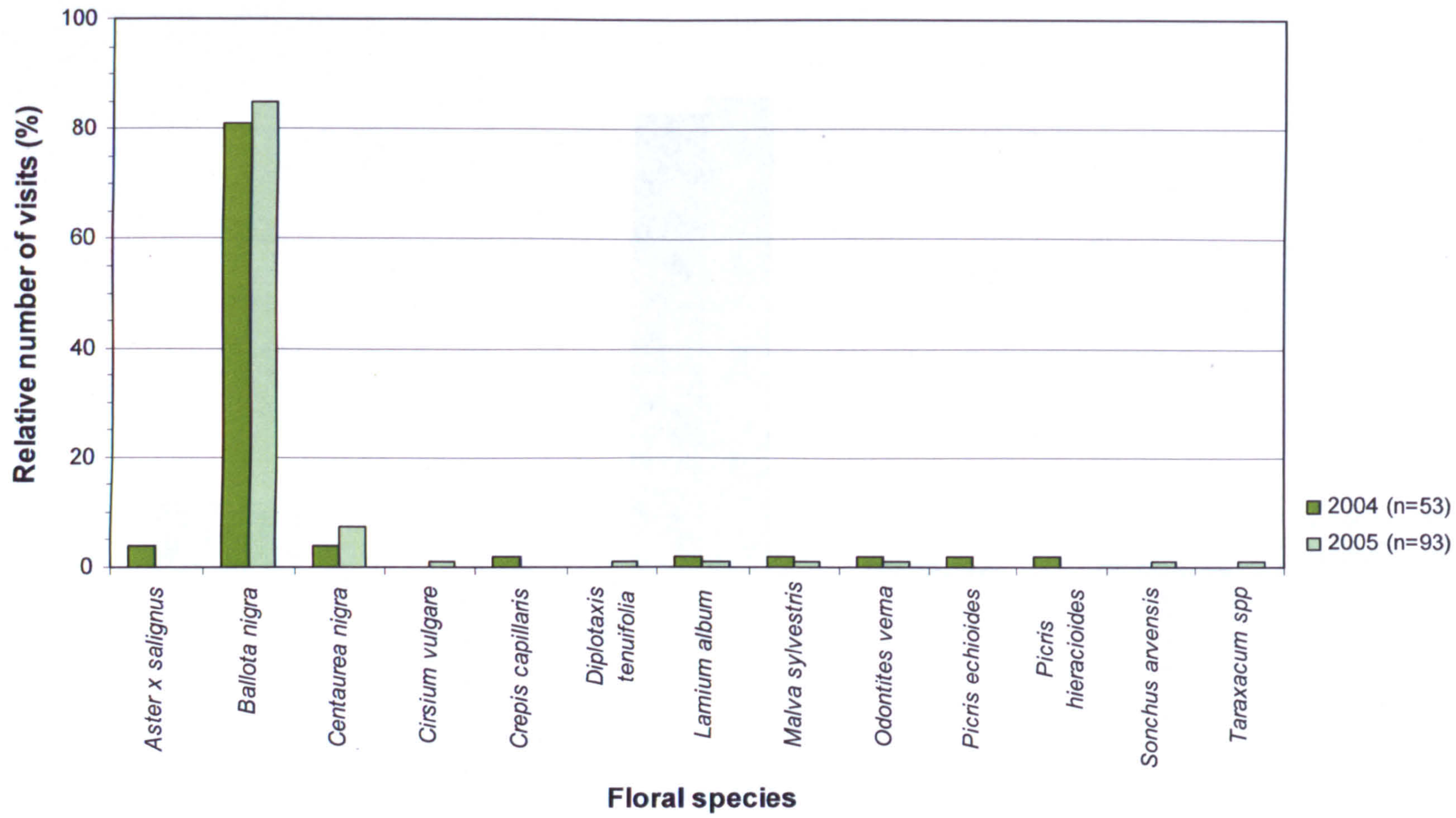


Figure 3.11. Relative plant species visits of *B. sylvarum* males 2004-2005.
Values represent the proportion of *B. sylvarum* males flower visits observed to each flower species across all sample sites. (2003 data not included as no males were recorded)

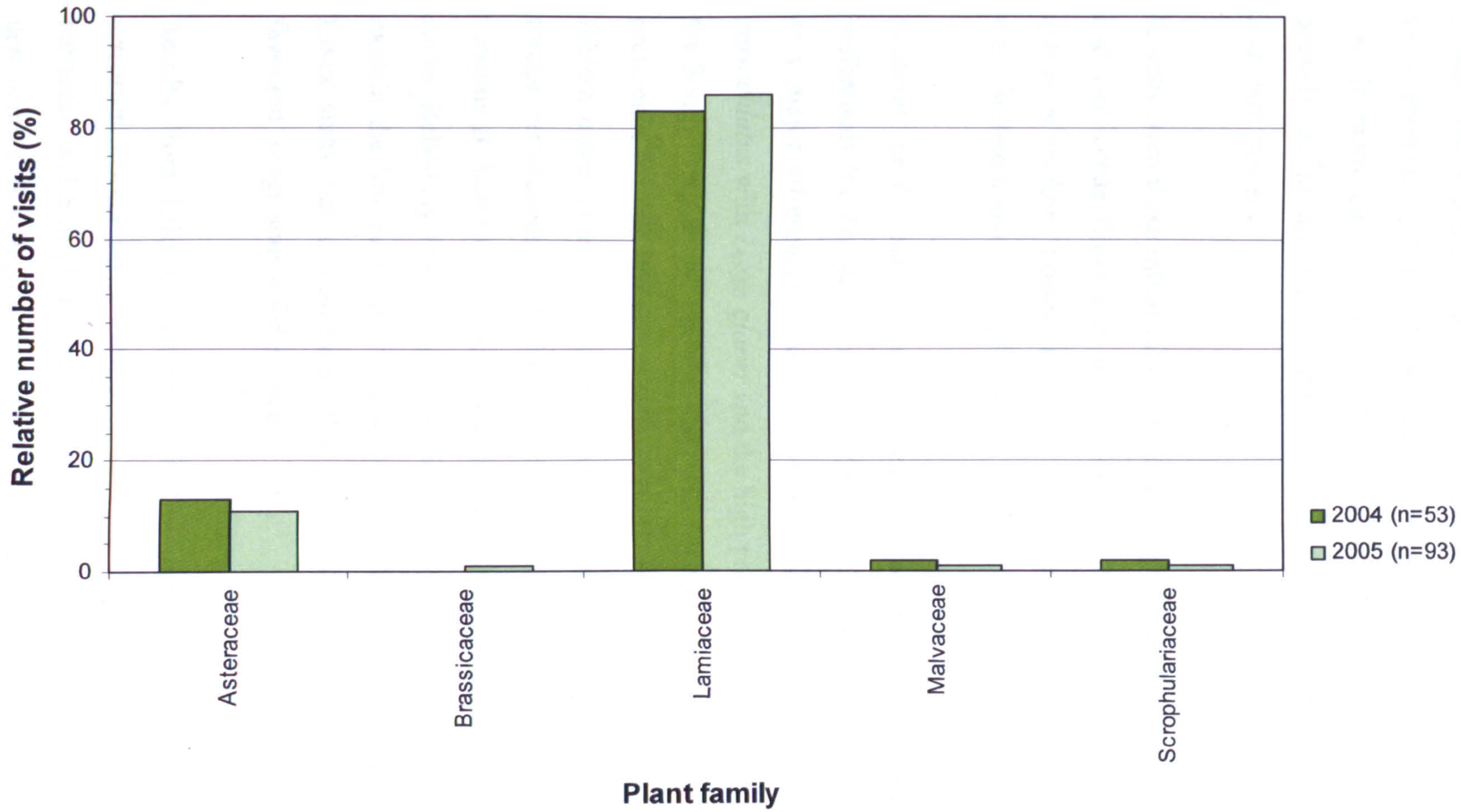


Figure 3.12. Relative plant family visits of *B. sylvarum* males 2003-2005.
Values represent the proportion of *B. humilis* male flower visits observed to each flower family across all sample sites. (2003 data not included as no males were recorded)

3.4 Discussion

It was observed that forage choices made by the bees were not proportional to floral abundance. A broad spectrum of forage was available to the bees on each site. In many circumstances both *B. humilis* and *B. sylvarum* were observed to actively avoid the more common flowering species to forage on other less abundant flowers.

Results from these initial foraging studies highlighted Fabaceae, Scrophulariaceae and Lamiaceae flowers as those most frequently visited by *B. humilis* and *B. sylvarum* workers. Species receiving the highest proportion of visits in the region were *Ballota nigra, Lotus glaber* and *Odontites verna.*

Findings for *B. humilis* corresponded to those of Carvell (2002) who recorded preferences for *Trifolium pratense, Odontites verna* and *Lotus corniculatus.* The only major differences between the two studies being the replacement of *Lotus corniculatus* with *Lotus glaber* and the high proportion of visits to *Ballota nigra* in the South Essex study. *Ballota nigra* was not recorded in the Carvell study so any preference for this flower by the bees could not have been assessed. Few visits to *Ballota nigra* were recorded in a Goulson and Darvill (2003) study of *B. humilis* forage preferences on Salisbury Plain. Findings from this study revealed that foraging *B. humilis* workers favoured several species from Fabaceae and *Odontites verna.* Relatively few *B. humilis* were recorded in this study however, which may explain the low proportions recorded on *Ballota nigra.* Nevertheless, the South Essex study has demonstrated that regionally *Ballota nigra* appeared to be a favoured forage source for the bees.

Results from Ellis (2006) revealed low forage visits to Lamiaceae with *B. sylvarum* worker visits mostly being made to Scrophulariaceae for nectar and Fabaceae and Scrophulariaceae for pollen. This difference in results may again demonstrate that regionally *Ballota nigra* is of value for *B. sylvarum* and *B. humilis* workers.

Findings from this South Essex study also corresponded with those of a *B. sylvarum* ecology study by Harvey (1999) who suggested that *Odontites verna* was particularly important for *B. sylvarum* populations. His report also considered the presence of a previous abundance of *Trifolium* species and *Lotus* species to be significant. The abundance of *B. sylvarum* visits to *Lotus glaber* and lack to *Trifolium* species and other *Lotus* species in this South Essex study however indicated that whilst *Lotus glaber* was a favoured forage source the same was not true for *Lotus corniculatus* and *Trifolium* species. Unlike *B. humilis*, *Trifolium pratense* received proportionally few visits from *B. sylvarum* workers, suggesting that the Harvey study might have identified site characteristics of the bees' preferred habitat rather than actual forage preferences.

The relative lack of visits recorded to *Trifolium pratense* by *B. sylvarum* should be taken into consideration for management prescriptions. Many conservation plans suggest *Trifolium pratense* as a favoured species for bumblebee conservation, particularly on agricultural field margins (Williams, 1989; Pywell *et al.*, 2005; Carvell *et al.*, 2006). Evidence from this study suggested that whilst *Trifolium pratense* might be a good management prescription for general bumblebee forage, when *B. sylvarum* is the target conservation species provision of *Trifolium pratense* may not be an effective forage source.

For *B. humilis* and *B. sylvarum* queens *Lathyrus latifolius* appeared to be the favoured forage source with other Fabaceae species such as *Colutea arborescens*, *Vicia sativa* and *Vicia villosa* also being frequently visited. Again, it is important to note that whilst *B. humilis* queens were recorded foraging on *Trifolium pratense*, *B. sylvarum* queens were not.

Forage use by *B. humilis* and *B. sylvarum* males was fairly general with *Ballota nigra* identified as the floral species receiving the majority of visits for both *Bombus* species. *Bombus* males only feed themselves not the colony (Goulson, 2003), so forage resources are not considered to be as important in terms of quality for them as for queens and workers. Results of floral visits were similar to those for workers of both species and with quality of forage not considered to be as

important for males, target floral species provided for *B. humilis* and *B. sylvarum* workers should also provide suitable forage for males of both species.

Whilst results from these surveys gave an insight into the foraging behaviour of *B. humilis* and *B. sylvarum* they did not provide any information on dietary breadths or dietary specialisations of the species or a comparison with the more nationally ubiquitous species that were also found on these sites. Dietary specialisation has been suggested as a cause of national declines in certain bumblebees including *B. sylvarum* and *B. humilis* (Goulson and Darvill, 2004; Goulson *et al.*, 2005) and is an important factor to be considered in the design of conservation management plans focused towards these two UK BAP species. Dietary breadth and specialization were further investigated and discussed in chapter 4.

In any habitat management for bumblebees it is also vital to ensure that suitable forage for both pollen and nectar collection is provided. This initial survey made no differentiation between flower visits for nectar and pollen. To assess any difference in requirements and to ensure that any forage provided for the bees is suitable as both a nectar and pollen resource a further study was carried out and was discussed in chapter 5.

Chapter 4

Dietary specialisation

4.1 Introduction

Declines in British bumblebees have often been attributed to reductions in forage sources caused by agricultural intensification (Williams, 1986; Osborne *et al.*, 1991; Osborne and Corbet, 1994; Goulson, 2003; Goulson *et al.*, 2005). Impacts of agricultural change have caused the loss of flower-rich field boundaries and hedgerows, the loss of fallow fields sown with Fabaceae such as red clover (*Trifolium pratense*), the switch from hay meadows (cut once a year) to silage production (cut several times a year) and increased herbicide application (Robinson and Sutherland, 2002). All of these factors have reduced the abundance and diversity of bumblebee forage on arable land (Carvell *et al.*, 2006) and could potentially be the driving force behind the declines currently being recorded in UK bumblebees.

It has been hypothesised that differential declines between bumblebee species can be at least partly attributed to differences between forage preferences, particularly those related to tongue length (Prŷs-Jones and Corbet, 1987; Goulson and Darvill, 2004; Goulson *et al.*, 2005). Tongue lengths vary between species (Table 4.1) and the bumblebees that remain abundant have been observed to be mostly the short-tongued species that emerge early in the season and are considered to have less specialised diets and tend to forage on flowers with shorter corollas (Inouye, 1980; Williams, 1989). These species are very common in suburban gardens where they are able to exploit a broad range of floral resources (Goulson *et al.*, 2005). It tends to be the long-tongued late emerging species such as *B. humilis, B. sylvarum, B. ruderatus* and *B. subterraneus* that are the bees suffering most from decline and these bees are considered to be more dependent on Fabaceae as a pollen resource and general forage resource (Edwards, 2000).

A study by Goulson and Darvill (2004) showed that longer-tongued bees tended to forage preferentially on Fabaceae flowers as a pollen resource, plants that have been particularly lost through agricultural change (Carvell *et al.*, 2006). It has been hypothesised that this preference occurs because species emerging later in the season are limited in their choice of available forage resources (Goulson *et al.*,

2005) and they need to raise their brood quickly, so specialize on more protein rich pollen (Ellis, 2006).

Table 4.1. Bumblebee tongue lengths. Measured as length of glossa plus prementum.

Bombus species	Mean tongue length (mm)	S.D. (mm)
**B. terrestris/lucorum*	7.9	+/- 0.5
**B. lapidarius*	7.8	+/- 0.4
**B. pascuorum*	8.5	+/- 0.6
**B. hortorum*	12.9	+/- 0.8
**B. pratorum*	7.3	+/- 0.4
**B. humilis*	8.4	+/- 0.5
°*B. sylvarum*	8.8	+/- 0.7

* - from Goulson and Darvill (2004)

° - from Goulson *et al.* (2005)

Bumblebees utilize flowers with corolla lengths most closely corresponding to their tongue lengths (Ranta and Lundberg, 1980). It is considered possible that longer-tongued bumblebees are thus more specialised and have a competitive advantage over shorter-tongued bumblebees when specialised plants with flowers with longer corolla lengths such as Fabaceae are abundant. This has been suggested as being the situation on machair (Goulson *et al.*, 2005) where red clover is a dominant member of the floral community and the long-tongued bumblebees *B. distinguendus*, *B. hortorum*, and *B. muscorum*, which have tongue lengths close to the optimum for extracting nectar from this plant, also appear to visit it for pollen and nectar with disproportionately high incidence (Edwards, 1998).

This concept of dietary specialisation among rarer bumblebee species has however been challenged. Williams (2005) questioned previous methods used to define and assess dietary specialisation. He stated that food-plant preference and specialization is an active selection of one forage species over another (whether

this is through choice or limitation), rather than a situation where bees visit a plant frequently simply because either the bees or particular plant species is abundant.

Williams (2005) investigated such food-plant specialisation by a combination of dietary breadth and dietary preference analysis. Dietary breadth analysis involves a comparison of the breadth of floral species or floral families that a particular *Bombus* species would be expected to visit during a defined number of floral visits. Contrastingly, dietary preference is an investigation of the extent to which certain *Bombus* species specialise on individual floral species when all bee visits to all floral species are compared for a particular site. These two methods therefore assess both the number of flower species each *Bombus* species is visiting during the same number of floral visits (e.g. an expected 6 floral species visited during 20 random visits) and whether the flowers being visited are the same for each *Bombus* species or whether different *Bombus* species are visiting different plants with respect to each other.

Williams (2005) also questioned whether quantitative assessments of bumblebee specialisation data had adequately taken into account the sources of their data for analysis. He argued that for forage data to be comparable it must be taken from a single survey with all bees being subjected to the same foraging options. Surveys carried out in different locations or at different temporal scales would not provide the bumblebees with the same floral choices and therefore would not be adequate assessments of floral preferences or specialisations. It was stated that as 'there are few sites where most bumblebee species occur together, where they might choose among the same plants, so there are correspondingly few data sets that permit genuine quantitative comparisons of forager choice. In this article Williams analysed a data set from Dungeness, Kent (Williams, 1989), a site with a broad bumblebee assemblage which was sampled intensively during July and August 1982. Results of floral preference, when adjusted appropriately for varying sample sizes amongst bumblebee species, were concluded as not being consistent with the relative rarity or decline of bumblebee species in Britain. The document did however express the importance of finding other such suitable data sets and comparing them for forage preference and more specifically for pollen preference.

If Williams' arguments were true, and the Goulson and Darvill (2004) data was unreliable, then there is no current evidence to support the link between foraging specialization and bumblebee declines. The South Essex sites provided an ideal location to further investigate dietary specialisation in bumblebees: a series of geographically close sites with a diversity of nationally rare and more ubiquitous bees all relatively abundant. The investigation therefore provided an opportunity for a similar data set to that in the Williams paper to be analysed. Although the surveys were carried out over a larger area than the Williams (1989) study, all sites were considered to be within the same metapopulations, forage assemblages were similar across sites (appendix 12.1) and all *Bombus* species recorded were present across all of the sites sampled. Therefore, under the conditions stated in the Williams article, data from the South Essex study were suitable for similar dietary comparison analysis.

As discussed in section 1.5.1, emergence times and colony cycle timings combined with habitat specialization have also been proposed as driving forces behind differential declines in British bumblebees (Edwards, 1999; 2000; 2001; 2002). General species colony timings are listed in Table 4.2 and are discussed in relation to the results of the dietary specialization analysis in the summary (section 4.4)

Table 4.2. *Bombus* species colony cycle timings. Emergence times are not precise, and depend greatly on latitude. For southern UK they are crudely classified as Early = March – April, Mid = April – mid May and Late = May onwards (Goulson *et al.* 2005).

Bombus species	Emergence time	Length of colony cycle
B. sylvarum	late	short
B. humilis	late	short
B. pascuorum	mid	long
B. terrestris	early	long
B. lapidarius	mid	long
B. hortorum	mid	long
B. pratorum	early	short

Based on data from Alford (1975), Prŷs-Jones and Corbet (1987), Edwards and Jenner (2006) and Goulson *et al.* (2005).

4.2 Methods

During 2003 and 2004, surveys were carried out at a number of sites throughout the South Essex area which were previously identified as supporting populations of the UK BAP species *B. humilis* and *B. sylvarum.*

Sites surveyed were the same as those in chapter 3 (Table 3.1). Surveys were carried out on each site to record flower visits by all worker *Bombus* species observed foraging on the sites. The species and number of bees observed were recorded. The species of flower each bee was seen foraging on and the position of the forage on the site were also recorded. Flower identification followed Stace (1997). Foraging visits made by *Bombus* workers for pollen and those for nectar were not differentiated due to the intrinsic problems involved in identifying which use the flower was being visited for. Instead a separate pollen analysis was carried out and is discussed in chapter 5.

The surveys comprised of a modified version of the bee walk transects used by Banaszak (1980) and Saville *et al.* (1997). Forage distribution on the sites was considered to be too patchy and discontinuous for linear walks to be used so non-linear walks encompassing the main flowering patches within the sites were used instead. The walks involved bumblebee counts whilst walking through areas of suitable forage. Observations were made approximately 2m either side of the observer and speed of walking was about 10m per minute. The walks were replicated for each visit to the same site both in terms of distance and approximate duration.

Bee walks were carried out from July to September in 2003 and 2004. The survey dates were based on the months reported in literature as being the main foraging period of *Bombus* workers (Prŷs-Jones and Corbet, 1987) and on observed timings from the surveys in 2003. All searches were conducted between 9:30 and 17:00 BST and during warm weather favourable to bumblebee activity (temperatures greater than 15°C).

Identification of the bees followed Prŷs-Jones and Corbet's Naturalist Handbook key (1987). Bees which could not be identified whilst foraging were captured using Thorne Supplies (Thorne, 2007) queen bee marking plunger cages (Kwak, 1987) and were identified by species morphology using a field lens. Problems of differentiating between *B. humilis*, *B. pascuorum* and *B. muscorum* were solved as discussed in chapter 2 (section 2.2). Due to the intrinsic problems distinguishing *B. terrestris* and *B. lucorum* in the field (Prŷs-Jones and Corbet, 1987), and because of the known similarities in ecological requirements of these two species, as in other studies (Fussell and Corbet, 1991; Croxton *et al.*, 2002; Dramstad *et al.*, 2003; Kreyer *et al.*, 2003; Goulson and Darvill, 2004; Walther-Hellwig *et al.*, 2006) foraging observations of these bees were recorded as *B. terrestris/lucorum*.

The validity of aggregating *B. terrestris* and *B. lucorum* worker records for dietary comparisons could be questioned. Combining results for the two species could mean that dietary breadth recorded would represent a combination of the different foraging choices of the two species. Throughout surveying in 2004 however, 42 *B. terrestris* queens were observed compared to only 14 *B. lucorum* queens and very few *B. lucorum* males were observed at the end of the season. It was therefore likely that the majority of workers observed in this study were *B. terrestris* workers. For this reason and due to similarities between the ecology and physiology of the two species (Sladen, 1912; Prŷs-Jones and Corbet, 1987), pooled results were considered to be an accurate representation of *B. terrestris* worker foraging.

The area surveyed was a series of sites across approximately 6km x 11km of fragmented habitat in South Essex comprising a mix of urban, semi-natural, agricultural and brownfield land. Sites known to support a broad mix of bumblebee species were included in the surveys. The floral assemblages were fairly consistent across all of the sites (appendix 12.1) and due to their proximity to each other in terms of the spatial scales that bumblebees are thought to operate over (Osborne *et al.*, 1999; Chapman *et al.*, 2003; Darvill *et al.*, 2004), it was decided that the bees recorded were operating under the same foraging options. Because of these factors, it was considered that this data set was suitable for analysis of dietary specialisation under the conditions stated in the Williams

(2005) article. The data obtained was therefore analysed using Williams (2005) methods for measuring dietary breadth and relative dietary preference:

Relative dietary breadth

Relative dietary breadth was measured using a rarefaction procedure (Heck *et al.*, 1975; Gotelli and Colwell, 2001). The rarefaction calculator (Rarefaction, 2007) was used to normalise the number of different plant species and plant families each bumblebee species would be expected to visit for a standardised number of flower visits. For this analysis, the mean number of plant species and plant families visited during 20 flower visits was calculated. This technique was used as it allowed comparison of bumblebee foraging choices without being influenced by differing sample sizes. It would be expected that greater sampling effort in terms of common bumblebee observations would yield a larger sample and identify more species visited than for rarer bumblebee species. Without standardisation, it would be easy to equate the fewer observations of flower visits by rarer bees with a dietary breadth of fewer flowers (Williams, 2005). This however may be an anomalous result and merely representative of a sample-size effect. Rarefaction standardisation of results was therefore used to remove this sample-size effect, creating sub-samples of 20 visits made from the observed frequency distribution of visits for each bumblebee species, but chosen at random without replacement 1000 times (Williams, 2005).

Relative dietary preference

Relative dietary preference was measured following the contingency table techniques used in the Williams studies (1989 and 2005): The frequency of visits that would be expected if foragers were unselective and encountered the same flowers, but in proportion to the total numbers recorded, were calculated from the products of the marginal totals divided by the total of all visits to all flowers. An index of preference for a species of food plant was then calculated by the deviation of observed from expected visits, divided by the expected frequency (Williams, 1989).

This method therefore calculated whether observed forage visits differed from those that would be expected if all bees were making the same floral foraging choices. Observations of floral foraging behaviour from one field season (June – September 2004) were used to calculate relative dietary preference.

4.3 Results

Relative dietary breadth

Tables of rarefied estimates of *Bombus* floral dietary breadths for each summer month during the years 2003 and 2004 including sample size (total number of flower visits observed) and standard deviation are given in the appendix (section 12.2). As sample sizes for rarefaction calculation of a population's dietary breadth were n=20, rarefaction values based on sample sizes of n<20 (where n=total observations) were considered to be unreliable for this study as they could bias estimates of dietary breadth towards lower values than those actually occurring. Species with sample sizes less than 20 were therefore not used in any further analyses (although values of n=17 for *B. terrestris/lucorum* in Aug 2003 were considered for inclusion due to their broad dietary variation when compared to other species and the lack of other data for this species). Rarefaction values based on n≥20 were considered to be reliable for this study. Samples with higher numbers of observations, however, were likely to generate more reliable estimated rarefaction values.

Table 4.3 summarises the results of monthly *Bombus* species rarefied foraging visits in terms of floral species visits. Rarefied estimates of bumblebee floral species dietary breadth based on annual observations were also included in the table. These were calculated by combining the July, August and September data for each *Bombus* species. A mean rarefied number of flower species was calculated for each bumblebee species by averaging all of the monthly rarefaction estimate totals for months in which n≥20 observations were made. These values

were plotted in Figure 4.1 and summary tables of values were included in the appendix (section 12.3).

Results in Figure 4.1 indicated that there was a difference between the dietary breadths of the *Bombus* species observed in this investigation in terms of the number of floral species visited. *Bombus sylvarum* had the lowest dietary breadth, with a rarefied mean of 3.81 (±0.24 S.E.) plant species expected to be visited during 20 random flower visits. The highest dietary breadth was *B. terrestris/lucorum*, with a rarefied mean of 8.96 (±0.79 S.E.) plant species expected to be visited during 20 random flower visits.

Rarefaction results were also calculated for monthly floral plant families visits. These results are presented in Table 4.4. Rarefied estimates of bumblebee floral family dietary breadth based on annual observations were also included in the tables. Mean monthly values were calculated and plotted in Figure 4.2. Summary tables of values are included in the appendix (section 12.3). Similarly to plant species, *B. sylvarum* had the lowest mean dietary breadth for floral families, with a rarefied mean of 3.19 (±0.16 S.E.) plant families to be visited during 20 random flower visits. The highest dietary breadth was *B. terrestris/lucorum*, with a rarefied mean of 5.83 (±0.30 S.E.) plant families expected to be visited during 20 random flower visits. Mean rarefied results for both floral species and floral families visited were relatively similar for *B. humilis*, *B. pascuorum* and *B. lapidarius*.

Values for *B. pratorum* and *B. hortorum* were included in Tables 4.3 and 4.4 but not in further analyses as greater than 20 bees were only observed during two of the months surveyed over the two years (2003 and 2004) and on these occasions the numbers were close to twenty observations. *B. hortorum* and *B. pratorum* were considered to be rare on the sites and further statistical analysis of observations was considered unreliable.

Table 4.3. Summary of rarefied number of plant species visited. Rarefied estimates of the number of flower species a bee would be expected to visit during 20 flower visits. Rarefied estimates were calculated using all recorded flower visits for each *Bombus* species for the time period stated. Annual totals were based on a combination of the sightings from each month (July, Aug. and Sept.) for each year.
[n>20 unless otherwise stated]

Date of sightings	*Bombus lapidarius*	*Bombus pascuorum*	*Bombus humilis*	*Bombus sylvarum*	*Bombus terrestris /lucorum*	*Bombus hortorum*	*Bombus pratorum*
July 2003	8.07	N.A.	8.03	1.95	N.A.	N.A.	N.A.
Aug 2003	7.74	6.11	7.29	3.91	11 (n=17)	N.A.	3.81
Sept 2003	4.67	5.45	4.87	4.16	N.A.	N.A.	N.S.
2003 total	8.85	6.04	6.61	4.16	13.73	N.A.	4.68
July 2004	8.20	6.78	8.25	4.45	7.39	9.54	N.A.
Aug 2004	4.41	6.65	6.56	4.56	10.53	N.A.	N.A.
Sept 2004	N.A.	7.14	7.03	N.A.	N.A.	N.A.	N.A.
2004 total	8.02	7.42	8.35	4.71	8.39	9.51	N.A.

N.A. – Insufficient sightings were made for the *Bombus* species in these months (n<20)

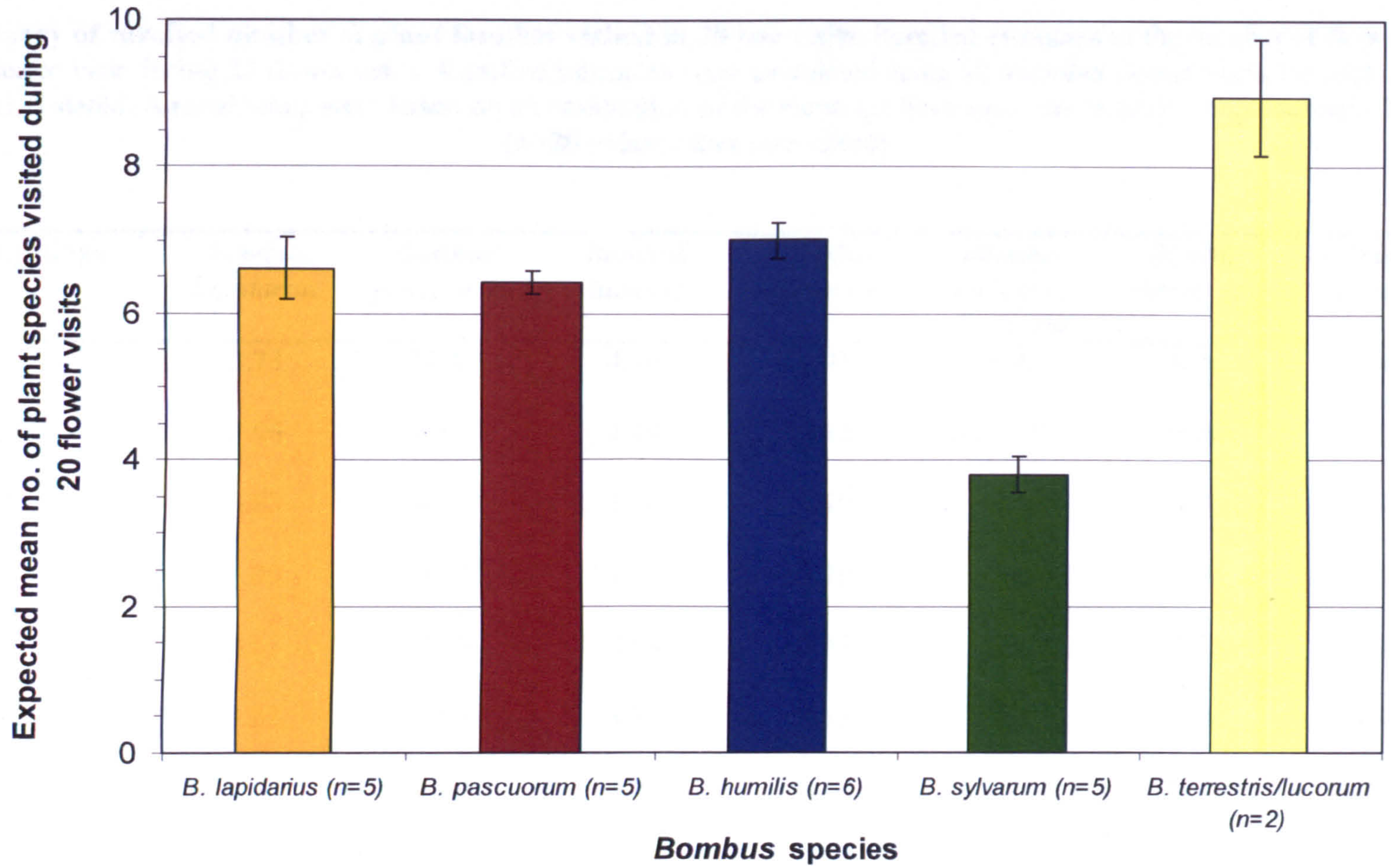


Figure 4.1. Mean rarefied estimates of monthly plant species dietary breadths. Means were calculated from rarefied estimates for all months in 2003 and 2004 for which >20 flower visits were observed. [Error bars represent the standard error of the mean]

Table 4.4. Summary of rarefied number of plant families visited in 20 bee visits. Rarefied estimates of the number of flower families a bee would be expected to visit during 20 flower visits. Rarefied estimates were calculated using all recorded flower visits for each *Bombus* species for the time period stated. Annual totals were based on a combination of the sightings from each month (July, Aug. and Sept.) for each year. [n>20 unless otherwise stated]

Date of sightings	*Bombus lapidarius*	*Bombus pascuorum*	*Bombus humilis*	*Bombus sylvarum*	*Bombus terrestris /lucorum*	*Bombus hortorum*	*Bombus pratorum*
July 2003	3.73	N.A.	4.46	1.95	N.A.	N.A.	N.A.
August 2003	3.96	4.69	4.19	3.42	6 (n =17)	N.A.	2.00
Sept 2003	3.83	4.43	3.63	3.79	N.A.	N.A.	N.A.
2003 total	4.05	4.58	4.22	3.70	5.91	N.A.	2.95
July 2004	4.65	3.43	4.09	3.35	4.89	4.72	N.A.
August 2004	3.22	4.03	3.75	3.43	6.76	N.A.	N.A.
Sept 2004	N.A.	4.60	4.00	N.A.	N.A.	N.A.	N.A.
2004 total	4.40	3.98	4.02	3.61	5.28	4.78	N.A.

N.A. – Insufficient sightings were made for the *Bombus* species this month (n<20)

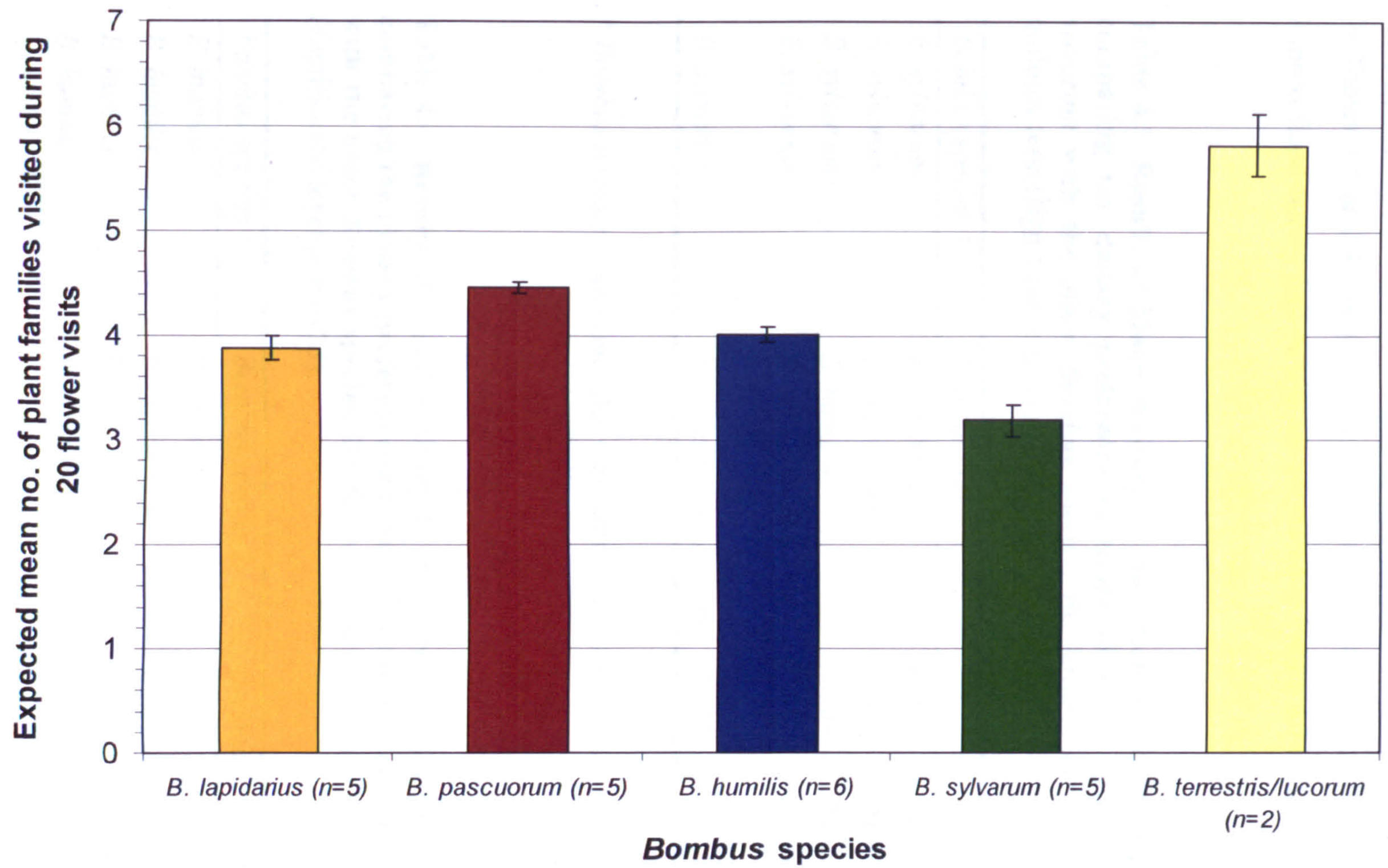


Figure 4.2. Mean rarefied estimates of monthly plant family dietary breadths. Means were calculated from rarefied estimates for all months in 2003 and 2004 for which >20 flower visits were observed. [Error bars represent the standard error of the mean]

Mann-Whitney U exact tests were carried out to assess whether there were significant differences between the rarefied dietary breadths of *B. humilis* and *B. sylvarum* from *B. lapidarius*, *B. pascuorum* and *B. terrestris/lucorum*. Results of these tests for *B. sylvarum* and *B. humilis* floral species preferences are presented in Tables 4.5 and 4.6 respectively. Results of these tests for *B. sylvarum* and *B. humilis* floral family preferences are presented in Tables 4.7 and 4.8 respectively.

Table 4.5. Results of Mann-Whitney U exact tests for independent samples comparing the dietary preference in terms of floral species visits of *B. sylvarum* with the other *Bombus* species. Bonferroni correction applied for multiple tests (significance level $p<0.0125$).

Bombus species 1	*Bombus* species 2	*p*-value	S/N.S.
B. sylvarum	*B. lapidarius*	0.032	Not significant
B. sylvarum	*B. pascuorum*	0.009	**Significant**
B. sylvarum	*B. humilis*	0.004	**Significant**
B. sylvarum	*B. terrestris/lucorum*	0.052	Not significant
B. sylvarum	* *B. terrestris/lucorum*	0.036	Not significant

* *Bombus terrestris/lucorum* values including sightings for August 2003 (n=17)

Table 4.6. Results of Mann-Whitney U exact tests for independent samples comparing the dietary preference in terms of floral species visits of *B. humilis* with the other *Bombus* species. Bonferroni correction applied for multiple tests (significance level $p<0.0125$).

Bombus species 1	*Bombus* species 2	*p*-value	S/N.S.
B. humilis	*B. lapidarius*	0.931	Not significant
B. humilis	*B. pascuorum*	0.273	Not significant
B. humilis	*B. sylvarum*	0.004	**Significant**
B. humilis	*B. terrestris/lucorum*	0.182	Not significant
B. humilis	* *B. terrestris/lucorum*	0.095	Not significant

* *Bombus terrestris/lucorum* values including sightings for August 2003 (n=17)

Table 4.7. Results of Mann-Whitney U exact tests for independent samples comparing the dietary preference in terms of floral family visits of *B. sylvarum* with the other *Bombus* species. Bonferroni correction applied for multiple tests (significance level $p<0.0125$).

Bombus species 1	*Bombus* species 2	*p*-value	S/N.S.
B. sylvarum	*B. lapidarius*	0.151	Not significant
B. sylvarum	*B. pascuorum*	0.021	Not significant
B. sylvarum	*B. humilis*	0.017	Not significant
B. sylvarum	*B. terrestris/lucorum*	0.095	Not significant
B. sylvarum	* *B. terrestris/lucorum*	0.025	Not significant

* *Bombus terrestris/lucorum* values including sightings for August 2003 (n=17)

Table 4.8. Results of Mann-Whitney U exact tests for independent samples comparing the dietary preference in terms of floral family visits of *B. humilis* with the other *Bombus* species. Bonferroni correction applied for multiple tests (significance level $p<0.0125$).

Bombus species 1	*Bombus* species 2	*p*-value	S/N.S.
B. humilis	*B. lapidarius*	0.537	Not significant
B. humilis	*B. pascuorum*	0.429	Not significant
B. humilis	*B. sylvarum*	0.017	Not significant
B. humilis	*B. terrestris/lucorum*	0.046	Not significant
B. humilis	* *B. terrestris/lucorum*	0.024	Not significant

* *Bombus terrestris/lucorum* values including sightings for August 2003 (n=17)

With a Bonferroni correction (Rice, 1989) applied to allow for multiple testing ($p=0.0125$), rarefied estimates for *B. sylvarum* dietary breadth in terms of expected number of floral species visited during 20 random flower visits were significantly lower than for *B. humilis* and *B. pascuorum*. This indicated that *B. sylvarum* workers would be expected to visit significantly fewer floral species during 20 floral visits than *B. humilis* and *B. pascuorum*.

Values were also low, although not significant, for *B. lapidarius* and *B. terrestris/lucorum*. If the n=17 value for *B. terrestris/lucorum* in August 2003 was included the *p*-value became even lower. August 2003 *B. terrestris/lucorum* sightings for forage visits were not originally included in the analysis as only 17 observations were made. However, the 17 observations for the month were on 11 different floral species and rarefaction values for this month would therefore be calculated as 11 floral species. Values for n<20 were not generally included in analyses as they were below the n≥20 threshold and therefore were considered to bias the result to one lower than the true value. For this analysis however, a lower than actual value would only bias the data towards being not significant, so analyses including this value were also carried out to assess what effect they would have on results.

No significant differences were found between the dietary breadths of *B. humilis* and the other *Bombus* species (*B. pascuorum*, *B. lapidarius* and *B. terrestris/lucorum*) at the p=0.0125 confidence level (Table 4.6).

Results of Mann-Whitney U exact tests comparing the mean rarefied plant family breadths were also calculated and results for *B. sylvarum* and *B. humilis* are presented in Tables 4.7 and 4.8 respectively. Values for *B. sylvarum* were substantially lower than those for *B. pascuorum*, *B. humilis* and *B. terrestris/lucorum*, as was *B. humilis* when compared to *B. terrestris/lucorum*. However, none of these differences were found to be statistically significant after a Bonferroni correction was applied for multiple tests (p=0.0125).

Relative dietary preference

Relative dietary preference is represented by the deviation of the observed frequencies of bumblebee flower visits from those that would be expected if all bees were making the same flower visiting choices (Williams, 1989). Tables of the *Bombus* worker sightings from the June to September 2004 field surveys which were used for dietary preference analyses are given in the appendix (section 12.4). The species *B. sylvarum*, *B. humilis*, *B. pascuorum*, *B. lapidarius* and the *B. terrestris/lucorum* group were analysed as these were the only species recorded

foraging on all of the sites throughout the survey period. *Bombus sylvarum* and *B. humilis* are regionally relatively abundant but nationally rare, whereas *B. pascuorum*, *B. lapidarius* and the *B. terrestris/lucorum* group are considered to be nationally ubiquitous species. All species were considered to be making foraging choices under the same foraging options.

Whilst Williams (2005) and Fitzpatrick *et al.* (2007) only represented the maximum values for dietary preference for each *Bombus* species, all values recorded were considered in this study. However, the maximum positive preference value for *B. sylvarum* in this study was 8.58, between the two values recorded in the Williams and Fitzpatrick *et al.* studies (4.93 and 10.07 respectively). No value was recorded for *B. humilis* in the Fitzpatrick *et al.* study, but values in this and the Williams study were also similar (3.55 and 4.27 respectively).

Overall, results of the dietary preference analysis (Table 4.9) revealed that none of the strong positive preferences for each *Bombus* species were associated with floral species that had received a large proportion of visits from that *Bombus* species (Table 12.4.1). In other words, the floral species which received substantially more foraging visits from one *Bombus* species than the others tended to be those that had received no (or very few) visits from other *Bombus* species and more (but still relatively few compared to overall visits to all flowers) from the *Bombus* species in question. This could indicate that the *Bombus* species had a broader flora that it visited compared to other *Bombus* species, but the fact that so few visits were recorded to the flowers did not indicate that the bee was specialising on that particular species.

Bombus sylvarum was however recorded as having the strongest positive preference for *Odontites verna* and *Ballota nigra* compared to the other *Bombus* species. *Odontites verna* and *B. nigra* were identified as being two of the most frequently visited floral species by *B. sylvarum*.

Table 4.9. Relative dietary preference. Contingency table comparing the deviations of the bees' observed frequencies of flower visits with those expected. Numbers highlighted in red represent the strongest positive correlation between bee species and flower selection for each bee species.

Floral species	*Bombus sylvarum*	*Bombus pascuorum*	*Bombus terrestris/ lucorum*	*Bombus humilis*	*Bombus lapidarius*
Achillea millefolium	-1.00	-1.00	-1.00	**3.55**	-1.00
Aquilegia spp	-1.00	-1.00	**4.16**	-1.00	-1.00
Arctium lappa	-1.00	-1.00	**4.16**	-1.00	-1.00
Ballota nigra	1.24	1.09	-0.93	0.27	-0.91
Brassicaceae spp	-1.00	-1.00	-1.00	-1.00	**2.75**
Brassica nigra	-1.00	-1.00	0.72	-1.00	1.50
Buddleja davidii	-1.00	-1.00	**4.16**	-1.00	-1.00
Calepina irregularis	-1.00	-1.00	-1.00	-1.00	**2.75**
Calystegia sepium	0.51	0.22	0.90	-1.00	-0.21
Carduus tenuiflorus	-1.00	**3.64**	-1.00	-1.00	-1.00
Centaurea nigra	-0.56	0.00	-0.84	-0.02	0.84
Cirsium arvense	-0.78	-1.00	0.06	-1.00	1.90
Cirsium spp	-1.00	**3.64**	-1.00	-1.00	-1.00
Cirsium vulgare	-1.00	-0.42	0.29	1.28	-0.53
Colutea arborescens	-1.00	-0.71	1.58	0.99	-1.00
Crataegus monogyna	-1.00	-1.00	**4.16**	-1.00	-1.00
Crepis capillaris	-1.00	1.32	-1.00	-1.00	0.87
Digitalis purpurea	-1.00	-1.00	**4.16**	-1.00	-1.00
Dipsacus fullonum	-1.00	-1.00	-1.00	**3.55**	-1.00
Echium vulgare	-1.00	0.55	2.44	-1.00	-1.00
Epilobium hirsutum	-1.00	-1.00	1.58	-1.00	0.87
Galega officinalis	-0.18	-0.21	0.83	-0.50	0.05
Geranium spp	-1.00	-1.00	-1.00	-1.00	**2.75**
Hypericum androsaemum	-1.00	-1.00	**4.16**	-1.00	-1.00
Hypericum perforatum	-1.00	-1.00	-1.00	**3.55**	-1.00
Inula crithmoides	-1.00	-1.00	**4.16**	-1.00	-1.00
Iris psuedacorus	-1.00	-1.00	**4.16**	-1.00	-1.00
Lamium album	**8.58**	-1.00	-1.00	-1.00	-1.00
Lathyrus hisutus	-1.00	-1.00	-1.00	-1.00	**2.75**
Lathyrus latifolius	-1.00	-0.81	1.87	0.84	-1.00
Lathyrus nissolia	-1.00	**3.64**	-1.00	-1.00	-1.00
Lathyrus pratensis	-1.00	1.32	0.29	0.14	-1.00
Lavandula spp	-0.21	0.63	-0.84	0.76	-0.44
Lilac spp	-1.00	-1.00	**4.16**	-1.00	-1.00

Table continued overleaf

Table 4.9. Relative dietary preference. (continued from previous page)

Floral species	*Bombus sylvarum*	*Bombus pascuorum*	*Bombus terrestris/ lucorum*	*Bombus humilis*	*Bombus lapidarius*
Linaria purpurea	-1.00	2.09	-0.14	-0.24	-1.00
Linaria vulgaris	-1.00	-1.00	-1.00	-1.00	**2.75**
Linum bienne	-1.00	-1.00	-1.00	-1.00	**2.75**
Lotus corniculatus	-1.00	-0.56	-0.97	-0.54	2.00
Lotus glaber	0.12	-0.79	-0.82	0.51	0.76
Lythrum salicaria	-0.13	0.69	-1.00	0.66	-0.32
Malva sylvestris	0.01	-1.00	0.09	-0.76	1.37
Medicago sativa	-1.00	-0.34	1.21	0.95	-1.00
Melilotus alba	3.79	-1.00	-1.00	-1.00	0.87
Melilotus officinalis	-0.36	-0.69	1.07	-0.70	0.50
Mentha spicata	-1.00	1.32	-1.00	1.28	-1.00
Misopates orontium	-1.00	-1.00	**4.16**	-1.00	-1.00
Odontites verna	1.49	0.67	-0.46	0.04	-0.82
Ononis spinosa	-1.00	-1.00	-1.00	1.28	0.87
Origanum vulgare	-1.00	-1.00	1.58	-1.00	0.87
Ornamental rose	**8.58**	-1.00	-1.00	-1.00	-1.00
Ornamental Thistle	-1.00	-1.00	**4.16**	-1.00	-1.00
Picris echioides	-1.00	-0.64	-0.21	-1.00	1.88
Picris hieracioides	3.79	-1.00	-1.00	1.28	-1.00
Potentilla reptans	-1.00	-1.00	-0.14	-1.00	2.12
Prunella vulgaris	-1.00	1.32	-1.00	1.28	-1.00
Pulicaria dysenterica	-1.00	-1.00	**4.16**	-1.00	-1.00
Rubus fruticosus	-0.96	-0.76	2.52	-0.81	-0.17
Sedum reflexum	-1.00	-1.00	-0.23	-1.00	2.19
Senecio jacobea	-1.00	-1.00	-1.00	**3.55**	-1.00
Senecio squalidus	-1.00	-1.00	-1.00	-1.00	**2.75**
Solanum dulcamara	-1.00	-1.00	-1.00	**3.55**	-1.00
Taraxacum spp	-1.00	-1.00	-1.00	-1.00	**2.75**
Trifolium arvense	-1.00	-1.00	-1.00	-1.00	**2.75**
Trifolium pratense	-0.88	0.88	-0.90	1.10	-0.62
Trifolium repens	-0.89	-0.36	-0.82	-0.45	1.61
Verbenaceae spp	-1.00	**3.64**	-1.00	-1.00	-1.00
Vicia cracca/villosa	-1.00	0.28	2.20	-0.92	-0.67
Vicia sativa	-1.00	1.32	1.58	-1.00	-1.00
Vicia tenuifolia	0.01	0.22	-0.73	1.40	-0.80

One result of note from the relative dietary breadth contingency table (Table 4.9) was the number of positive preferences (>1) and number of negative preferences (= -1) for each species (Table 4.10).

Table 4.10. Total positive and negative dietary preferences for each *Bombus* species. Summary based on an index of preference for food plant species calculated by the deviation of observed from expected floral visits to each floral species.

Bombus species	Total no. of positive preferences >1	Total no. of negative preferences = -1
B. sylvarum	6	50
B. pascuorum	11	38
B. terrestris/lucorum	22	27
B. humilis	12	39
B. lapidarius	17	33

Bombus terrestris/lucorum showed the highest number of positive preferences >1. In other words it was recorded visiting more flower species a greater than expected number of times than other *Bombus* species when all visits by all species were compared. On these flower species, *B. terrestris* was recorded either in greater numbers than would be expected compared to other bees or to flowers which were not being visited by other bees. This provided further evidence of the broad dietary breadth of *B. terrestris/lucorum* compared to the other species recorded.

Bombus lapidarius also demonstrated a large number of dietary positive preferences. However, the dietary breadth analyses did not record *B. lapidarius* as being significantly different from *B. pascuorum* and *B. humilis* suggesting that whilst it might have had a similar dietary breadth to *B. pascuorum* and *B. humilis*, some of its dietary preferences may have been different.

Bombus sylvarum, the bee recorded as having a significantly narrower dietary breadth than other bees, was also recorded as having the fewest positive preferences with respect to the other bees.

A relative dietary preference table was also calculated for floral family visits (Table 4.11)

Table 4.11. Relative dietary preference for floral families. Contingency table comparing the deviations of the bees' observed frequencies of flower visits with those expected grouped by floral families. Numbers highlighted in black represent positive correlations between bee species and flower selection for each bee species. Numbers highlighted in red represent negative correlations between bee species and flower selection for each bee species.

Floral Family	*Bombus sylvarum*	*Bombus pascuorum*	*Bombus terrestris /lucorum*	*Bombus humilis*	*Bombus lapidarius*
Asteraceae	-0.67	-0.36	-0.33	-0.35	1.08
Boraginaceae	-1.00	0.55	2.44	-1.00	-1.00
Brassicaceae	-1.00	-1.00	0.43	-1.00	1.71
Buddlejaceae	-1.00	-1.00	4.16	-1.00	-1.00
Clusiaceae	-1.00	-1.00	1.58	1.28	-1.00
Convolvulaceae	0.51	0.22	0.90	-1.00	-0.21
Crassulaceae	-1.00	-1.00	-0.23	-1.00	2.19
Dipsacaceae	-1.00	-1.00	-1.00	3.55	-1.00
Fabaceae	-0.52	-0.31	-0.04	0.09	0.40
Geraniaceae	-1.00	-1.00	-1.00	-1.00	2.75
Iridaceae	-1.00	-1.00	4.16	-1.00	-1.00
Lamiaceae	0.42	0.81	-0.84	0.52	-0.63
Linaceae	-1.00	-1.00	-1.00	-1.00	2.75
Lythraceae	-0.13	0.69	-1.00	0.66	-0.32
Malvaceae	0.01	-1.00	0.09	-0.76	1.37
Oleaceae	-1.00	-1.00	4.16	-1.00	-1.00
Onagraceae	-1.00	-1.00	1.58	-1.00	0.87
Plantaginaceae	-1.00	-1.00	4.16	-1.00	-1.00
Ranunculaceae	-1.00	-1.00	4.16	-1.00	-1.00
Rosaceae	-0.93	-0.77	2.46	-0.82	-0.12
Scrophulariaceae	1.46	0.67	-0.44	0.03	-0.82
Solanaceae	-1.00	-1.00	-1.00	3.55	-1.00
Verbenaceae	-1.00	3.64	-1.00	-1.00	-1.00

Of particular interest from the comparison of relative dietary preference at the floral family level was the number of positive and negative preferences for each species. Similarly to the species level analysis, *B. terrestris/lucorum* had the most positive preferences (n=12) and therefore the most floral families that it visited a

disproportionate amount when compared to the other *Bombus* species. *Bombus sylvarum* had the fewest positive preferences (n=4). Interestingly one of the four positive preferences was recorded on Scrophulariaceae flowers, indicating a degree of preference for these flowers greater than for the other *Bombus* species. *Bombus humilis* demonstrated a similar number of positive preferences to *B. pascuorum* and *B. lapidarius*. Both *B. humilis* and *B. lapidarius* recorded positive preferences for Fabaceae flowers, whereas the other *Bombus* species recorded negative preferences.

4.4 Discussion

Relative dietary breadth

Given that surveys were all carried out across the same sites and same time periods, results indicated that *B. sylvarum* workers had narrower floral species dietary breadth than the other *Bombus* species surveyed. With the same foraging options *B. sylvarum* foraged on a narrower breadth of flower species and families than *B. pascuorum, B. humilis, B. lapidarius*, and *B. terrestris/lucorum*. Whilst values for *B. humilis* were similar to those for *B. pascuorum* and *B. lapidarius*, all three were generally lower than those for *B. terrestris/lucorum*.

Mann-Whitney U exact tests revealed significant differences between the floral species dietary breadth of *B. sylvarum* and the two species considered to be ecologically most similar to *B. sylvarum, B. humilis* and *B. pascuorum* (Sladen, 1912; Prŷs-Jones and Corbet, 1987). Calculations of mean rarefied foraging estimates revealed substantial differences between *Bombus* species for other floral species and family comparisons (Figures 4.1 and 4.2) but, despite *p*-values being low, Mann-Whitney U exact tests were not significant after Bonferroni corrections had been applied to allow for multiple tests (Rice, 1989). It seemed likely however, that results of these statistical tests were limited by the small sample sizes of the monthly dietary breadths (with 'n' ranging from 2 months to 6 months) and that if more rarefied estimates had been generated by continuing surveys for another year, further statistical significance would have been recorded between

Bombus species. This was particularly the case for the rarefied means of *B. terrestris/lucorum* which were substantially greater than the other *Bombus* species for floral species and family dietary breadth particularly when yearly totals were compared (Tables 4.3 and 4.4). Despite having differences between means greater than those found to be significant between *B. sylvarum* and *B. pascuorum*, comparison of *B. terrestris/lucorum* dietary breadth with other *Bombus* species failed to reveal significant difference. This was even the case when compared to those of *B. sylvarum*, the lowest values recorded. The small sample size of *B. terrestris/lucorum*, with only two months or yearly totals available for comparison, appeared to be the cause of the lack of statistical significance.

Bombus humilis dietary breadth was consistently similar to that of *B. pascuorum* and *B. lapidarius* at both the floral species and family levels. This contradicted the theory that declining species have narrower dietary specialisations as *B. pascuorum* and *B. lapidarius* are both considered to be nationally ubiquitous in the UK. However this is only a study of floral visits, a more in depth analysis of foraging behaviour, for example pollen collection, might reveal further differences with respect to resource partitioning and was investigated further in chapter 5.

Results for *B. pratorum* and *B. hortorum* were included in Tables 4.3 and 4.4 but were only observed in small numbers during two of the months surveyed over the two years (2003 and 2004) and were therefore not included in further analysis. *Bombus hortorum* and *B. pratorum* were both considered to be ubiquitous species distributed throughout the British Isles (Edwards and Jenner, 2006) but even during the two months that the bees were recorded, numbers of these two species were very low on the sites. *Bombus pratorum* is an early emerging bumblebee (Prŷs-Jones and Corbet, 1987) therefore by July few workers would be foraging probably explaining the low numbers recorded in this study.

Bombus hortorum is also a fairly early emerging bumblebee (Prŷs-Jones and Corbet, 1987), but not to the same extent as *B. pratorum*. It would be expected that *B. hortorum* workers would still be foraging in large numbers during this survey. It is a long-tongued species and is commonly associated with gardens but is rarely abundant where it is found (Prŷs-Jones and Corbet, 1987). The lack of

observations in this study was fairly surprising due to the abundance of other longer-tongued species such as *B. humilis*, *B. pascuorum*, and *B. sylvarum* particularly when the favoured food source of *B. hortorum*, *Trifolium pratense* (Goulson and Darvill, 2004), was present across the sites. The low numbers of *B. hortorum* must therefore be explained by something other than presence of suitable forage, perhaps lack of forage or nest sites earlier in the year when *B. hortorum* queens were nest searching or *B. hortorum* being out-competed by the other successful long-tongued species on the sites.

In the two months that *B. hortorum* and *B. pratorum* workers were observed however, *B. hortorum* had a broad dietary breadth similar to that of *B. terrestris/lucorum*, whereas *B. pratorum* appeared to have a much narrower dietary breadth, more similar to that of *B. sylvarum*. When surveyed at sites where these bees were found in greater abundance, Goulson and Darvill (2004) found that *B. hortorum* was the most specialised species in terms of flower visits and that *B. pratorum* was the most polylectic, visiting 13 different species of plant for nectar. The unexpectedly opposing results for these two species in the South Essex study may be representative of the small number of observations in the study. Lack of further analysis of the dietary breath of *B. hortorum* and *B. pratorum* workers meant that further conclusions about the dietary preferences of these two species could not be made.

Relative dietary preference

Whilst no obvious preferences were revealed for the *Bombus* species investigated, results did indicate that the species with the broadest dietary breadth, *B. terrestris/lucorum*, also had the highest number of positive floral preferences compared to the other *Bombus* species and the lowest number of negative preferences. Contrastingly, *B. sylvarum* the species with the narrowest dietary breadth also demonstrated the least positive preferences for floral species and the highest number of negative preferences. This indicated that *B. sylvarum* under-visits more of the floral species in the study than the other *Bombus* species, again providing evidence for its narrower dietary preference.

When dietary preference at the floral family level was compared, *B. sylvarum* again showed the least positive preferences when compared to the other *Bombus* species. But, at both the family and species level, *B. sylvarum* was demonstrated as visiting the Scrophulariaceae flower, *Odontites verna*, proportionally more often than would be expected if all *Bombus* species were making the same foraging choices.

Summary

Overall, results indicated that *B. sylvarum* were either more specialised in their foraging choices or that they were limited in forage choices by the presence of other bumblebee species (competition). This was particularly apparent when compared to *B. terrestris/lucorum* which appeared to have a much broader dietary breadth than the other *Bombus* species and showed a fairly strong negative correlation with all of the floral target species identified previously in this study as being favoured by *B. sylvarum* and *B. humilis* (*Lotus glaber*, *Odontites verna*, *Ballota nigra* and *Trifolium pratense*). However, both *B. humilis* and *B. sylvarum* had similar dietary preferences to *B. lapidarius* and *B. pascuorum* indicating that resource partitioning, at least in terms of floral species visited, was not occurring.

The field based nature of this study meant that sufficient controls for the assessment of all aspects of bumblebee resource partitioning (scent marking, spatial partitioning, etc) were not possible, so further conclusions could not be drawn. Either way, the evidence gathered from this investigation indicated that knowledge of *B. sylvarum* foraging requirements when foraging with other *Bombus* species is required if attempts are to be made to halt and reverse declines in *B. sylvarum* distribution in the British Isles.

Whilst *B. sylvarum* was consistently recorded as having the narrowest dietary breadth, *B. humilis* was recorded as having a similar dietary breadth to the nationally ubiquitous species *B. lapidarius* and *B. pascuorum*. *Bombus humilis* did however appear to have a narrower dietary breadth than the nationally ubiquitous *B. terrestris/lucorum*, although not statistically significantly so.

One theory put forward for the decline of bumblebees in the UK has been the link between the loss of long corolla flowers associated with traditional agricultural techniques and the associated decline of long-tongued bumblebees adapted to specialise on these flowers (Inouye, 1980; Ranta and Lundberg, 1980; Prŷs-Jones and Corbet, 1987; Goulson and Darvill, 2004; Goulson *et al.*, 2005). Of the species surveyed, *B. sylvarum* was the rarest in the UK (Edwards, 1999), it had the narrowest dietary breadth and also has the longest tongue length of those bees surveyed (Table 4.1). No attempt was made in this study to correlate dietary preference with flower corolla lengths and, due to an absence of floral abundance surveys in these studies, no statistical analysis of the preferred floral species of the bees was possible. However, floral species identified as receiving the majority of visits by *B. sylvarum* workers in this study are all known to have fairly long corollas (*Lotus glaber*, *Odontites verna* and *Ballota nigra*).

Bombus terrestris/lucorum had the broadest dietary breadth and the most positive and least negative correlations with forage flowers from dietary preference analysis. It is considered to be one of the most ubiquitous UK bumblebees and had the shortest tongue-length of the bees analysed in this study. Some evidence from this study therefore supported the theory that tongue length is related to dietary breadth and that longer-tongued bees may be more specialised in the flower choices than bees with shorter tongues (Goulson *et al.*, 2005).

Bombus pascuorum, B. humilis and *B. lapidarius* however all have similar tongue lengths, differ greatly in their UK distributions and yet showed no significant difference in dietary breadth or dietary preference in these analyses. This suggested that whilst it might be possible that extremes of dietary breadth are contributing to declines in certain species, there must also be other factors influencing the overall declines in British bumblebees.

An alternative explanation for the differential declines in UK bumblebees could be related to a combination of dietary breadth and colony cycles (Table 4.2). Both *B. humilis* and *B. sylvarum* are late emerging species and have relatively short colony cycles which demand abundant high quality forage late in the season which might explain their narrow dietary breath (Ellis, 2006). *Bombus pascuorum* and *B.*

lapidarius emerge earlier and have longer colony cycles, but exhibited a similar dietary breadth to *B. humilis*. *Bombus humilis* and *B. sylvarum* are both localised and relatively rare in the UK, whereas *B. pascuorum* and *B. lapidarius* are numerous and widespread. Results of this study could indicate that dietary specialisation is a beneficial life strategy for bumblebees with longer colony cycles, but species with shorter colony cycles are not as successful. This could be explained by *Bombus* species with shorter life cycles and narrow dietary breadths being impacted to a greater extent by the loss of wildflowers due to agricultural intensification due to their increased requirements for high quality forage to provide the protein rich pollen necessary to raise their brood quicker as suggested by Goulson *et al.* (2005) and Ellis (2006).

The results of this study by no means provide conclusive evidence of this, but do indicate that further research may be beneficial to investigate the theory, perhaps comparing these results with those of other species undergoing similar declines in the UK. Also, a study on the continent where *B. sylvarum* and *B. humilis* are more common might reveal further information on dietary breadth and preference of these bees.

What the results did indicate however is that whilst *B. humilis* had a similar dietary breadth to the ubiquitous species *B. pascuorum* and *B. lapidarius*, *B. sylvarum* had a much narrower dietary breadth and thus might be more limited than some of the more ubiquitous UK species, particularly when the species are foraging together. Without further experimentation it is impossible to know whether this difference is caused by competition or dietary preference. Whichever the driving factor however, conservation efforts targeted towards bumblebees in general might not be effective for *B. sylvarum*. The study demonstrated that individual bumblebee species might require the provision of more specific flora for nectar and pollen forage than more ubiquitous species. The study also indicated that conservation management for *B. sylvarum* should also be beneficial for the seemingly more generalist *B. humilis*.

Results of the family level dietary preference data were consistent with previous studies of foraging behaviour for *B. humilis* and *B. sylvarum*. *Bombus sylvarum*

has consistently been recorded foraging on *Odontites verna* (Edwards, 1999; Harvey 1999; Harvey 2000a) and this preference for Scrophulariaceae was confirmed with a positive dietary preference recorded in this study when compared to the other *Bombus* species. *Bombus humilis* on the other hand has been reported to be most closely associated with flowers from the family Fabaceae (Edwards, 1999; Harvey, 1999; Carvell, 2002; Goulson and Darvill 2003; Chapman 2004) and Lamiaceae (Chapman 2004). In the South Essex study, *B. humilis* demonstrated a positive dietary preference for Fabaceae and Lamiaceae compared to the other *Bombus* species.

This study made no differentiation between foraging for nectar and pollen. Bees forage on both pollen and nectar, so any habitat management should attempt to provide sources of both. Methods to assess which floral species are utilised by *B. humilis* and *B. sylvarum* for pollen collection are discussed in chapter 5.

Chapter 5

Pollen analysis

5.1 Introduction

Bumblebees are central place foragers (Bronstein, 1995; Dramstad and Fry, 1995), collecting both nectar and pollen for their brood to feed on (Goulson and Darvill, 2004). Nectar is known to be an energy source and is therefore important throughout a bee's life cycle (Heinrich, 1979). Pollen however, is protein-rich and is known to be particularly important to developing larvae within a colony (Heinrich, 1979). The first larvae in a colony develop into workers which take over foraging duties for new larvae. More workers are then produced until production is switched to males and queens. The success of a colony is dependent on the production of these sexuals, both males and gynes (young queens), without which the colony would not be able to reproduce (Goulson, 2003). Availability of forage resources is known to affect the success of a colony. In a field experiment in the Quebec City area (Canada), colonies of *Bombus impatiens* and *Bombus ternarius* whose nectar and pollen supplies were increased regularly throughout a season reached larger sizes (in number of workers) and had a higher reproductive success than control colonies, by 51% and 86% respectively (Pelletier and McNeil, 2003). In particular, food supplementation increased the number of males produced and the probability of producing gynes. Evidence from the study indicated that when nectar and pollen were limited, colonies invest relatively more in cheaper to produce males, and that they only increase their investment in gynes when resources are available (Pelletier and McNeil, 2003). For a bumblebee population to be viable, it must be producing queens as well as males. This means that adequate nectar and pollen availability are vital for a healthy bumblebee population.

It has been suggested that one of the main factors causing the decline of bumblebees across the British Isles is the loss of suitable forage plants largely due to agricultural intensification (Goulson *et al.*, 2005). This theory corresponds with the evidence of colony success dependent on resource availability provided by the Canadian study. Recent studies have indicated that providing a suitable forage matrix can increase the number of bumblebees foraging on areas of land (Carvell *et al.*, 2004; Pywell *et al.*, 2005). In general these studies were designed to provide forage for the more ubiquitous British bumblebee species as many of the study

areas were not known to support populations of rarer bees. Surveys have not therefore recorded the benefits of these management practices for rare bumblebees.

Before management plans can be designed to address the population declines that have been reported in these bees over the last fifty years (Williams, 1982), investigation of whether forage provision in areas supporting populations of rare bees is beneficial to these bees needs to be carried out. As demonstrated in chapters 3 and 4, *B. sylvarum* appears to have more specific forage requirements and narrower dietary breadths than some of the more ubiquitous species. So, if habitat management plans for forage provision are to be effective, an understanding of any specific foraging needs is required. This includes their requirements both in terms of nectar and pollen. Thus far in this investigation floral species and plant families which are being used by *B. humilis* and *B. sylvarum* for forage visits in the South Essex area have been identified, but no attempt has been made to distinguish which flowers are important just for nectar and which for nectar and pollen. Pollen requirements are considered to be more specific than nectar (Goulson and Darvill, 2004) and its importance for the development of colonies (Heinrich, 1979) means that provision of suitable pollen sources is a key target for any habitat management for bumblebee conservation. The following investigation attempted to identify which flowers were specifically favoured by the bees as a pollen source.

5.2 Methods

Observation of bumblebee behaviour on flowers has been used as an indication of whether a bee is foraging for nectar or pollen (Goulson *et al.*, 2005; Carvell, 2002). This method can however, be relatively unreliable as it may be that the observer only witnesses part of the bees behaviour pattern on a particular flower, or that the bee collected pollen from the previous flower of the same species, but is only observed collecting nectar. This method of pollen analysis also does not provide information on whether the bee is collecting pollen from a single or several plant species or families. A more accurate method to assess which plant

species the bumblebees are using for pollen is to collect pollen from the hind tibia (pollen basket) of individual bumblebees during or after foraging flights (Figure 5.1). Pollen grain morphology varies between plant species so, in many cases, the plant of origin of pollen grains can be identified using microscopy (Ranta & Lundberg, 1981).

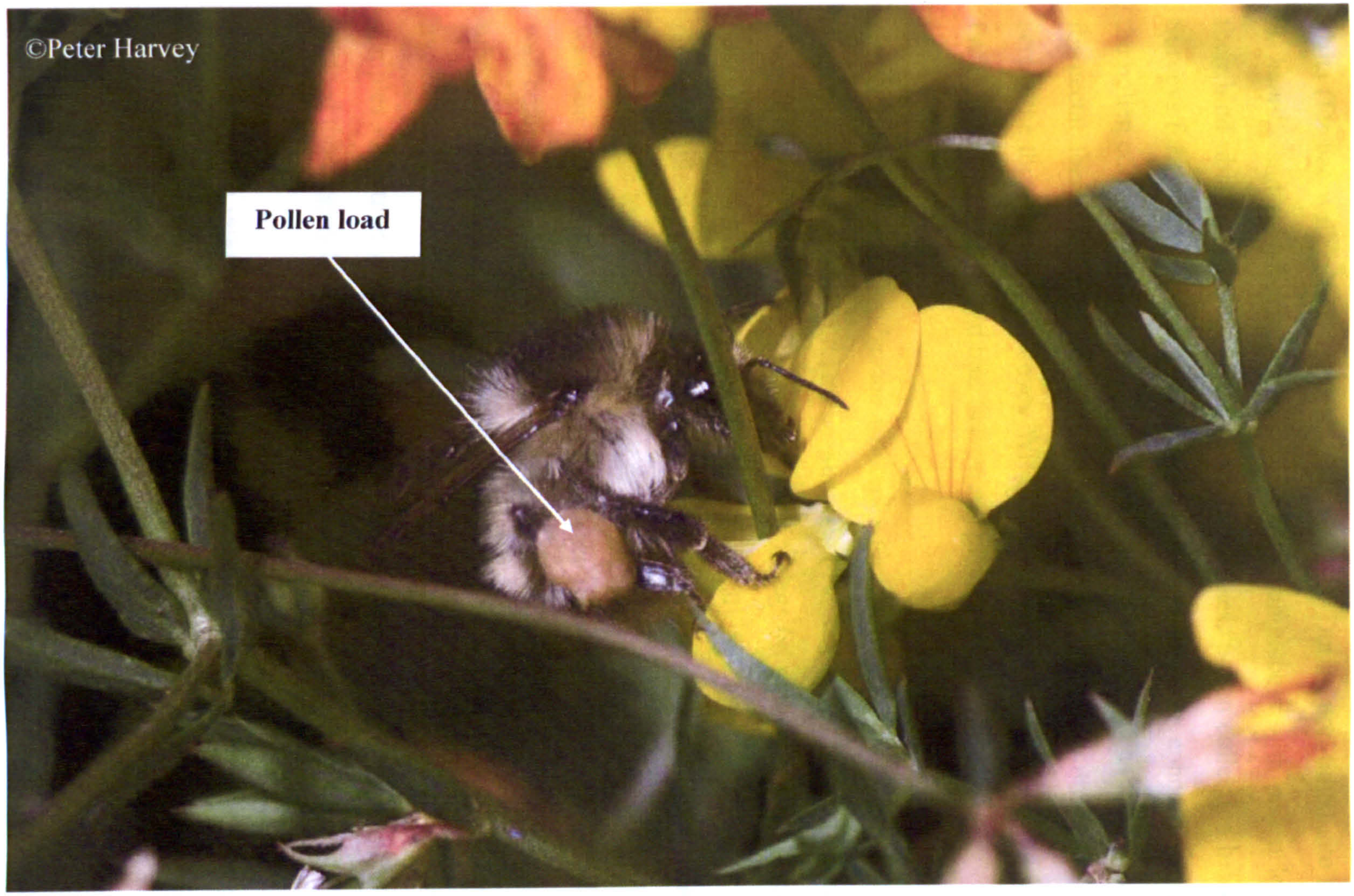


Figure 5.1. ***Bombus sylvarum*** **worker with pollen load on hind tibia.**

Bombus humilis and B. sylvarum foraging behaviour

Pollen collection was carried out during the 2004 site bee walks to assess floral visits (for bee walk methodology see section 3.2). During non-timed site surveys *B. humilis* and *B. sylvarum* worker and queen bumblebees with pollen loads on their hind tibias (Figure 5.1) were captured to assess pollen collection behaviour. Surveys for floral visits and pollen sampling were carried out concurrently so that the pollen collecting bumblebees had the same floristic choices as those monitored for nectar foraging flower visits. Bees were sampled randomly during bee walks across the three key sites (Canvey Wick, Wat Tyler and Hadleigh Castle Country Park) in an attempt to avoid bias to a certain plant species or site.

Bombus humilis and *B. sylvarum* workers with large quantities of pollen on their hind tibia were captured in Thorne Supplies (Thorne, 2007) queen bee marking plunger cages (Kwak, 1987). One of their hind legs (at random – left or right, not both) was extended through the mesh of the marking cage using tweezers. The pollen sample was then removed using a small plastic spatula. Care was taken to avoid damage to the bumblebee's leg or wing. Only one of the two pollen baskets was sampled from any one bee, the bees were released as soon as the collection was completed. The pollen collected was placed into a small labelled sampling tube and holes were made in the lid of the tube to allow aeration of the sample to avoid mould developing which could affect pollen grain identification. The samples were then stored at room temperature prior to analysis. The spatula was cleaned thoroughly between samples.

Analysis of the samples was carried out by Paul Westrich (Institut für Biologie und Naturschutz, Kusterdingen, Germany), and generously funded by Mike Edwards (Bumblebee Working Group/Bee Wasp and Ant Recording Society (BWARS)). The pollen was identified to family level, and where possible, to genus or species level. In some cases identification was not possible. Pollen collected was quantified by counting approximately 200 pollen grains and calculating the proportions of each grain type from those 200 grains. In certain cases, when too few pollen grains were collected, this quantification was based on the number of

grains present. The results are presented here and were included in the BWARS national bumblebee pollen analysis database.

Comparison of foraging behaviour

Following the initial study it was decided that the investigation should be expanded to include a comparison of pollen foraging behaviour between *B. sylvarum*, *B. humilis* and the more nationally ubiquitous *Bombus* species which foraged on the same sites. Three of the more ubiquitous bumblebee species were identified for further investigation; the common carder bee (*B. pascuorum*), the red-tailed bumblebee (*B. lapidarius*) and the buff-tailed/white-tailed bumblebee group (*B. terrestris/lucorum*). These species were selected as they were all identified in previous floral surveys as being abundant in the South Essex area and were present on all of the sites being used by *B. humilis* and *B. sylvarum* in the region. It could therefore be assumed that these species had the same foraging options in terms of pollen collection as *B. humilis* and *B. sylvarum*.

Pollen collection was carried out during the 2005 floral survey bee walks (section 3.2) using the same methods as the previous year. Thirty pollen samples were collected from each *Bombus* species across the sites. Once collected the samples were again sent to Paul Westrich for identification. Pollen identification and quantification followed the same procedures as for the initial analysis.

5.3 Results

Bombus humilis and B. sylvarum foraging behaviour

The 2004 pollen analysis study assessed the individual foraging behaviour of *B. sylvarum* and *B. humilis* in terms of pollen collection. Complete results for the analysis of pollen grain composition of each pollen sample are included in the appendix (section 12.5). A summary of the percentage composition of pollen

samples in terms of plant families present are presented in Figure 5.2 and are summarised for each species and caste below:

Bombus sylvarum queens

The sample size for *B. sylvarum* queens was very small (n=2). In the two samples analysed however Fabaceae was the only plant family from which pollen had been collected (Figure 5.2). From within Fabaceae, *Colutea arborescens*, and *Trifolium pratense* were identified as the species visited. Vicia-type pollen was also identified in one sample. The sample containing the Vicia-type pollen was collected from a *B. sylvarum* queen on *Lathyrus latifolius* so it was concluded that the pollen came from this species.

Bombus humilis queens

Only Fabaceae and Rosaceae pollen were present in the samples from *B. humilis* queens (Figure 5.2). From within these families *Vicia* species, *Lathyrus latifolius*, *Trifolium pratense*, *Colutea arborescens* and *Rubus fruticosus* were the sources of the pollen. Sample sizes were fairly small (n = 9), but appeared large enough to give a fairly good representation of queen behaviour for this species on the South Essex sites.

Bombus sylvarum workers

The families Scrophulariaceae and Fabaceae made up over 90% of the pollen collected from *B. sylvarum* workers and appeared to be the preferred pollen sources on the sites for these bees (Figure 5.2). Smaller proportions of pollen came from Lamiaceae, Rosaceae, Asteraceae and an unidentified pollen source (Figure 5.2).

Bombus humilis workers

Unlike *B. sylvarum* workers, *B. humilis* workers showed relative constancy to one plant family. Fabaceae pollen comprised 83% of all the pollen in the *B. humilis*

worker pollen samples (Figure 5.2) and was found in 97% of all samples taken from these workers (Figure 5.5). It therefore appeared to be the favoured forage source for the workers. Other plant families utilised were Scrophulariaceae, Rosaceae and Asteraceae, each comprising approximately 5% of the total pollen collected. Lamiaceae pollen and some unknown pollen were also present but both constituted less than 2% of the total pollen collected.

Due to the nature of microscopic analysis of pollen samples it was not always possible to identify pollen to species level. Therefore, quantification of pollen collected in terms of floral species was not possible. From the pollen which was identified at species level and from comparisons of pollen types with the particular flower the bee was foraging on when pollen was sampled (e.g. Lotus type pollen identified when bee was sampled foraging on *Lotus glaber*) it was possible to identify the most abundant floral species in the pollen samples. These species were summarised for each *Bombus* species and caste in Table 5.1.

A comparison of the relative number of pollen samples containing pollen from only one plant family and those containing pollen from multiple plant families was also calculated (Figure 5.3). This behaviour was roughly equal for both *Bombus* species with just under 50% of *B. sylvarum* worker samples and just over 50% of *B. humilis* worker samples being collected from a single plant family. However, comparison of the families being visited for these samples containing pollen from a single plant family showed marked differences between the bees (Figure 5.4). Greater than 75% of *B. sylvarum* pollen samples from a single plant family were collected from Scrophulariaceae flowers. For *B. humilis* samples, Scrophulariaceae pollen made up less than 5% of these samples, with Fabaceae pollen making up greater than 95% of these single plant family samples.

Table 5.1. Floral species identified as being collected by *Bombus* workers and queens, 2004. Most abundant pollen types in samples collected from foraging *B. humilis* and *B. sylvarum* workers and queens across the South Essex sites.

Bombus species and caste	Floral family	Floral species
B. sylvarum queens (n=2)	Fabaceae	*Colutea arborescens*
		Trifolium pratense
		Lathyrus latifolius
B. humilis queens (n=9)	Fabaceae	*Lathyrus latifolius*
		Trifolium pratense
		Vicia species
	Rosaceae	*Rubus fruticosus*
B. sylvarum workers (n=28)	Fabaceae	*Galega officinalis*
		Lamium species
		Lotus glaber
		Trifolium pratense
	Scrophulariaceae	*Odontites verna*
	Rosaceae	*Rubus fruticosus*
B. humilis workers (n=37)	Fabaceae	*Galega officinalis*
		Lotus glaber
		Trifolium pratense
		Trifolium repens
		Vicia species
	Scrophulariaceae	*Odontites verna*
	Asteraceae	*Centaurea nigra*
	Rosaceae	*Rubus fruticosus*

The final analysis performed on the pollen sample data was a comparison of the percentage of worker pollen samples from each *Bombus* species containing pollen from each plant family (Figure 5.5). In *B. sylvarum* worker pollen samples Fabaceae and/or Scrophulariaceae pollen was present in all of the samples analysed (see combined data, Figure 5.5), with each separately being recorded in over 60% of samples. In *B. humilis* worker pollen samples Fabaceae pollen was present in >95% of the samples analysed. The percentage of samples containing Scrophulariaceae pollen was <15%, similar to that of Rosaceae and Asteraceae.

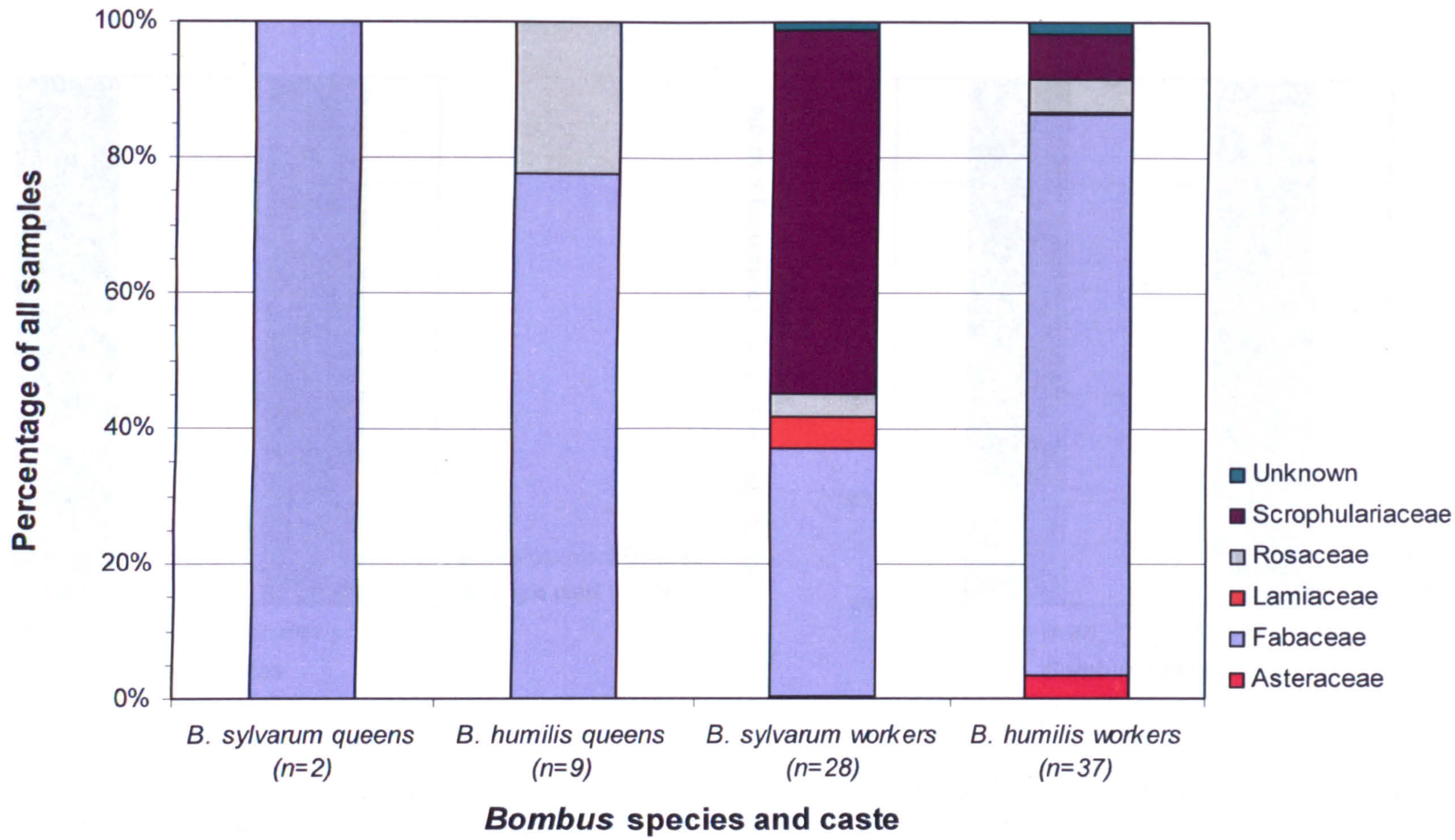


Figure 5.2. Total percentage of pollen from each floral family in all *B. humilis* and *B. sylvarum* pollen samples, 2004. Percentage values calculated as the proportion of pollen grains from each floral family in the total pollen collected for each *Bombus* species. Pollen samples were collected across all sites.

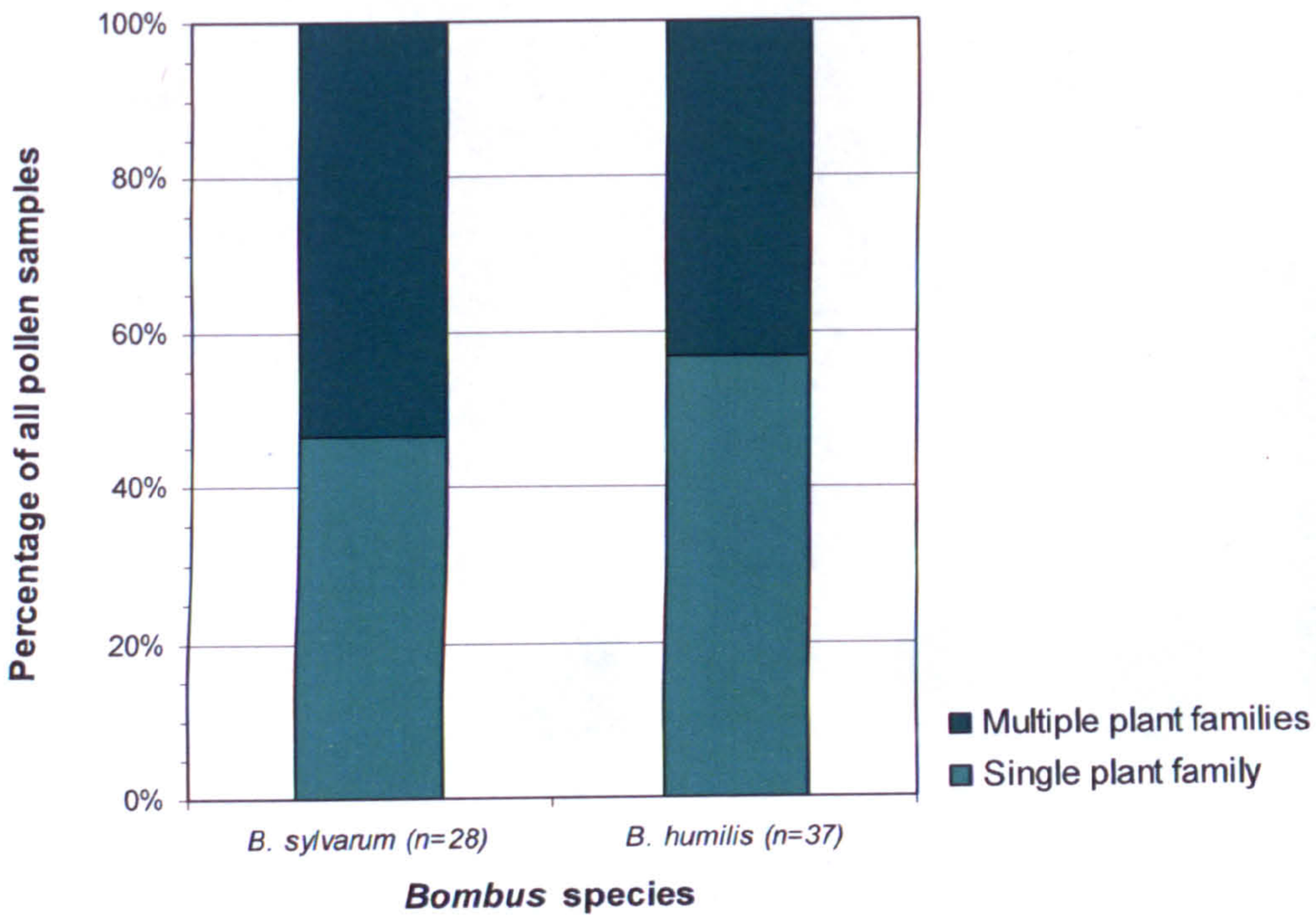


Figure 5.3. Percentage of *B. humilis* and *B. sylvarum* worker pollen samples containing pollen from single and multiple plant families, 2004. Pollen samples collected across all sites.

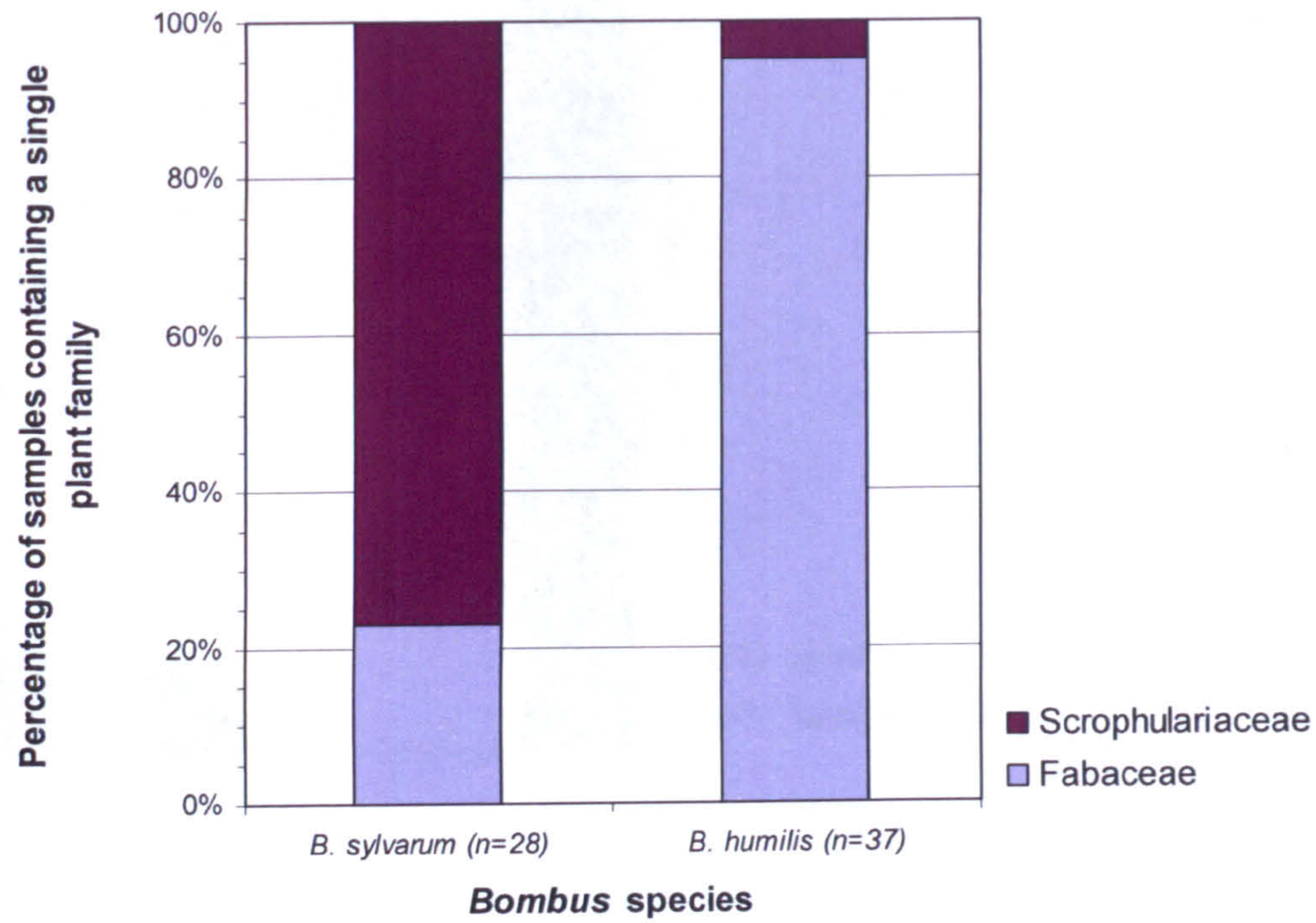


Figure 5.4. Plant families of *B. humilis* and *B. sylvarum* worker pollen samples containing pollen from a single plant family, 2004. Pollen samples collected across all sites.

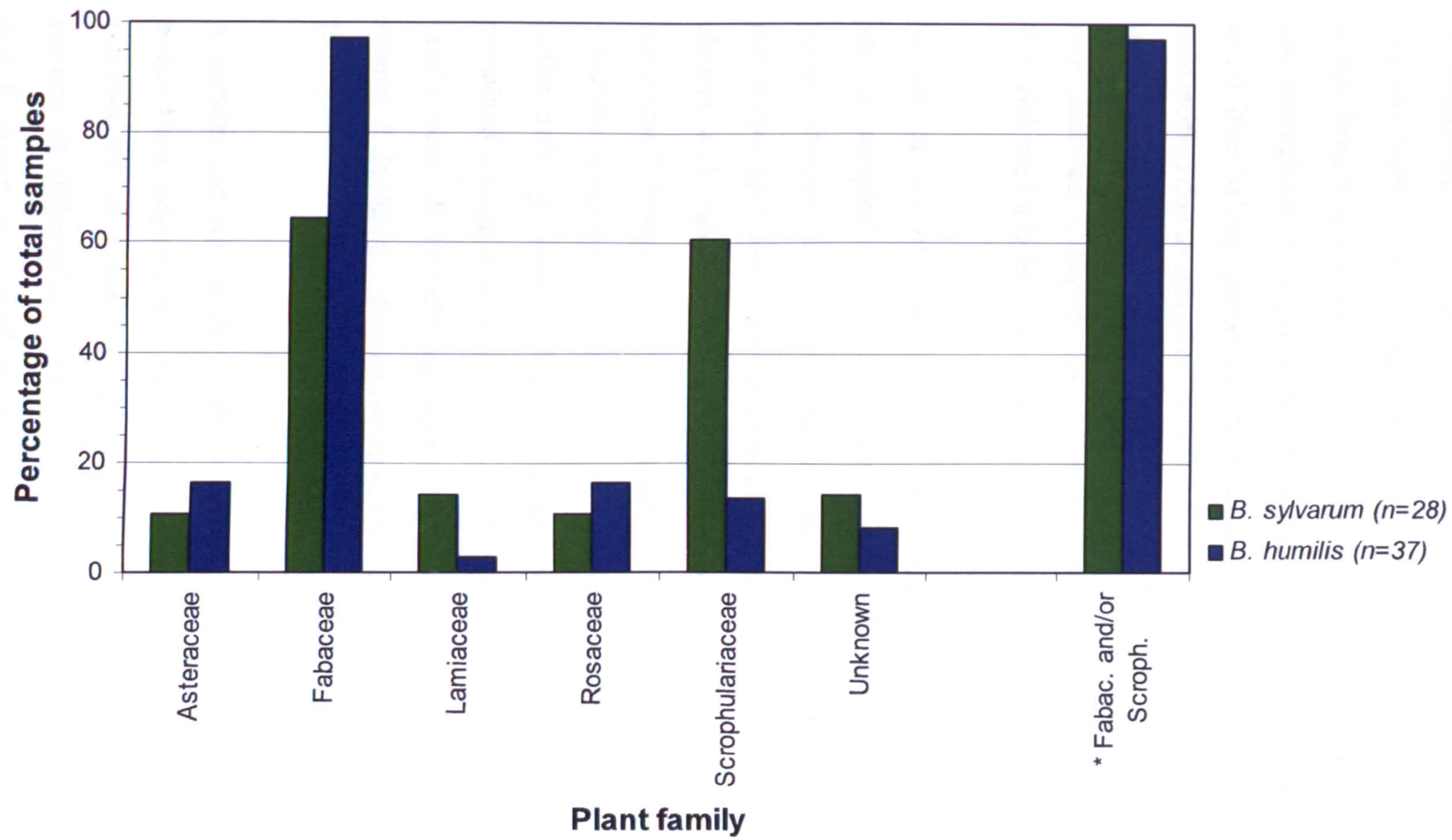


Figure 5.5. Percentage of all *B. humilis* and *B. sylvarum* worker samples containing pollen from each plant family, 2004. Pollen samples collected across all sites. (* Fabac. and/or Scroph. - represents a combined total for percentage of samples containing Scrophulariaceae and/or Fabaceae pollen)

Comparison of foraging behaviour

For the comparison of pollen foraging behaviour between *B. humilis*, *B. sylvarum*, *B. lapidarius*, *B. pascuorum* and *B. terrestris/lucorum* only pollen samples from *Bombus* workers were taken. Pollen sampling involved random collection of pollen from workers observed during bee walks. Pollen was collected across sites and throughout the main worker foraging period (July-August) in an attempt to avoid bias to any particular plant species or site. Some of the samples were unidentified due to mould or were lost in transit so sample sizes were not 30 for all *Bombus* species. This was a rare occurrence however and the majority of samples were analysed. Complete results for the 2005 pollen analysis carried out by Paul Westrich are included in the appendix (section 12.5).

Results of overall composition of pollen samples were varied for all *Bombus* species sampled (Figure 5.6). Fabaceae and Scrophulariaceae were the major pollen sources in all five *Bombus* species but to differing degrees. *Bombus pascuorum* appeared to be the most general species in terms of its pollen foraging choices with Fabaceae, Scrophulariaceae and Rosaceae all being almost equally represented. Assuming that the bees all faced the same foraging options, *Bombus sylvarum* appeared to demonstrate the strongest preference for Scrophulariaceae pollen although samples from the ubiquitous species *B. terrestris/lucorum* also contained a large proportion of pollen from this family. Similarly to the previous year's study, *B. humilis* appeared to favour Fabaceae pollen, but the ubiquitous species *B. lapidarius* showed the most frequent collection of pollen from this plant family.

A comparison was made of the relative number of pollen samples containing pollen from only one plant family and those containing pollen from multiple plant families for all *Bombus* species (Figure 5.7). Results demonstrated interesting patterns of differentiation between the *Bombus* species sampled. *Bombus humilis* and *B. sylvarum* showed the least consistency of the five *Bombus* species for collecting pollen from only one plant family. Values for *B. humilis* were much lower than the previous year's study (2004 - 57% and 2005 - 25%) perhaps indicating a reduction in the availability of Fabaceae pollen or increased

competition for it. *Bombus lapidarius* showed the highest constancy with 80% of samples from this species containing only a single plant family.

The plant families making up these pollen samples containing only one plant family also showed variability between *Bombus* species (Figure 5.8). *Bombus sylvarum* constancy was almost identical to that in the previous year with the majority of pollen samples being Scrophulariaceae pollen with a small proportion of Fabaceae samples. Contrastingly *B. humilis* showed variability between years. Similarly to *B. humilis* 2004 survey results, Fabaceae was the preferred choice for pollen constancy, but Scrophulariaceae and Asteraceae made up over 40% of the samples containing pollen from only one plant family.

The percentage of worker pollen samples from each *Bombus* species containing pollen from each plant family was represented in Figure 5.9. Scrophulariaceae and Fabaceae pollen appeared to be the favoured pollen sources for all of the *Bombus* species except for *B. pascuorum* which had a large proportion of samples containing Rosaceae pollen. Of particular interest from this graph was the proportion of samples containing Fabaceae and/or Scrophulariaceae pollen. As in 2004, 100% of *B. sylvarum* samples contained pollen from one or both of these two plant families. These consistent results demonstrated a strong affinity by *B. sylvarum* workers for these two floral families. The value for *B. humilis* was also similar to the previous year (~95%). Interestingly the values for the 3 nationally ubiquitous species were lower than for *B. humilis* and *B. sylvarum*. As all the bees were foraging on the same sites with the same flora available, these differences in frequency of collection of Fabaceae and Scrophulariaceae pollen might be indicative of a broader dietary preference amongst the more ubiquitous bees. However, experimentation with bees foraging with controlled forage options would be required to prove such a theory.

Floral species visited by *B. humilis* and *B. sylvarum* workers for pollen collection in the 2005 survey were largely similar to the 2004 survey, although *Centaurea nigra* pollen was present in greater quantities in the 2005 *B. sylvarum* samples. The only newly recorded pollen sources were a *Salvia* species for *B. humilis* and a

Cirsium species and *Knautia* species for both *B. humilis* and *B. sylvarum*. However, these appeared in few samples for either bee species.

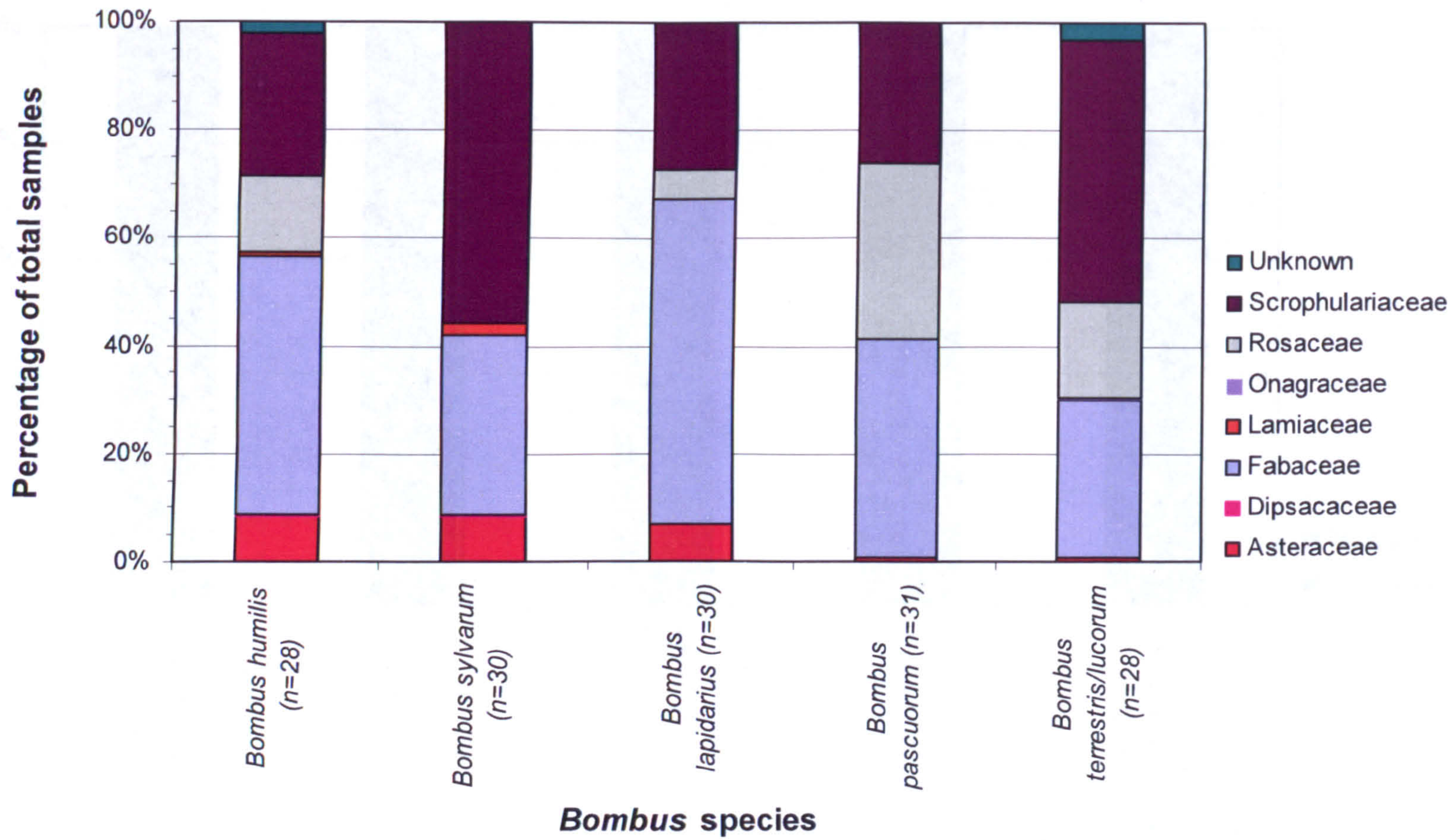


Figure 5.6. Total percentage of pollen from each floral family in all *Bombus* species pollen samples, 2005. Overall percentage calculated as the sum of the percentage of pollen grains from each floral family in a 200 grain sub-sample from all of the samples collected. Pollen samples collected across all sites.

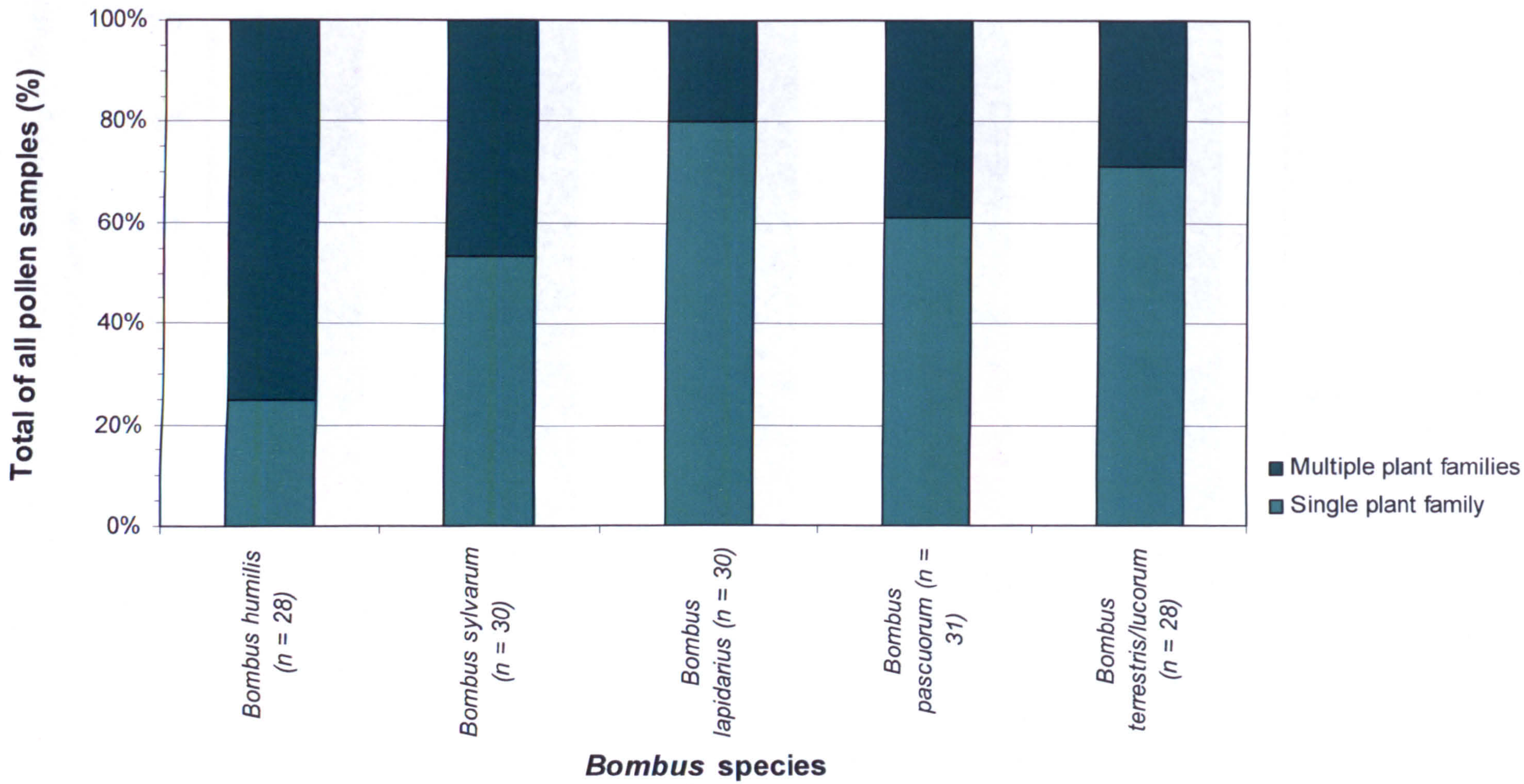


Figure 5.7. Percentage of *Bombus* worker pollen samples containing pollen from single and multiple plant families, 2005. Pollen samples collected across all sites.

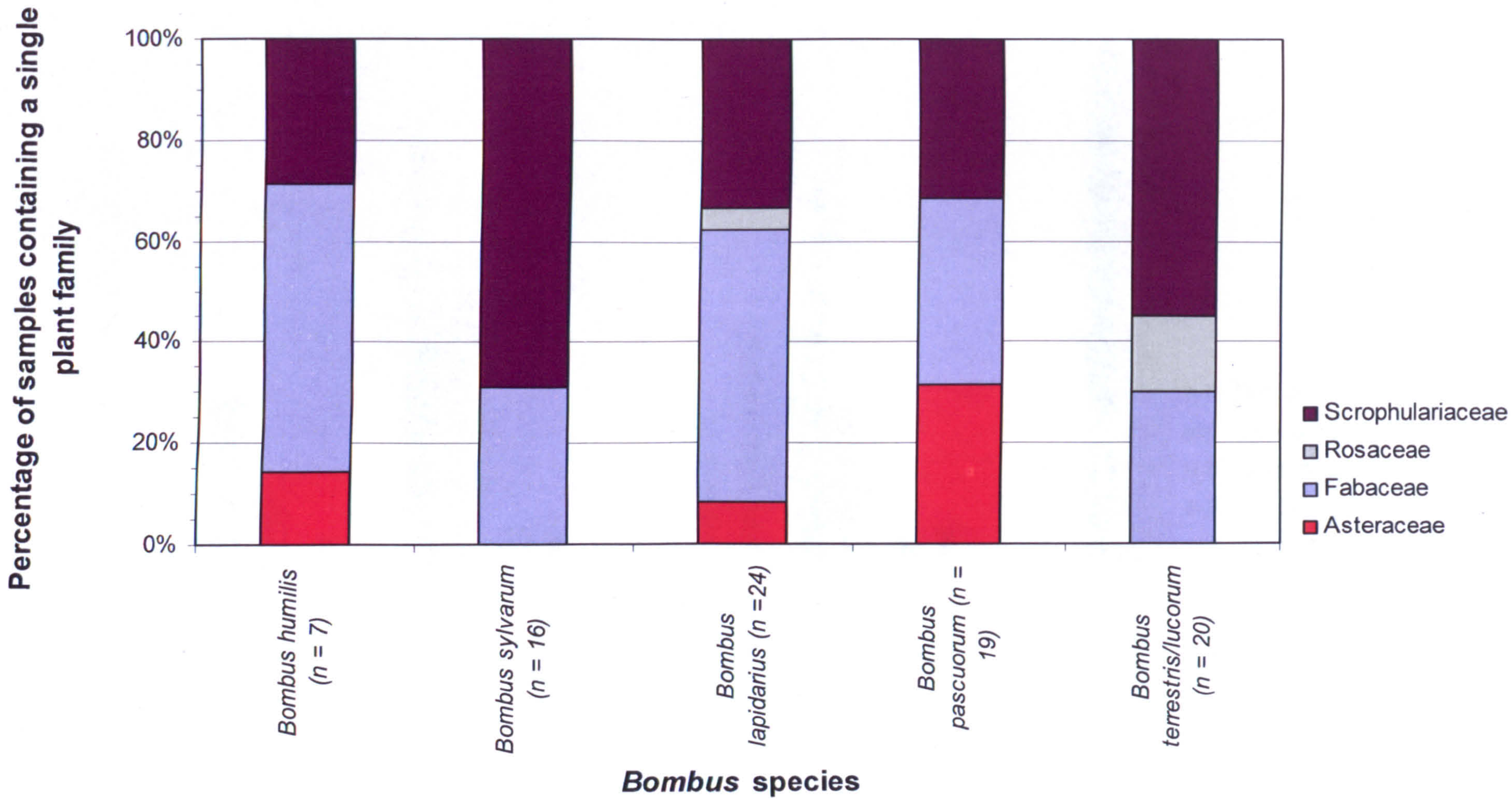


Figure 5.8. Plant families of *Bombus* worker pollen samples containing pollen from a single plant family, 2005. Pollen samples collected across all sites.

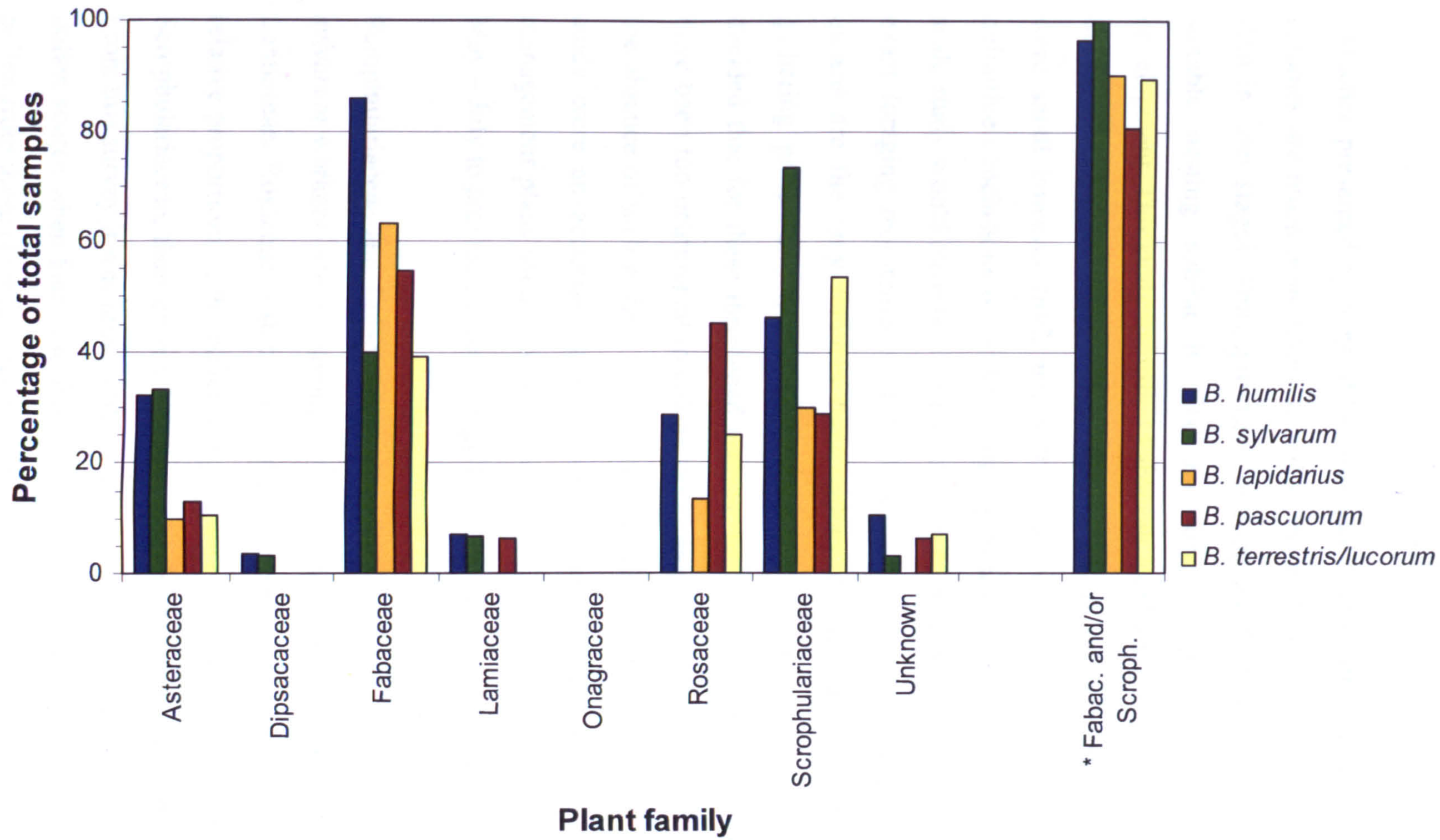


Figure 5.9. Percentage of all *Bombus* worker samples containing pollen from each plant family, 2005. Pollen samples collected across all sites. (* Fabac. and/or Scroph. - represents a combined total for percentage of samples containing Scrophulariaceae and/or Fabaceae pollen)

5.4 Discussion

Bombus humilis and B. sylvarum foraging behaviour

Evidence presented by Schmid-Hempel and Durrer (1991) has demonstrated that colonies are much more likely to fail in the early stages of colony development than in later stages. Thus, provision of adequate food supply in the vicinity of suitable nesting habitat is vital for foraging queens to facilitate colony development. This study identified Fabaceae species and *Rubus fruticosus* as being the favoured pollen collected by *B. humilis* and *B. sylvarum* queens. Sample sizes were small however (n=2 and n=9 respectively), so it was difficult to draw definitive conclusions on pollen sampling behaviour from these results. A larger scale study would have been required to make a more accurate assessment of the exact foraging requirements of *B. humilis* and *B. sylvarum* queens. However, queens are the only foragers for a colony during early colony development, gathering pollen and nectar single-handedly (Alford, 1975). It was therefore decided that for these threatened species, large scale sampling of queens would have been too detrimental to colony development for inclusion in this project. In the absence of such a study it must be assumed that the species identified in this study were an accurate representation of foraging behaviour and thus habitat management plans should include the cultivation of these species on sites during May – July to provide suitable forage for *B. humilis* and *B. sylvarum* queens.

Scrophulariaceae and Fabaceae made up over 90% of the pollen collected by *B. sylvarum* workers. The remaining 10% was made up of pollen from the families Lamiaceae, Rosaceae, Asteraceae and an unidentified pollen source. Whilst their relative proportions in the pollen samples were much lower than for Fabaceae and Scrophulariaceae, their presence indicated an importance to *B. sylvarum* colonies. From this survey it was impossible to tell whether this importance was as a reserve pollen source when Fabaceae and Scrophulariaceae pollen was limited or whether pollen from these plant families is nutritionally different from that of Fabaceae and Scrophulariaceae and therefore necessary to the bees. Past studies have provided evidence for both sides of this argument: Studies of colonies of commercially

reared bumblebees in greenhouses given a diverse mix of pollen performed no better than those feeding on only tomato (Solanaceae) pollen (Whittington and Winston, 2003), indicating that nutritionally one source of pollen was as valuable as another. Goulson and Darvill (2004) have however argued that longer-tongued bees tended to forage preferentially on Fabaceae flowers as a pollen resource and that this may be a response to needing to raise their brood quickly, so specializing on more protein rich pollen. Whilst variation in the protein quality of pollen has been investigated (Standifer, 1967), benefits for bumblebees have received very little attention (Goulson *et al.*, 2005).

Without the possibility of greenhouse-based studies for *B. sylvarum* and *B. humilis* to investigate differential values of pollen from different plant families, the importance of different pollen sources to the bees cannot be assessed. Therefore providing a mix of all of these pollen sources should be considered to be of the greatest benefit to the development of *B. sylvarum* colonies. Priority should be given to Fabaceae and Scrophulariaceae however as not only did these two families make up the majority of *B. humilis* and *B. sylvarum* pollen collected, but also were recorded in all of the 28 *B. sylvarum* worker pollen samples analysed and >95% of those from *B. humilis* workers (Figure 5.5).

The proportion of Scrophulariaceae pollen collected by *B. humilis* workers was substantially less than for *B. sylvarum* workers. Scrophulariaceae pollen made up only approximately 7% of the *B. humilis* pollen samples compared to more than 50% of the *B. sylvarum* samples. This might be indicative of resource partitioning occurring between the two *Bombus* species and may also partially explain the differences observed in the national declines of these two species. If this is the case, it highlights the importance of providing Fabaceae pollen for both species, but also Scrophulariaceae flowers and in particular *Odontites verna* specifically for *B. sylvarum* conservation.

Comparison of the proportion of pollen samples which contained pollen from only one plant family were approximately equal for *B. humilis* workers and *B. sylvarum* workers (Figure 5.3). The families being visited for this constancy however revealed marked differences between the bees (Figure 5.4) *B. sylvarum* collected

mostly from Scrophulariaceae flowers and *B. humilis* from Fabaceae. These results indicated that *B. sylvarum* and *B. humilis* workers differed in their pollen collecting behaviour. With workers foraging on the same sites with a broad variety of floral available, assuming that the bees are foraging with the same foraging options, differences between pollen collected represented variation in foraging behaviour and might therefore be further evidence of resource partitioning between the two species.

Comparison of foraging behaviour

Evidence generated in chapter 4 indicated that some species may have a narrower dietary breadth than others which may be contributing to their decline in the UK. This aspect of rare bumblebee ecology in the UK has been much debated by Goulson and Darvill (2004), Goulson *et al.* (2005) and Williams (2005) and an accurate knowledge of it is required if habitat management for bumblebee conservation is to benefit the rare as well as the more ubiquitous species. The 2005 investigation was designed to assess any dietary variation of *B. humilis* and *B. sylvarum* in terms of pollen collecting behaviour compared to more ubiquitous UK bumblebees.

Both the *B. humilis* and *B. sylvarum* 2005 samples contained larger proportions of Asteraceae pollen than in 2004 samples. This pollen appeared to have come mostly from *Centaurea nigra*. It is likely that these differences were due to differences in floral availability between years but, due to limitations inherent in such field based study and randomisation of sampling, it was impossible to conclude definitively why this change had occurred between the two sample years.

Other than the increase in Asteraceae pollen, *B. sylvarum* worker samples were relatively similar between the two study years. *Bombus humilis* samples varied quite considerably however, particularly in the proportions of Fabaceae pollen collected. In 2004, Fabaceae pollen made up ~80% of the pollen collected, this dropped to ~50% in the 2005 samples. *Bombus humilis* also collected much fewer samples from a single plant family (~60% in 2004 compared to ~25% in 2005) and the floral families featured in these samples changed from ~95% Fabaceae in 2004

to ~55% Fabaceae in 2005 with the rest being Asteraceae and Scrophulariaceae. This variability of *B. humilis* in terms of forage between years may have been a reaction to reduced availability of Fabaceae pollen in 2005, perhaps indicating an ability to be able to adapt to changes in available flora. It also may have been indicative of the broader dietary breadth the species was demonstrated as having when compared to *B. sylvarum* in chapter 4 (sections 4.3 and 4.4). Further experimentation assessing floral availability with pollen collected would be required investigate why *B. humilis* pollen collection varied so considerably between years whereas *B. sylvarum* was relatively constant. If *B. humilis* was demonstrated as being more 'opportunistic' (i.e. better able to change pollen collecting behaviour based on available pollen), it might explain why *B. humilis* populations are still relatively more successful in the UK than *B. sylvarum*. It would also coincide with the observations of the BWG that *B. humilis* populations can survive in smaller habitat fragments than *B. sylvarum* (Edwards, 1998), as it would mean that they are better able to exploit diminishing resources.

Of the more ubiquitous species *B. pascuorum* was the most general in terms of plant family constancy, being almost equally divided between Scrophulariaceae, Fabaceae and Asteraceae. *Bombus terrestris/lucorum* was also divided between these three plant families. Single family constancy for *Bombus lapidarius*, the species showing the highest proportion of pollen samples containing only one plant family, was divided between Scrophulariaceae, Fabaceae, Rosaceae and Asteraceae.

Scrophulariaceae and Fabaceae pollen appeared to be the most common pollen source for all of the *Bombus* species. *Bombus pascuorum* was the only species which had an equally large proportion of pollen from a different plant family, Rosaceae. Of all 5 *Bombus* species, *B. sylvarum* and *B. humilis* appeared to be the most strongly related to pollen collection from Fabaceae and Scrophulariaceae with all *B. sylvarum* samples containing pollen from one or both of these two plant families for the second year running. The value for *B. humilis* was also similar to the previous year (96% in 2005 and 97% in 2004), demonstrating the consistently high frequency of pollen samples from *B. humilis* and *B. sylvarum* containing pollen from these two floral families. Interestingly the values for the 3 nationally

ubiquitous species were lower than for *B. humilis* and *B. sylvarum*, *B. pascuorum* being the most different with ~20% of samples from this species containing no Fabaceae or Scrophulariaceae pollen.

Like *B. sylvarum* and *B. humilis*, *B. pascuorum* is from the sub-genus Thoracobombus (the carder bees) and is considered to be the most ecologically similar to *B. sylvarum* and *B. humilis* in terms of its life cycle, tongue length and nesting requirements (Sladen, 1912; Prŷs-Jones and Corbet, 1987). Unlike *B. sylvarum* and *B. humilis*, *B. pascuorum* is still considered to be nationally ubiquitous (Williams, 1982). Results for *B. pascuorum* workers throughout the investigation demonstrated a broader dietary breadth in terms of pollen collection than *B. humilis* and *B. sylvarum*. Analysis of *B. pascuorum* pollen samples recorded large proportions of Asteraceae pollen in total pollen loads (Figure 5.6), in pollen samples containing a single plant family (Figure 5.8) and was present in a high proportion of all samples (Figure 5.9). This seemingly broader dietary preference for pollen than *B. humilis* and *B. sylvarum* might partly explain the different fortunes of the three species in the UK.

Summary

Fabaceae and Scrophulariaceae pollen were by far the most collected pollen by both *B. sylvarum* and *B. humilis* throughout both years of the survey. *Bombus sylvarum* appeared to select Scrophulariaceae pollen whilst *B. humilis* foraged on Fabaceae pollen providing evidence for the possibility that resource partitioning occurs between the two species. Plant species of particular importance from these two families were *Odontites verna*, *Lotus glaber*, *Trifolium pratense*, *Vicia* species and *Galega officinalis* (see Table 5.1).

Care must be taken with the provision of *Trifolium pratense* as a forage resource for *B. sylvarum* populations as it made up relatively little of the Fabaceae pollen collected by *B. sylvarum*. Correspondingly, few visits by *B. sylvarum* to *Trifolium* species were recorded in the foraging surveys in chapters 3 and 4. In the dietary preference analysis carried out on these species (chapter 4, Figure 4.5) *B. sylvarum* had a negative value for *Trifolium pratense* and *Trifolium repens*, indicating that

B. sylvarum was recorded making fewer visits to these flowers than would be expected based on the number of visits made by the other *Bombus* species. However, whilst *Trifolium pratense* does not appear to be an important flower for *B. sylvarum,* results from this study have demonstrated that it is a favoured forage source of other long-tongued bees in this survey, particularly *B. humilis.*

Other species favoured for pollen collection by *B. humilis* and *B. sylvarum* were *Rubus fruticosus* and *Centaurea nigra.* The importance of *Rubus fruticosus* to these bees was also noted by Peter Harvey (1999) but its value was considered to be as scrub for nesting sites or merely as associated with areas managed to provide the best flower-rich herbage. This study has demonstrated that its importance may also be as a source of pollen to foraging bees, perhaps as a back-up source when other sources are limited or as a supplement to Fabaceae and Scrophulariaceae pollen.

It was difficult to draw conclusions for *B. sylvarum* queens due to the small sample size, but from these results and those for *B. humilis* queens it would appear that Fabaceae was the preferred pollen source, particularly *Vicia* species, *Lathyrus latifolius, Trifolium pratense* and *Colutea arborescens. Rubus fruticosus* pollen was also present in *B. humilis* queen samples again possibly indicating its value as an early forage source.

Of particular interest in this study was the lack of Lamiaceae pollen found in pollen samples compared to the frequency of visits recorded to this family during the floral surveys in chapters 3 and 4. This indicated that the value of Lamiaceae flowers may have been as a nectar source rather than a pollen source. The main Lamiaceae flowers on the sites were *Ballota nigra* and these flowers tended to support the bees later in the season when other flowers had disappeared (personal observations). The lack of Lamiaceae pollen in this study might therefore be caused by the sampling time being relatively early in the season for *B. humilis* and *B. sylvarum* workers to be foraging on *Ballota nigra.* Sampling of pollen from bees foraging on *Ballota nigra* later in the season would need to be carried out to substantiate this theory.

Whilst the results of this study were somewhat limited by its field-based nature, they did indicate that interspecies differences occurred during pollen collection. Pollen samples were collected randomly, and on all of the sites a similarly broad range of pollen sources were available. The ability of bumblebees to forage over relatively large distances (Osborne *et al.*, 1999; Chapman et al., 2003; Darvill *et al.*, 2004) meant that bees should not have been limited in choice to the nearest flowers to their nest. As all of the bees appeared to have the same foraging options, it could be concluded that differences in pollen composition recorded between species were not related to floral availability but to an unidentified aspect of foraging behaviour, such as interspecies resource partitioning or specialisation. A study with controlled foraging options would be required however to substantiate this.

This investigation and the two previous chapters have identified the most visited forage sources in terms of nectar and pollen for *B. humilis* and *B. sylvarum* in South Essex. Conservation management for the bees should target these flowers alongside suitable nesting habitat on a landscape level across appropriate temporal and spatial scales. Best practice for the creation of such habitat and the spatial dynamics for such habitat creation have been investigated in the following chapters.

Chapter 6

Forage patch design

6.1 Introduction

In previous chapters the plant families Fabaceae, Scrophulariaceae and Lamiaceae were recorded as being plant families visited most frequently by both *B. humilis* and *B. sylvarum* for foraging visits. Of these, Fabaceae and Scrophulariaceae were recorded as receiving the majority of pollen foraging visits by workers and queens. The floral species *Odontites verna, Lotus glaber, Lotus corniculatus, Ballota nigra, Trifolium pratense* and *Lathyrus latifolius* were identified as being the most important for the bees from these plant families.

Declines in bumblebee habitat and forage availability have been generally accepted as being the main driving forces behind bumblebee declines nationally (Williams, 1986; Osborne *et al.*, 1991; Osborne and Corbet, 1994; Goulson, 2003; Goulson *et al.*, 2005) Decreases in the abundance of a number of highly preferred bumblebee forage plants, especially members of Fabaceae and Lamiaceae (Rich and Woodruff, 1996; Pywell *et al.*, 2005; Carvell *et al.*, 2006) are thought to have significantly impacted bumblebee colony success particularly on arable land. Many of the floral species identified in the South Essex study as being favoured forage species of *B. humilis* and *B. sylvarum* have been shown to have declined on a national scale (Preston *et al.*, 2002).

A key target for conservation habitat management is therefore the provision of the floral species identified in this study as being the preferred forage species of the two *Bombus* species. The Bumblebee Working Group (BWG) identified the need for areas of at least 10km^2 of suitable habitat matrix to support populations of both these bee species (Edwards, 2002). The need for conservation habitat management on a landscape scale is therefore imperative. The first step towards such landscape scale conservation is to identify best practice for habitat creation.

This study was an investigation of whether *B. sylvarum* and *B. humilis* are able to locate and utilize new forage patches in areas where previously no forage was available. It also investigated effective methods to provide suitable forage as part of the restoration of a capped landfill site.

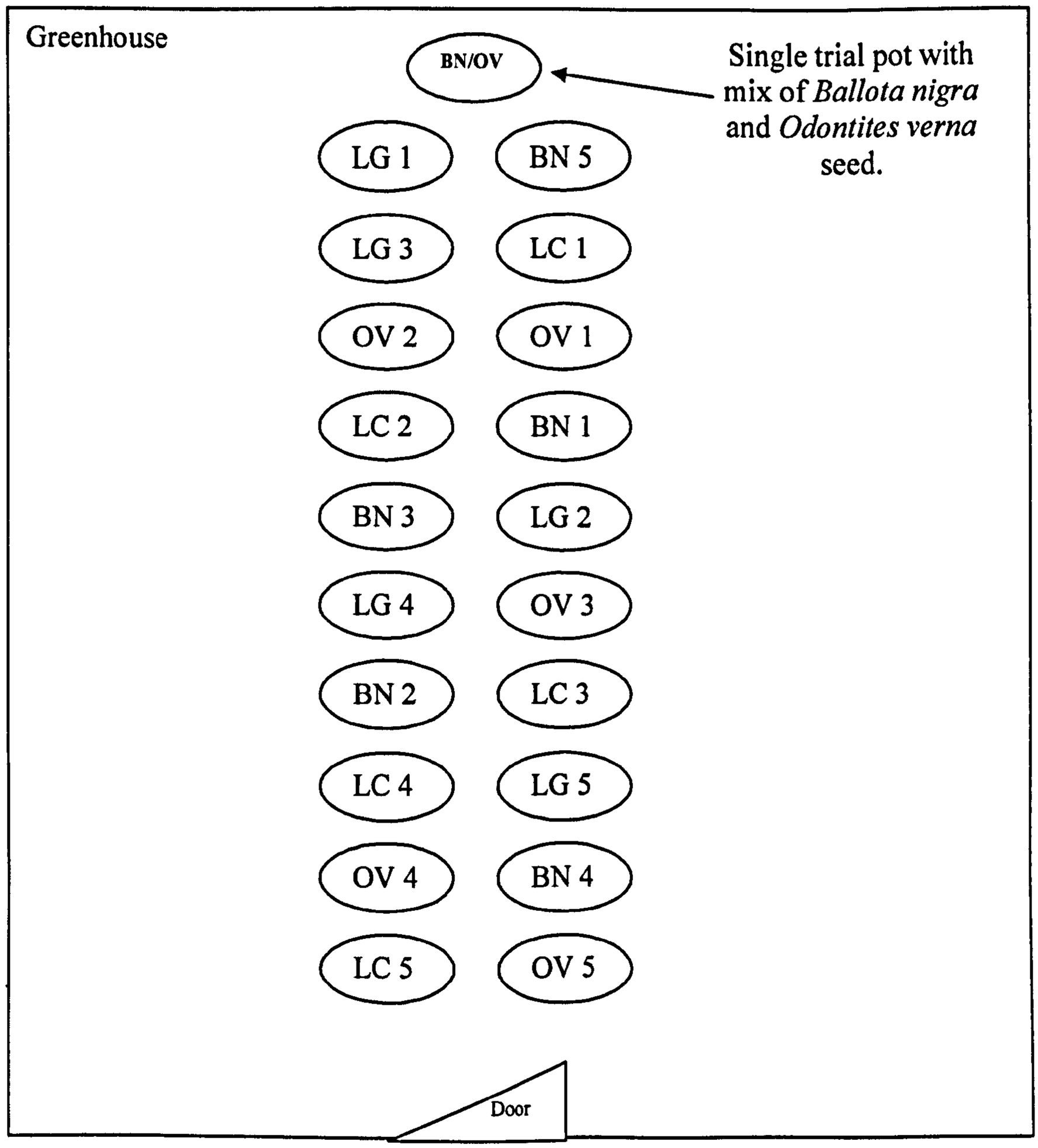
6.2 Methods

Greenhouse trials

Following the identification of the preferred forage plants of the *B. humilis* and *B. sylvarum* workers, greenhouse trials were carried out to assess the potential for growing forage plants from seed and for the production of seed banks for these species. Growth trials of *Lotus corniculatus*, *Lotus glaber*, *Odontites verna* and *Ballota nigra* seeds were initiated in January 2004 at Cranfield University greenhouses.

Lotus corniculatus, *Ballota nigra* and *Odontites verna* seeds were purchased from wildflower seed retailer Nickys Seeds (Nickys Nursery, 2007). Due to the limited availability of *Lotus glaber* seeds from suppliers, seeds for this species were collected from the South Essex sites. Five pots were prepared for each wildflower species each containing 50 seeds. *Odontites verna* is widely reported as being hemiparasitic on other plant species (Govier *et al.*, 1968) so a further trial was carried out mixing *Ballota nigra* and *Odontites verna* to assess whether *Odontites* verna performed better when grown with other species. Due to limited seed availability only one trial pot of this seed mix was created.

The pots were filled with John Innes loam-based compost No.3. The seeds were sprinkled on top then covered lightly with more compost and watered. The pots were placed in a random arrangement (Figure 6.1) and watered regularly. Percentage of successful germination was monitored weekly. The trial was run for 26 weeks.



<u>Key</u>
LG = *Lotus glaber*
LC = *Lotus corniculatus*
BN = *Ballota nigra*
OV = *Odontites verna*
BN/OV = *Ballota nigra/Odontites verna* mix 20:50 seed mix

Figure 6.1. Plan of greenhouse growth trials for *Lotus glaber*, *Lotus corniculatus*, *Ballota nigra* and *Odontites verna*. Pots were arranged randomly and were watered regularly. 50 seeds in each pot, cultivated for 26 weeks.

Cleanaway Pitsea Landfill Trials

Greenhouse trials of favoured forage plants were followed by field trials. Experimental forage patches were created at the Cleanaway Pitsea Landfill, Essex (TQ740857) (Figure 2.1). Being situated within the known area of both *B. humilis* and *B. sylvarum* East Thames Corridor metapopulations, the landfill site provided an ideal opportunity for the creation of new forage. Prior to the creation of these forage patches, the area in question on the site was an active landfill and was only recently capped with topsoil. Forage created on the site would therefore be completely new and the ability of *B. humilis* and *B. sylvarum* to find and exploit new forage resources could be assessed.

Phase 1

In November 2003, a trial plot was created on a recently capped area of the landfill site. An area of approximately $100m^2$ of bare soil on the capped landfill was marked out. The area was cultivated then subdivided into quarters (Figure 6.2). Each area was sown with a different wildflower species. The species used; *Lotus corniculatus*, *Lotus glaber*, *Odontites verna* and *Ballota nigra*, were selected as those being most frequently visited by both *B. sylvarum* and *B. humilis* workers (section 3.3) during the summer 2003 surveys.

The area immediately surrounding the trial plot was bare topsoil during the summer of 2003 and was subsequently sown with *Lolium perenne*. This area contained no known bumblebee forage sources in 2003 and therefore no bumblebees were present on this part of the site. The nearest bumblebee forage patch recorded in this study was at least 1km away from the site of the trial plot. Small unsurveyed patches of suitable forage were previously available on other areas of the landfill site but, as the trial forage patch was created centrally in a newly capped area, these were estimated as being at least 250m away from the plot. This meant that the trial plot was created in an area which previously did not support bumblebee populations. The following year, although a small amount of natural colonisation of flowering plants occurred on the *Lolium perenne* seeded

areas of the site, the experimental area represented an 'island' forage resource of wildflowers favoured by the bees.

The trial plot was monitored during the summer of 2004 to provide information on whether these wildflower species could successfully be grown on a landfill site and to assess the ability of *B. sylvarum* and *B. humilis* to locate new 'island' resources. Percentage cover of the target forage species on each sown area was calculated using $1m^2$ quadrat measurements on the forage patches. Five quadrats were place randomly on each forage patch. Timed bumblebee counts following the same surveying methods as the site indices in chapter 2 (section 2.2) were used to assess whether the target bees were using the site, whether numbers of the bees on the forage patch were different from those on surrounding areas of the site which had been sown with *Lolium perenne* and how numbers on the trial plot compared to suitable forage patches across other South Essex sites. Surveys on surrounding areas were carried out randomly on areas of approximately equal size within fifty metres of the trial plot.

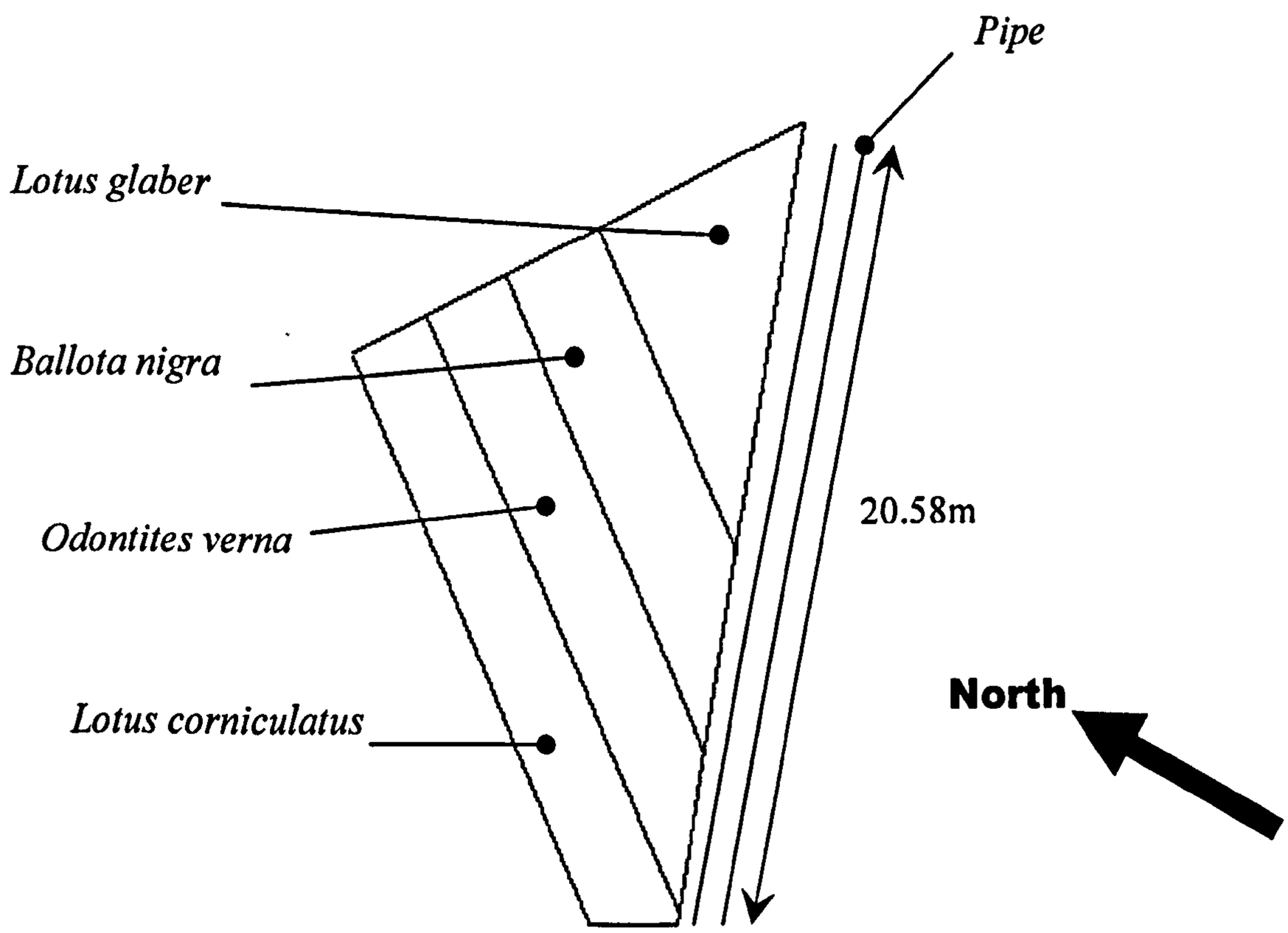


Figure 6.2. Design plan for the seeded forage plot at Pitsea Landfill site, 2003. Seeded sections were all of equal area.

Phase 2

Following the creation of the initial trial plot, permission was granted for the use of a larger area of the landfill site. This enabled further investigation of best practice for trial plot creation. In 2004, a series of discrete forage plots were created on another newly capped area of landfill. Six 5m x 5m plots were measured out with buffer zones of at least 5m of bare topsoil between each plot. Each area was cultivated and sown with a different wildflower species known to be favoured by *B. humilis* and *B. sylvarum*. The four original wildflower species were sown: *Lotus corniculatus*, *Ballota nigra*, *Odontites verna* and *Lotus glaber*. Two further species were also sown: *Trifolium pratense* and *Lathyrus latifolius*. *Trifolium pratense* was selected following the results of floral surveys which indicated that *B. humilis* regularly utilised the species as forage, whilst *B. sylvarum* was recorded under-visiting the species compared to other bees (section 4.4). *Lathyrus latifolius* was sown as an early forage source for foraging queens. All seeds were purchased from Nickys Seeds (Nickys Nursery, 2007) except the *Lotus glaber* seeds which were harvested from the Hadleigh Castle Country Park site, the phase 1 landfill trial plot and the greenhouse growth trials. 400g of seed was sown on the *Lotus corniculatus, Odontites verna* and *Trifolium pratense* plots. Due to seed availability only 300g was sown on the *Lathyrus latifolius* plot, 100g on the *Lotus glaber* plot and 50g on the *Ballota nigra* plot.

Due to the trial plots being created late in 2004, no target forage species were present when surveyed in 2005. Management was carried out in November 2005 to remove any vegetation not known to be visited by *B. sylvarum* and *B. humilis* and the sites were then monitored in 2006. During 2006 bee surveys were carried out on the site to assess whether *B. humilis* and *B. sylvarum* were able to locate these new forage plots. Timed bumblebee counts following the same surveying methods as the site indices in chapter 2 (section 2.2) were carried out on the plots to compare the number of bees on these plots with those on the mixed seed trial plot created in 2003. Percentage cover of the target forage species on each forage patch was calculated using $1m^2$ quadrat measurements on the forage patches. Five quadrats were place randomly on each trial plot.

6.3 Results

Greenhouse trials

Results of the greenhouse growth trials of *Lotus glaber*, *Lotus corniculatus*, *Ballota nigra* and *Odontites verna* are presented in Figure 6.3. *Lotus glaber* and *Lotus corniculatus* recorded the highest germination rates. *Ballota nigra* germination rates were low, but the few plants that did grow produced numerous flower heads and seed. *Odontites verna* had very low germination and few flowers. The single trial pot with *Odontites verna* and *Ballota nigra* seed produced no plants of either species and results were not plotted.

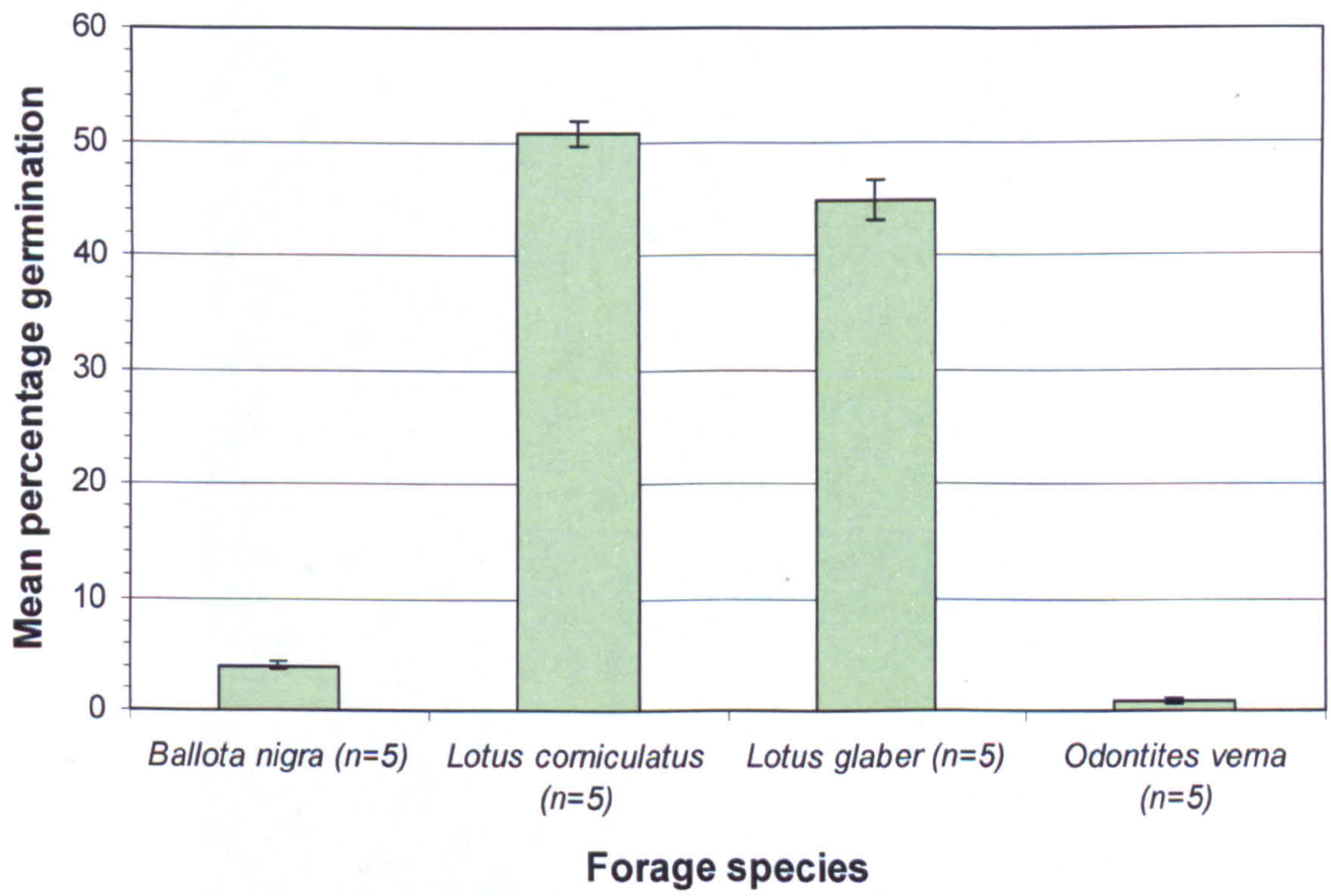


Figure 6.3. Average germination rates of greenhouse forage trials. Germination rates measured as percentage of 50 seeds sown which had germinated after 26 weeks. Germination monitored weekly to record maximum.
[Error bars represent the standard error of the mean]

Cleanaway Pitsea Landfill Trials

Phase 1

As can be seen from Figure 6.4, target forage species performed well on the 2003 trial forage patch on the landfill site. Similarly to the greenhouse trials, *Lotus glaber* and *Lotus corniculatus* were the most successful of the forage species. *Odontites verna* and *Ballota nigra* were also successfully grown on the forage plot (Figure 6.5).

Figure 6.4. Pitsea Landfill site phase 1 forage trial plot, August 2004. Comparison of the area sown with *Lotus glaber* seed with the surrounding area sown with *Lolium perenne*. In the centre of the picture is a pipe (represented in the site plan, Figure 6.2).

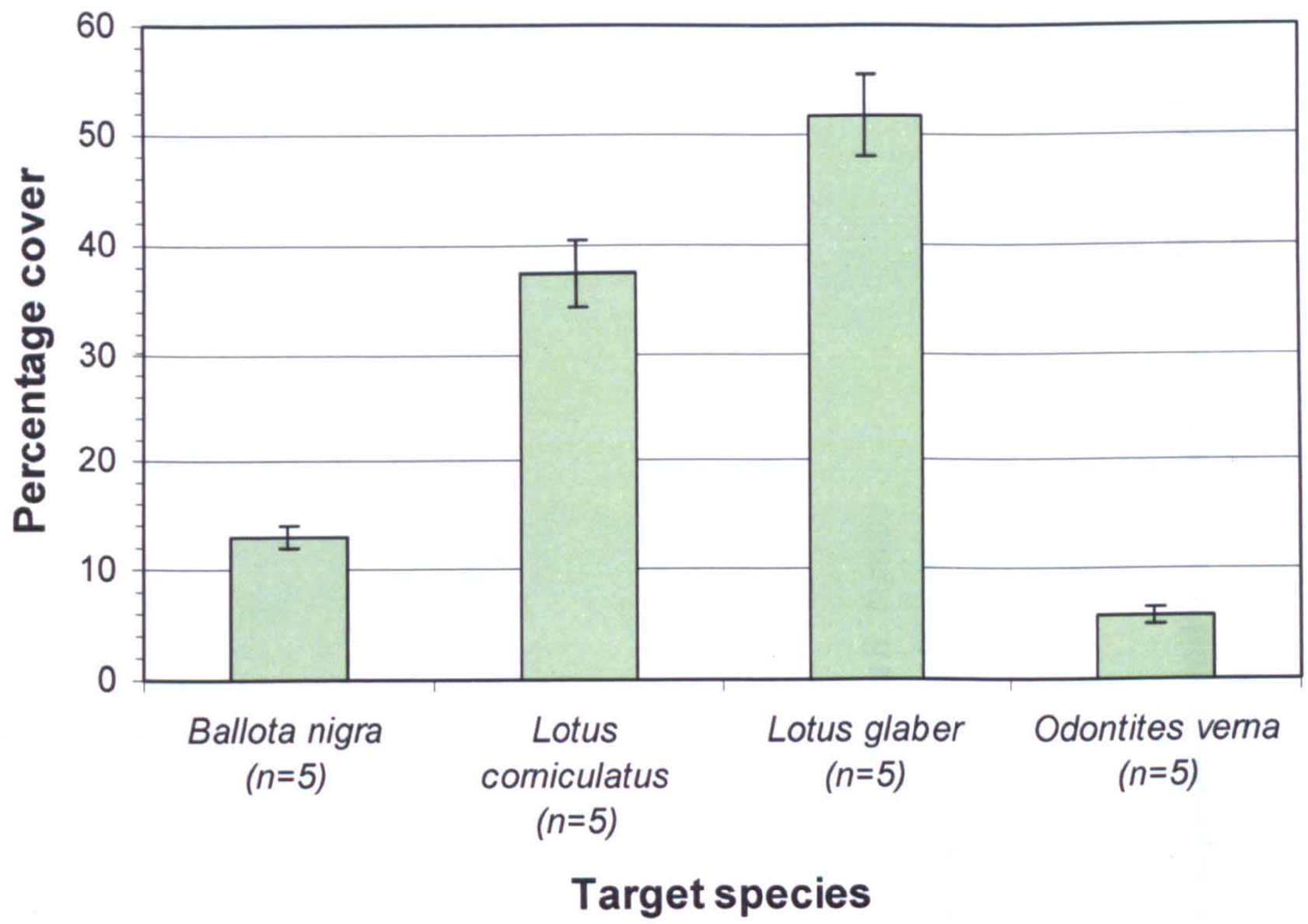


Figure 6.5. Mean percentage cover of target forage species on phase 1 trial plot at Pitsea Landfill site, August 2004. Average percentage cover calculated from 5 randomised $1m^2$ quadrats in each seeded area. [Error bars represent the standard error of the mean]

Bumblebee surveys recorded both *B. sylvarum* and *B. humilis* individuals foraging on the plot indicating that both species were able to locate and utilise new forage. Floral surveys of surrounding *Lolium perenne* dominated areas found none of the target forage species sown on the plot. *Picris echioides*, *Cirsium arvense*, *Cirsium vulgare, Malva sylvestris* and occasional *Trifolium pratense* appeared to be the only available flower heads on these areas of the site.

Timed counts on the trial plot and on equivalent areas of the surrounding *Lolium perenne* sown area recorded higher numbers of both *B. humilis* and *B. sylvarum* on the trial plot (Figure 6.6). A comparison of the mean timed counts on the trial plot with those across other known forage patches on the South Essex sites revealed that numbers foraging on the trial plots were similar to those on other sites (Figure 6.7).

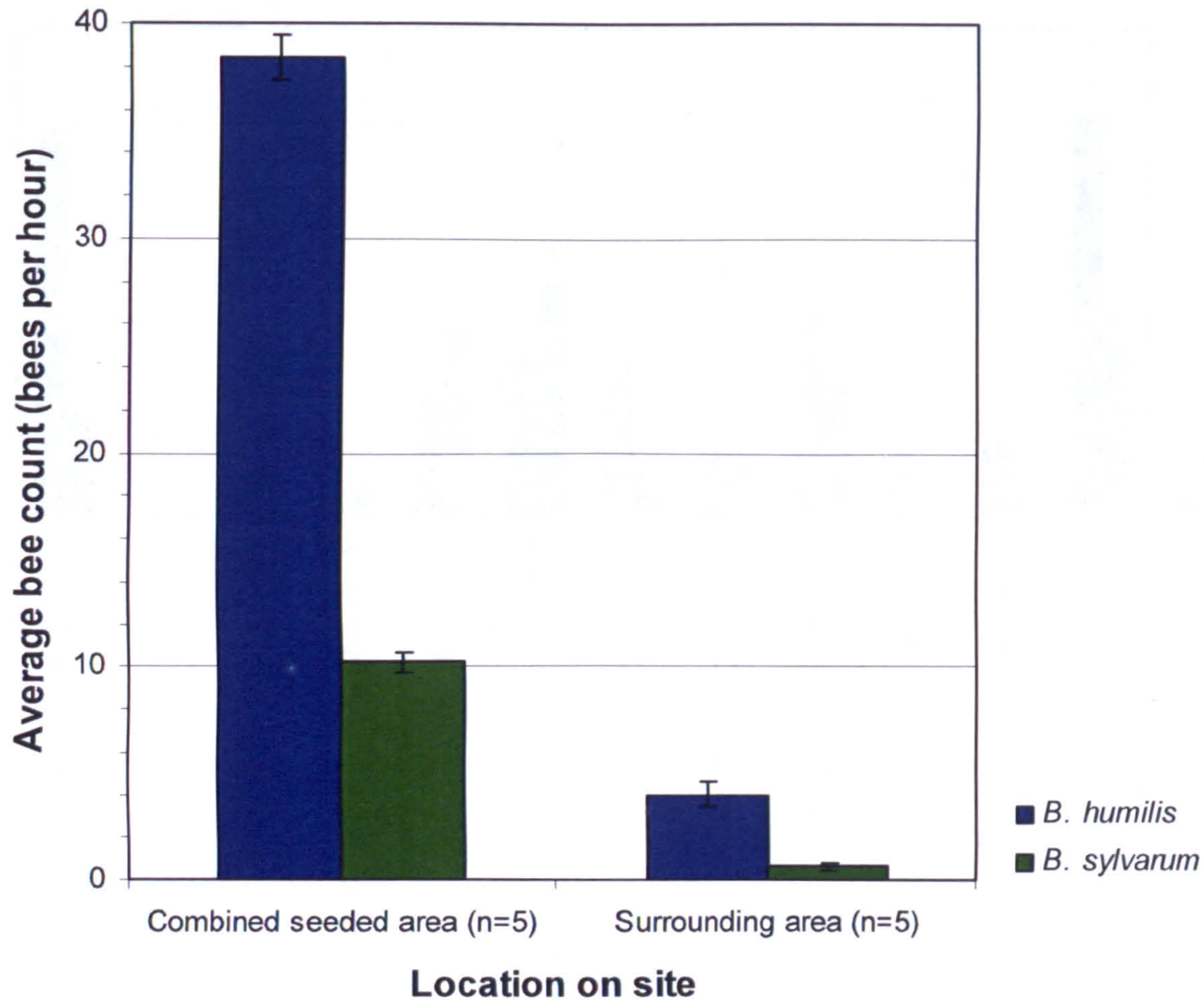


Figure 6.6. Average timed bee counts of *B. humilis* and *B. sylvarum* on the trial forage patch and grass seeded area of the Pitsea Landfill site, August 2004. Surveys on the trial plot were a combination of all the seeded areas. All surveys on the surrounding area were carried out within 50m of the trial plot. [Error bars represent the standard error of the mean]

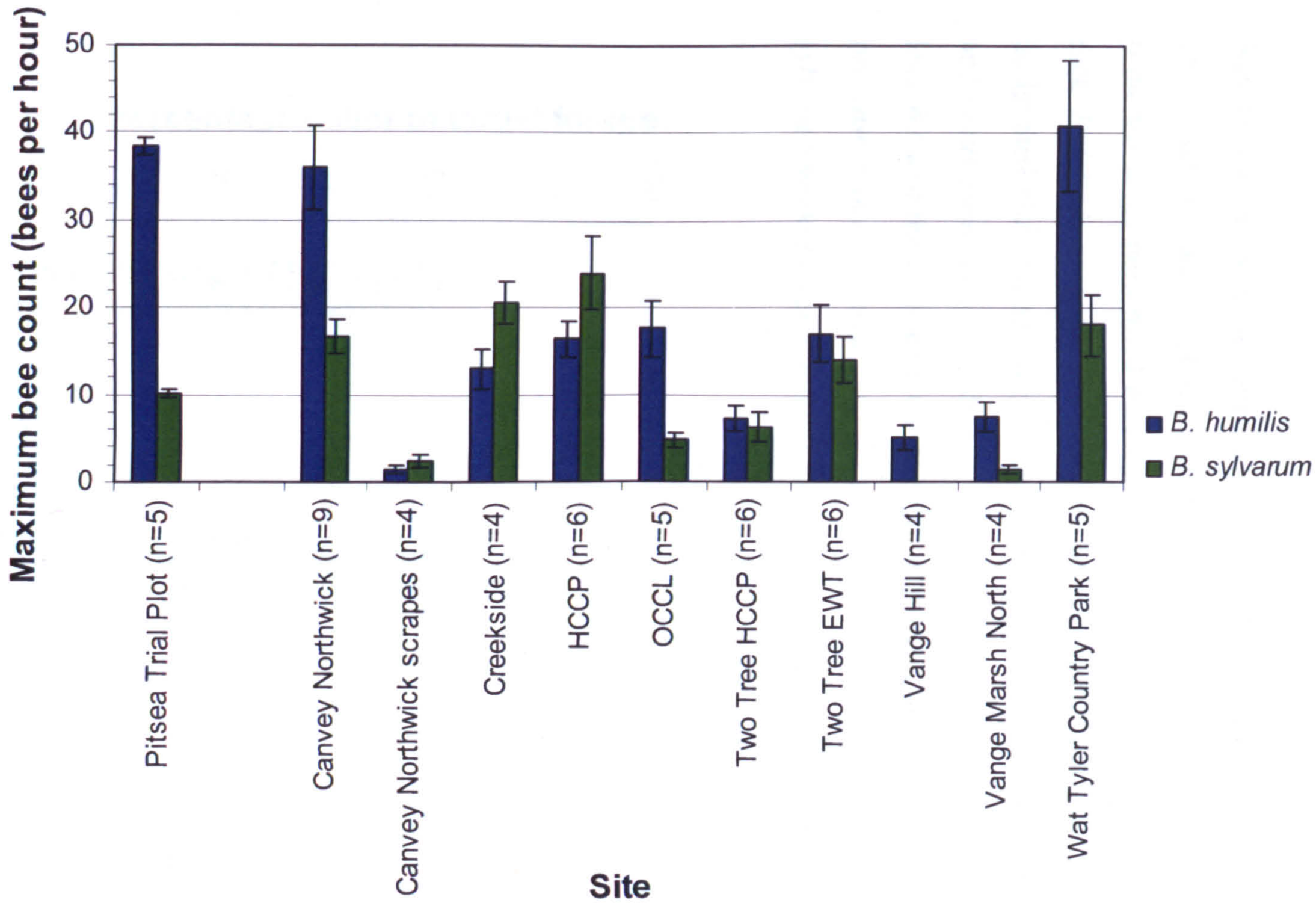


Figure 6.7. Mean timed bee counts on the trial forage plot and South Essex survey sites, 2004. A comparison of the mean counts of *B. humilis* and *B. sylvarum* over approximately equally sized forage rich areas of each site in August and September 2004. [Error bars represent the standard error of the mean]

Results of Mann-Whitney U exact tests revealed that significantly more *B. humilis* and *B. sylvarum* were foraging on the trial plot than on equivalent surrounding areas sown with *Lolium perenne* (p=0.008 for both tests).

Phase 2

Results for the average percentage cover of the seeded forage species on each 25m^2 trial plot during phase 2 seeding trials were varied (Figure 6.8). *Trifolium pratense* seeds performed best with an average of 70% cover of the trial plot. In fact, *Trifolium pratense* flowers were so successful that they spread outside the designated plot and across the neighbouring *Lathyrus latifolius* plot. *Lotus glaber* and *Lotus corniculatus* seeds also performed well both covering an average of at least 40% of their respective trial plots. However, the *Lotus* species and *Trifolium pratense* plots flowered early in the season and by the time *B. humilis* and *B. sylvarum* workers were foraging the flowering was mostly over.

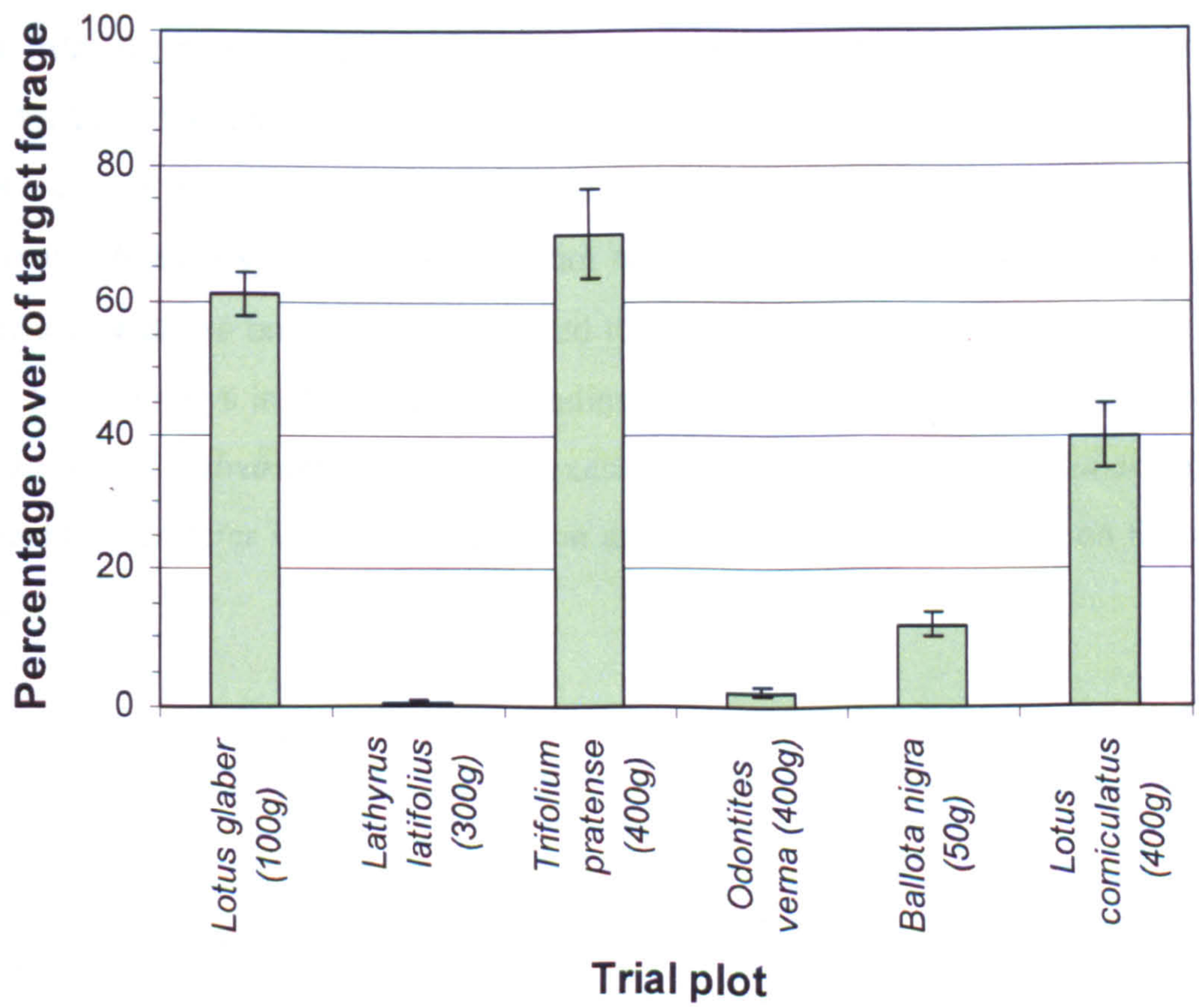


Figure 6.8. Percentage cover of target forage species on the Pitsea Landfill site trial plots, August 2006. Mean values calculated from the results of five quadrat surveys. [Error bars represent the standard error of the mean]

Lathyrus latifolius and *Odontites verna* were unsuccessful. Very few *Odontites verna* plants germinated and no *Lathyrus latifolius* flowers were available for *Bombus* queens in June/July. By September several *Lathyrus latifolius* plants were observed growing on the plot, so it was hoped that these would provide flowers in spring 2007. *Ballota nigra* seeds also had limited success, but the plants that were produced flowered at the appropriate time for *B. humilis* and *B. sylvarum* workers and it was these flowers that accounted for most of the bees recorded on the timed trial plot counts (Figure 6.9).

A comparison of the results of timed bee counts during the first year of growth following seeding on the phase 1 and 2 demonstrated that both plots successfully attracted foraging *B. humilis* and *B. sylvarum* (Figure 6.9). Results from discrete seeded forage patches combined together (phase 2) did not perform as well for *B. humilis* as the mixed forage patch created in 2003. Results for *B. sylvarum* were fairly similar across the two plots. As the forage patches were created in different years it was difficult to draw definitive conclusion from the results as to which technique produced the more attractive forage for the bees. However, what was clear from the phase 2 study was that forage was not available at the appropriate time for the bees. The majority of *B. sylvarum* and *B. humilis* sightings on the trial plots were on *Ballota nigra* with almost all of the remaining sightings being on *Cirsium vulgare* a species which was not seeded in these trials. *Lotus* species and *Trifolium pratense* on the plots flowered too early for the bees. Evidence for this came from surveys in the areas surrounding the trial plots in which sightings of *B. humilis* and *B. sylvarum* were almost exclusively recorded on *Lotus glaber* which flowered much later in mixed vegetation and was no longer flowering on the trial plots.

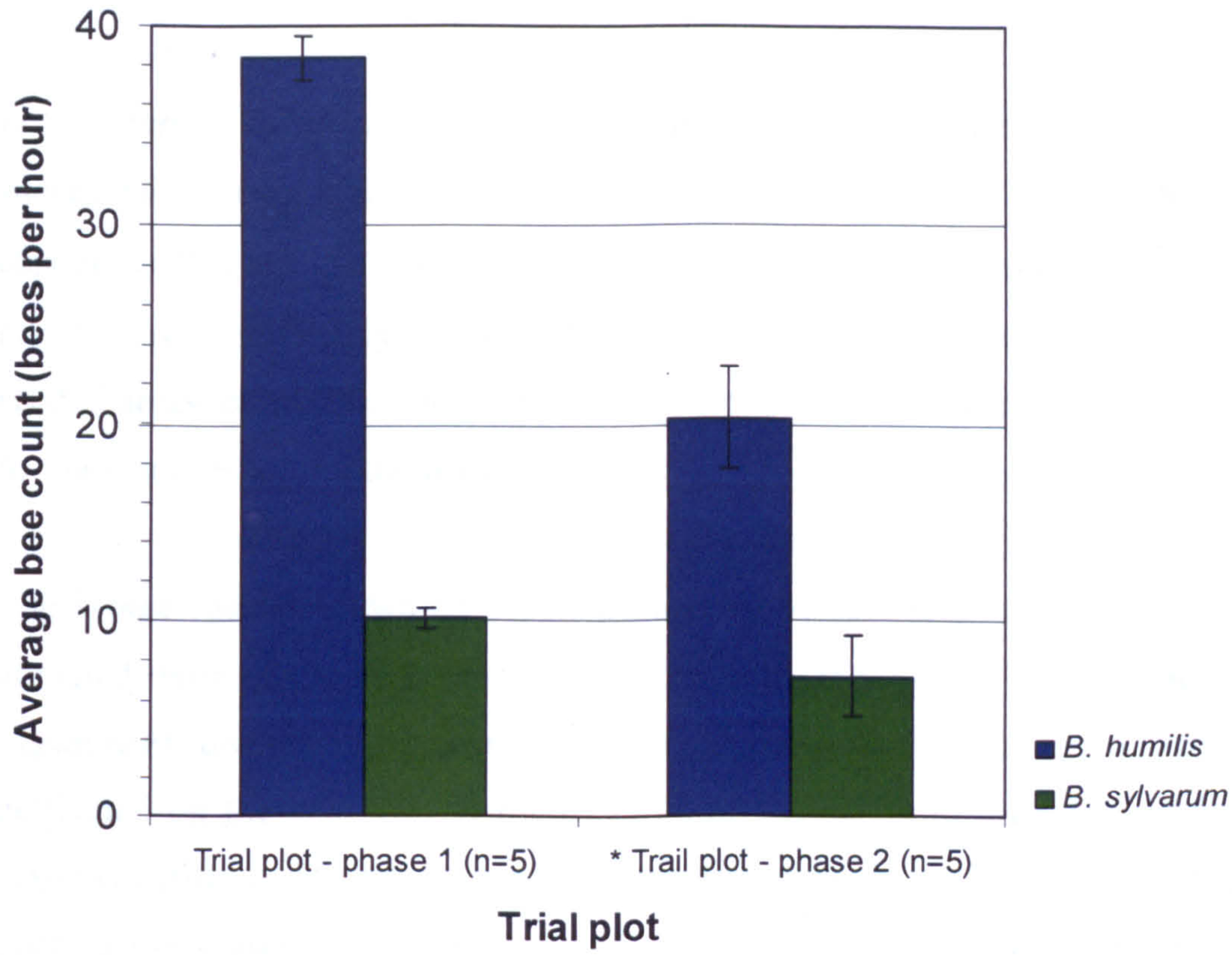


Figure 6.9. Average timed bee counts on phase 1 and 2 trial plots, Pitsea Landfill site. Results represent the average number of *B. humilis* and *B. sylvarum* recorded on the trial plots during the first year of forage availability after seeding (2004 and 2006 respectively). Surveys were carried out across the whole of the phase 1 trial plot and a combined survey of all of the discrete phase 2 plots (* as the areas involved were not equal phase 2 results were divided by 1.5 to compare equivalent areas of trial plots). [Error bars represent the standard error of the mean]

6.4 Discussion

Greenhouse trials

Greenhouse trials were successful in growing *Lotus glaber*, *Lotus corniculatus* and *Ballota nigra* from seed and would be an effective method for growing plants for greenhouse based studies or to produce seed banks of these floral species for site management.

Lotus glaber, Lotus corniculatus, Ballota nigra and *Odontites verna* were all successfully grown in the first phase of the forage plot creation experiments on the capped landfill site. Numbers of *B. humilis* and *B. sylvarum* recorded on the forage patch were significantly higher than those on the surrounding *Lolium perenne* seeded areas, confirming that the floral species sown were suitable forage species for habitat creation for the bees.

The forage species grown on separate plots in the second phase experiment also attracted both *B. humilis* and *B. sylvarum* to the plots. The second phase experiment demonstrated that *Trifolium pratense* could also be successfully cultivated on the site. Few *Lathyrus latifolius* seeds germinated and no flowers were available for foraging queens in the following spring, but plants observed later in the season indicated that seeding was an effective method to introduce *Lathyrus latifolius* to the site. It was hoped that the trial plot would develop and that *Lathyrus latifolius* flowers would be available for foraging queens in later years. Unfortunately such monitoring was beyond the scope of this project.

Odontites verna was unsuccessful in the phase 2 study when seeded on its own. The plants performed relatively better when sown next to other target species. This may have been a result of the hemiparasitic requirements of the species (Govier *et al.*, 1968). Of the two seeding trials, the continuous phase 1 plot with target species sown next to each other attracted higher mean numbers of bees than an equivalent area of the discrete patches (phase 2). This result, combined with that of *Odontites verna* being more successful on the phase 1 trial plot and *Lotus* species and *Trifolium pratense* flowers flowering too early for foraging workers on the 2005 plots, indicated that seeding bumblebee forage plants in larger continuous patches may be the better method for providing suitable bumblebee forage. However, due to the lack of controls and inter-year design of the plots caused by the limitations of working on an active landfill site, these conclusions can only be speculative at best.

A definitive conclusion that could be drawn from the study was that sowing wildflower seeds of species known to be regularly visited by *B. sylvarum* and *B. humilis* was an effective way to supplement forage resources and could be a potential reclamation management technique for post-operational landfill sites.

The presence of *B. humilis* and *B. sylvarum* on the trial plots indicated that the bumblebees were able to locate and utilise newly created forage sources, but such provision can only be effective if created over an appropriate spatial scale for existing populations. The trial plots in this investigation were created within the existing population of both *B. humilis* and *B. sylvarum* in South Essex (chapter 2, Figure 2.1) but on areas where previously no forage was available. Prior to the creation of the forage patch, the nearest available forage was approximately 250m away from the plot, it can therefore be estimated that the bees must have travelled at least this distance to locate the new forage. This indicated that forage creation over such distances is effective for existing populations. Further research on the distances these bees can travel to locate new forage is required however, to ensure that the spatial scales over which bumblebee habitat is created for the bees is appropriate for existing populations. Appropriate spatial scales are discussed further in chapter 9.

Assuming that patches are created over appropriate distances this study demonstrated that in one year forage patches could be produced which attract both *B. humilis* and *B. sylvarum* in numbers comparable to the best forage patches of other South Essex sites. In fact, during the 2004 timed surveys mean numbers on the trial plot were higher than those recorded on the Old County Council Landfill site, Vange Marsh North and Vange Hill for both species. For *B. humilis,* numbers on the trial plot were also higher than at Hadleigh Castle Country Park, Two Tree Island and Creekside.

Whilst this appeared to be a successful technique for supplementing the forage resources of both *B. humilis* and *B. sylvarum*, a recently capped landfill is a fairly atypical environment compared to many South Essex sites. With the majority of land in South Essex being privately owned, intensively farmed or post-industrial and under pressure for development (Harvey, 2000b), most potential for habitat

creation in the area would be on Country Parks and SSSI sites. On such sites there are regulations for appropriate habitat management so, after it had been demonstrated that bumblebee forage could be created and that *B. humilis* and *B. sylvarum* were able to locate this forage if provided within the ranges of current populations, it was important to assess best practice for forage creation on sites with stricter habitat management regulations. This is discussed further in chapter 7.

This study has demonstrated that providing forage for *B. humilis* and *B. sylvarum* can attract the bees and increase their numbers on a site. It was not however, able to distinguish whether bees were merely concentrated on the new forage patches by being draw from other areas or whether the provision of forage actually benefited the bumblebee populations in terms of number of reproductives produced. In other words, no evidence was provided to indicate whether forage was in fact a limiting factor for the bees or whether some other factor (such as nesting habitat) was limiting. To analyse this, a measure of colony number or colony success (production of reproductives) would be required before and after forage provision. At present no such method exists, but a potential technique is discussed in section 9.4 and its application for further experimentation is proposed in section 10.3.

Chapter 7

Habitat manipulation

7.1 Introduction

If declines in UK bumblebees (Williams, 1982), are to be halted and reversed suitable forage sources should be provided for the bees (Edwards, 1998). For forage provision to be effective, the specific foraging requirements of individual bumblebee species must be understood (Edwards, 1998). Knowledge of *B. humilis* and *B. sylvarum* foraging behaviour generated from this study will be used to increase forage availability in the South Essex area with an aim of conserving, consolidating and enhancing these nationally important populations. For landowners and managers to carry out habitat improvement however, effective methods for the provision of these forage sources need to be assessed.

The East Thames Corridor comprises of a matrix of urban areas, arable land, semi-natural country parks and brownfield sites (Harvey, 2000b). Within urban areas, parks and gardens are known to be of benefit to some of the UK's more ubiquitous bumblebee species (Chapman *et al.*, 2003; Goulson *et al.*, 2005; Osborne *et al.*, 2007). There seems no reason why parks and gardens could not also benefit *B. humilis* and *B. sylvarum* populations if these sites are located within a matrix of suitable semi-natural habitats and contain suitable forage. On such sites, publicity and education are the only routes for habitat improvement. For this reason, presentations to community groups, press releases and the production of a website (Connop, 2007) with information and links on bumblebee conservation were outputs from this project.

Sites with perhaps the greatest potential for habitat improvement in the East Thames Corridor are arable land, semi-natural country parks and brownfield sites. Many studies have investigated best practice for the creation of bumblebee forage on arable land (Kells *et al.*, 2001; Carvell *et al.*, 2004; Pywell *et al.*, 2005), so further investigation was not considered necessary in this study. It is hoped that by dissemination of this research, favoured forage species identified in this study will be targeted for growth on arable field margins in the East Thames Corridor through partnerships between Natural England, DEFRA and local arable landowners.

Investigations in chapter 6 demonstrated that when suitable forage was grown on brownfield sites (in the case of this study, an area of recently capped landfill) both *B. humilis* and *B. sylvarum* were able to locate and exploit these new forage sources if created on the appropriate spatial scales. The majority of brownfield sites however do not offer this possibility of habitat management and it is their unmanaged history that has made them so suitable for supporting diverse invertebrate faunas (Harvey, 2004). The brownfield sites identified as supporting *B. humilis* and *B. sylvarum* in this study (chapter 2) tended to be those abandoned for 10-20 years or more with infertile, minerally deficient substrates supporting sparsely vegetated patches of early successional and ruderal habitat (Harvey, 2004). With many of these sites being under imminent threat of development (English Partnerships, 2005), protection and conservation is an immediate requirement for them (Harvey, 2000b). Whilst best practice management for general brownfield conditions is yet to be investigated, potential lies in the designation of the Canvey Wick site, Canvey Island (TQ768835) as Britain's first brownfield SSSI (English Nature, 2005) and the increasing development of brown roofs (Echoschemes, 2003).

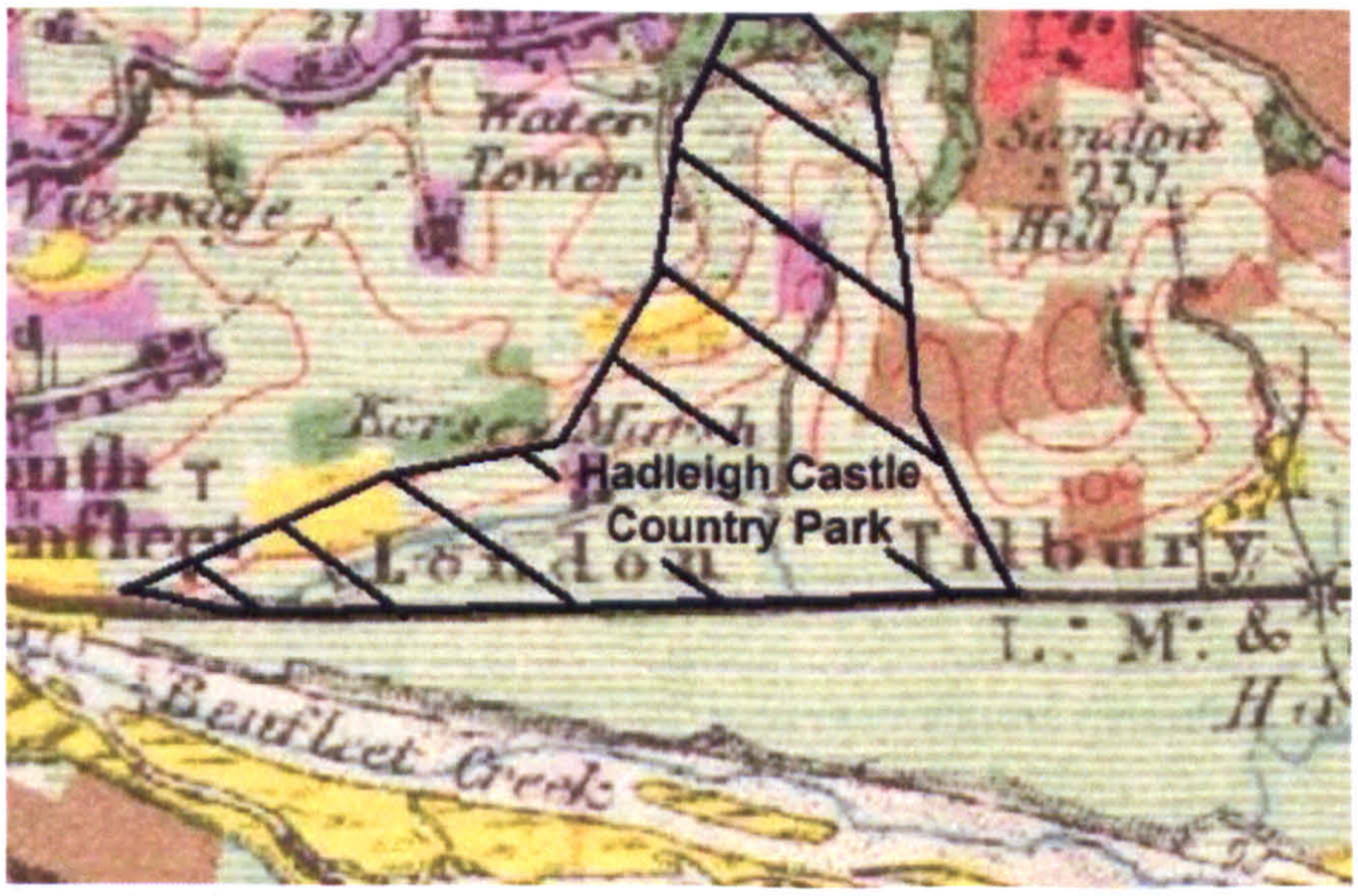
In this chapter an effective method for the creation of bumblebee forage patches on semi-natural SSSI sites was investigated. Regulations prohibit the introduction of seed onto such sites (JNCC, 1981) so methods used to produce forage patches on the Cleanaway Pitsea Landfill site (chapter 6) were not applicable here.

Hadleigh Castle Country Park SSSI (TQ800869) was identified as a suitable site for a bumblebee habitat improvement program. The site runs between South Benfleet and Hadleigh in South Essex and is a mix of woodland, hedgerows, grassland and marsh with ponds and ditches. Historically the area was used for agriculture and much of the site was open grassland (Figure 7.1). In recent years management has led to the development of substantial areas of scrub and loss of much bumblebee foraging habitat. Following the successful demonstration of the ability of *B. humilis* and *B. sylvarum* to locate new forage patches at the Cleanaway Pitsea Landfill site a ten-year program of scrub clearance was proposed to generate new bumblebee forage patches at the park.

i) Historical land use on the site of Hadleigh Castle Country Park, Essex (1931-1935).

Key:

- Meadow and permanent grassland
- Rough marsh pasture
- New housing and allotments
- Housing and industry
- Commons and rough pasture
- Arable land
- Forest and woodland



ii) Extent of Hadleigh Castle Country Park, 2007

Figure 7.1. Maps of i) historical land use on the site of Hadleigh Castle Country Park, Essex (1931-1935) and ii) Extent of Hadleigh Castle Country Park, 2007. Land use map - 1:63,360 (EDINA, 2007).

Site surveys revealed both *B. humilis* and *B. sylvarum* were already present on the site due to existing management creating several areas of suitable forage containing *Odontites verna*, *Lotus glaber*, *Trifolium pratense*, *Centaurea nigra*, *Ballota nigra* and *Cirsium* species. These areas are generally managed for rough hay crops, and were previously cut by mower, but management changed to grazing by cattle in 2003. Target forage patches have improved considerably since the change in management. The fields are grazed twice a year, for approximately two months starting in March and again in September/October when bumblebee forage plant flowering is over. Scrub bands are cut and several paths are mown on these sites along the edges of which much of the *Odontites verna* is found. Areas of tall rough grassland with grass tussocks are also present on the site which might act as nesting habitat. This is discussed further in chapter 8.

The scrub clearance program comprised of an area of approximately 0.5ha of scrub being cleared annually to increase the area of suitable bumblebee forage and nesting habitat for *B. humilis* and *B. sylvarum* populations. Scrub, in particular *Rubus fruticosus*, has been recognised as being of importance to *B. humilis* and *B. sylvarum* both in this study (section 5.4) and by Peter Harvey (1999). Due to the abundance of scrub on the site compared to semi-natural grassland however, it was decided that removal of 0.5ha a year for ten years would still leave abundant scrub on the site whilst at the same time increasing the area of semi natural grassland vital for many invertebrates (Harvey, 2000b). Scrub clearance began in 2006. Following the clearance of the first 0.5ha, an effective method for promoting the recolonisation of these cleared patches by floral species known to be favoured by foraging *B. humilis* and *B. sylvarum* workers was assessed.

This initial study comprised of a comparison of natural recolonisation, to assess any existing seed bank, with a green haying method developed by Trueman and Millet (2003). Surveys of the site were carried out in 2007 to monitor the establishment of forage species such as *Lotus glaber*, *Odontites verna*, *Trifolium pratense* and *Centaurea nigra* on each treatment and to assess whether *B. humilis* and *B. sylvarum* were able to locate and utilise the new patches.

7.2 Methods

In April 2006, an area of 0.5ha of scrub at Hadleigh Castle Country Park was cut (Figure 7.2). Scrub was cut by chainsaw and roots were removed using a grab on an excavator. In September of the same year, the area was surveyed to assess what vegetation if any had naturally recolonised the site. Due to the very sparse nature of the vegetation, it was concluded that quadrat sampling would be an inappropriate method to assess the vegetation cover. Instead, a general walked survey creating a list of species present was carried out.

Figure 7.2. Area cleared of scrub at Hadleigh Castle Country Park, September 2006.

Following the vegetation survey, the cleared area was divided in half to create a recolonisation experiment comparing a control treatment, left to colonise naturally, with a seeded treatment. To generate seed stock, green hay was cut from a nearby area of the park rich in the target floral species using a Ryetec flail mower collector (Figure 7.3). A list of the floral species present in the hay was recorded (Table 7.2). The green hay was spread onto the eastern most half of the site (Figure 7.4) following the methodology of Trueman and Millet (2003).

Figure 7.3. Green hay cutting at Hadleigh Castle Country Park, September 2006.

Figure 7.4. Green hay spreading on the cleared scrub patch at Hadleigh Castle Country Park, September 2006.

The eastern side was selected in an attempt to reduce the amount of seed blown onto the non-hay half of the site by the local predominant winds (Figure 7.5).

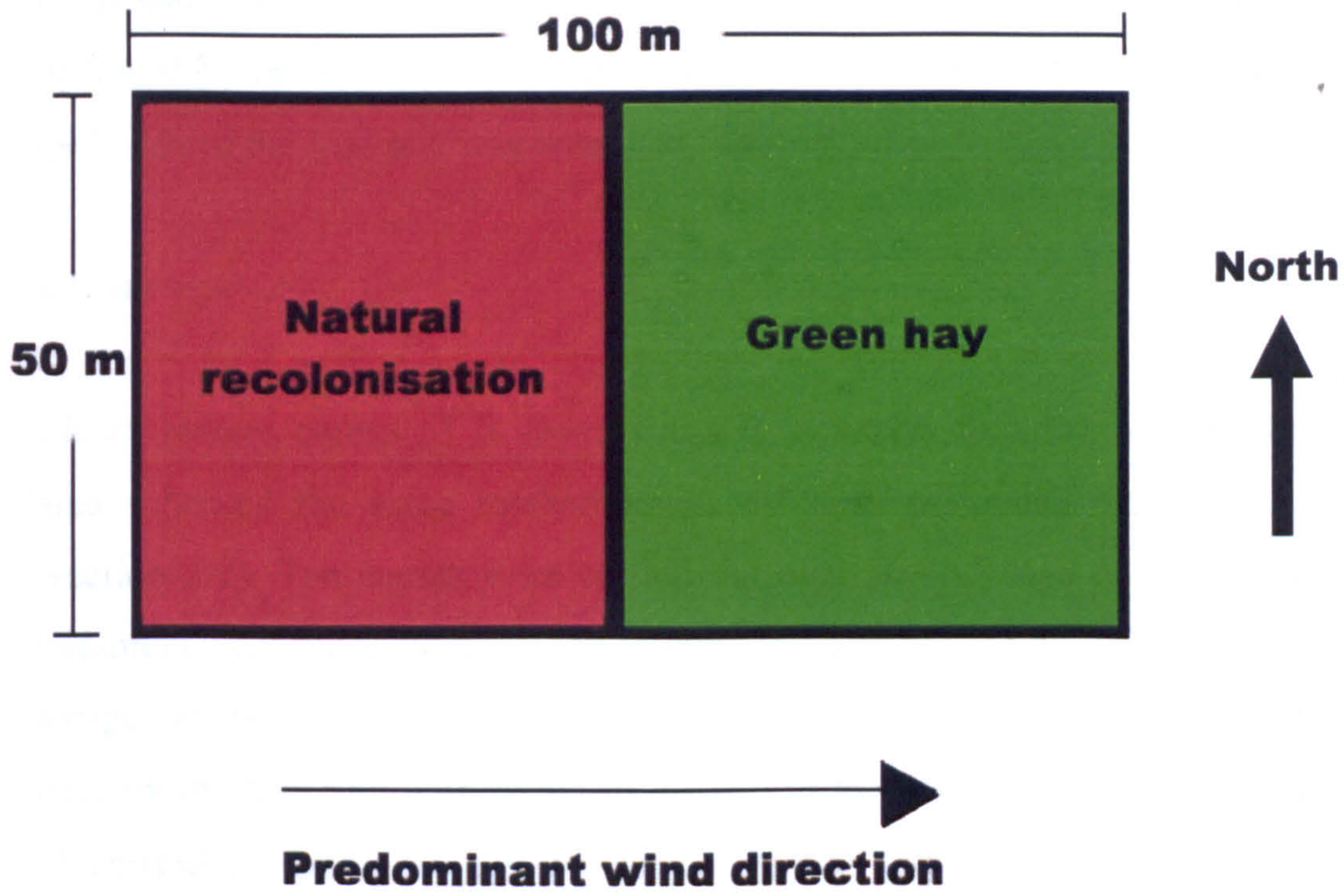


Figure 7.5. Plan of forage patch experiment, Hadleigh Castle Country Park 2006. Half of the plot was covered in green hay the other half left to colonise naturally.

During August 2007, the site was surveyed to compare the abundance of the target forage plants and the number of *B. humilis* and *B. sylvarum* foraging on the experimental areas. Surveys were carried out in August as it was identified as the main foraging time for *B. humilis* and *B. sylvarum* workers during bee walk surveys (chapter 2).

Floral surveys

Thirty 1x1m quadrats were randomly placed across each treatment area. Each quadrat was subdivided into 10x10cm squares. The squares were used to estimate the percentage of vegetation cover, relative abundance of each floral species in terms of percentage cover and the number of flowers/inflorescences of each flowering plant species within each quadrat. For flower counts, only open flowers were recorded as these were representative of availability to foraging bumblebees.

One flower 'unit' was counted as a head (e.g. *Trifolium* species), spike (e.g. *Prunella vulgaris*), capitulum (e.g. *Centaurea nigra*), umbel (e.g. *Achillea millefolium*) or individual flower (e.g. *Ranunculus acris*) (Bowers, 1985b; Dramstad and Fry, 1995; Carvell, 2002; Carvell *et al.*, 2004). Flower identification followed Stace (1997). A list of all of the floral species recorded in the quadrats of each side of the trial plot was compiled (Table 7.3).

Bee surveys

Timed indices counts of *B. humilis* and *B. sylvarum* foraging on each treatment area followed the same methodologies as those previously used in chapter 2 (section 2.2). Ten counts were carried out over several days on each of the two treatments. On the same days, ten counts were also carried out on areas of suitable forage across the rest of Hadleigh Castle Country Park to compare numbers of the bees on the trial plot with those on the rest of the site. These timed surveys were all carried out during August to record *B. humilis* and *B. sylvarum* numbers during their peak foraging time. The methodology and timing of the surveys repeated those carried out on the site in previous years (chapter 2, section 2.3). Repeating methodology meant that it was possible to compare numbers of *B. humilis* and *B. sylvarum* on the site between years.

7.3 Results

Survey of the cleared scrub patch

The September 2006 walked vegetation survey recorded the flora which had colonised the cleared patch between April and September 2006 (Table 7.1). Of these species only *Rubus fruticosus* was recorded as being a target forage species following *B. humilis* and *B. sylvarum* forage surveys (section 3.3).

The floral assemblage of the area cut for green hay was recorded (Table 7.2). This included four species which were considered priority target species for *B. humilis*

and *B. sylvarum*: *Centaurea nigra*, *Lotus glaber*, *Odontites verna* and *Trifolium pratense*. There were also five other species which have been recorded as bumblebee forage plants in this study.

Table 7.1. Initial vegetation survey of cleared scrub plot, September 2006. Results of a walked survey recording presence of species only.

Species.	Common name
Anagallis arvensis	Scarlet pimpernel
Bryonia dioica	White bryony
Chenopodium album	Fat hen
Chenopodium polyspermum	Many-seeded goosefoot
Cirsium arvense	Creeping thistle
Cirsium vulgare	Spear thistle
Cornus sanguinea	Dogwood
Crataegus monogyna	Hawthorn
Picris echioides	Bristly ox-tongue
Rubus fruticosus	Bramble
Sambucus nigra	Elder
Stachys recta	Yellow woundwort

Table 7.2. Floral assemblage of the green hay. Occurrence of bumblebee visits to each floral species during the South Essex surveys (2003-2006) also recorded.

Species	Common name	Recorded as bumblebee forage plants	Target species for *B. humilis* and *B. sylvarum*
Achillea millefolium	Yarrow	-	-
Agrimonia eupatoria	Agrimony	Yes	-
Anagallis arvensis	Scarlet pimpernel	-	-
Centaurea nigra	Common Knapweed	Yes	Yes
Lotus glaber	Narrow-leaved birdsfoot trefoil	Yes	Yes
Odontites verna	Red bartsia	Yes	Yes
Picris echioides	Bristly ox-tongue	Yes	-
Potentilla reptans	Creeping cinquefoil	-	-
Prunella vulgaris	Self-heal	Yes	-
Rubus fruticosus	Bramble	Yes	-
Senecio jacobaea	Common ragwort	Yes	-
Trifolium pratense	Red clover	Yes	Yes

2007 Floral surveys

Records of the floral species recorded on each treatment in the August 2007 vegetation surveys are listed in Table 7.3.

Table 7.3. List of flora recorded in trial plot vegetation surveys, August 2007.
x = floral species recorded on site, - = floral species not recorded on site.

Floral species	Green hay	Natural recolonisation
Anagallis arvensis (scarlet pimpernel)	x	x
Brassica napus (rape)	-	x
Brassica nigra (black mustard)	-	x
Bryonia cretica (white bryony)	x	x
Centaurea nigra (common knapweed)	x	x
Centaurium erythraea (common centaury)	x	x
Cerastium alpinum (alpine mouse-ear)	x	x
Chenopodium polyspermum (many-seeded goosefoot)	x	x
Cirsium arvense (creeping thistle)	x	x
Cirsium vulgare (spear thistle)	x	x
Crataegus monogyna (hawthorn)	x	x
Dipsacus fullonum (teasel)	x	x
Epilobium hirsutum (great willowherb)	-	x
Galium aparine (common cleavers)	-	x
Geranium robertianum (herb robert)	x	-
Kickxia spuria (sharp-leaved fluellen)	-	x
Lotus glaber (narrow-leaved birdsfoot trefoil)	x	-
Medicago lupulina (black medick)	x	x
Myosotis arvensis (field forgetmenot)	x	-
Odontites verna (red bartsia)	x	x
Picris echioides (bristly ox-tongue)	x	x
Plantago lanceolata (ribwort plantain)	x	x
Plantago media (hoary plantain)	x	x
Potentilla reptans (creeping cinquefoil)	x	x
Prunella vulgaris (self-heal)	x	x
Ranunculus acris (meadow buttercup)	x	x
Reseda luteola (weld)	-	x
Rubus fruticosus (bramble)	-	x
Rumex conglomeratus (clustered dock)	-	x
Sambucus ebulus (dwarf elder)	x	x
Senecio jacobeae (common ragwort)	x	x
Solanum villosum (hairy nightshade)	-	x
Taraxacum spp. (dandelion spp.)	x	x
Trifolium campestre (hop trefoil)	x	x
Trifolium pratense (red clover)	x	x
Trifolium repens (white clover)	x	-
Urtica dioica (common nettle)	x	x

More floral species were recorded on the natural recolonisation treatment than the green hay side (Table 7.3) but, as can be seen in Figure 7.6, the area with the green hay treatment produced a more substantial floral cover suitable for bumblebee foraging.

Figure 7.6. Habitat manipulation experiment at Hadleigh Castle Country Park, July 2007.

The green hay area had a more comprehensive vegetation cover and less bare ground than the area left to colonise naturally. Values of the average percentage of vegetation cover from the quadrat surveys are displayed in Figure 7.7 and showed that the average percentage of total vegetation cover on the green hay side was higher than that for the natural recolonisation. A Mann-Whitney U exact test of independent samples showed that the percentage cover of vegetation on the green hay plot was significantly greater than that on the naturally recolonised treatment (p=0.024). This demonstrated that the vegetation cover was more complete on the green hay plot, although there was still some bare ground, a habitat feature that has been recognised as important for a number of rare species found on semi-natural grasslands in the East Thames Corridor (Harvey, 2000b).

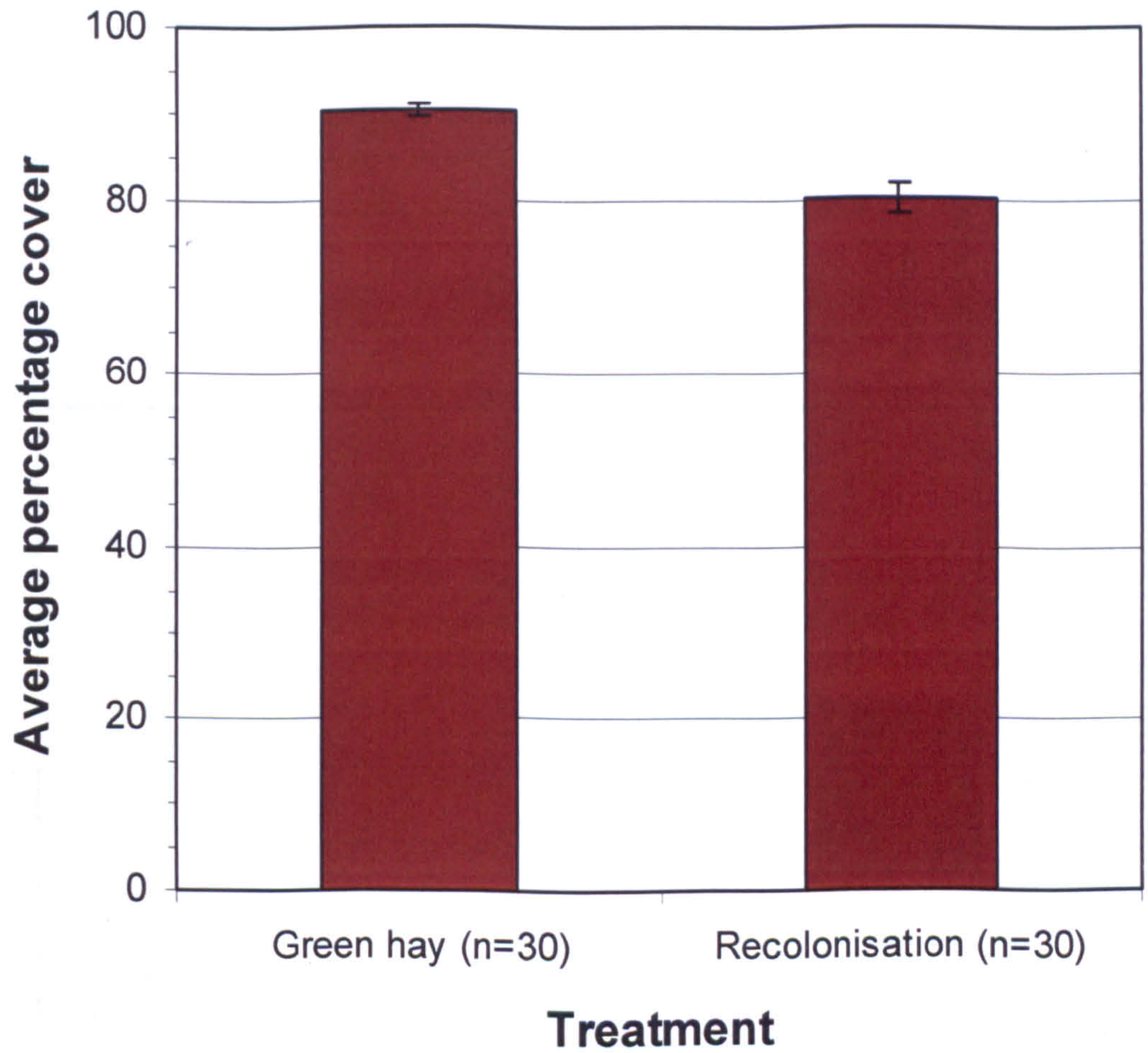


Figure 7.7. Average percentage cover of vegetation on the Hadleigh Castle Country Park trial plots, August 2007. Values calculated from 1x1m quadrat surveys of trial plots. [Error bars represent the standard error of the mean]

There were also more flower heads per quadrat on the green hay side particularly those of *Odontites verna*, a species recognised in this study as of great value to the bees both for nectar and pollen (Figure 7.8).

Mann-Whitney U exact tests of independent samples were carried out to compare the mean number of flowers in each treatment area for each floral species recorded as being visited by *B. humilis* or *B. sylvarum* during the timed surveys. Results are displayed in Table 7.4.

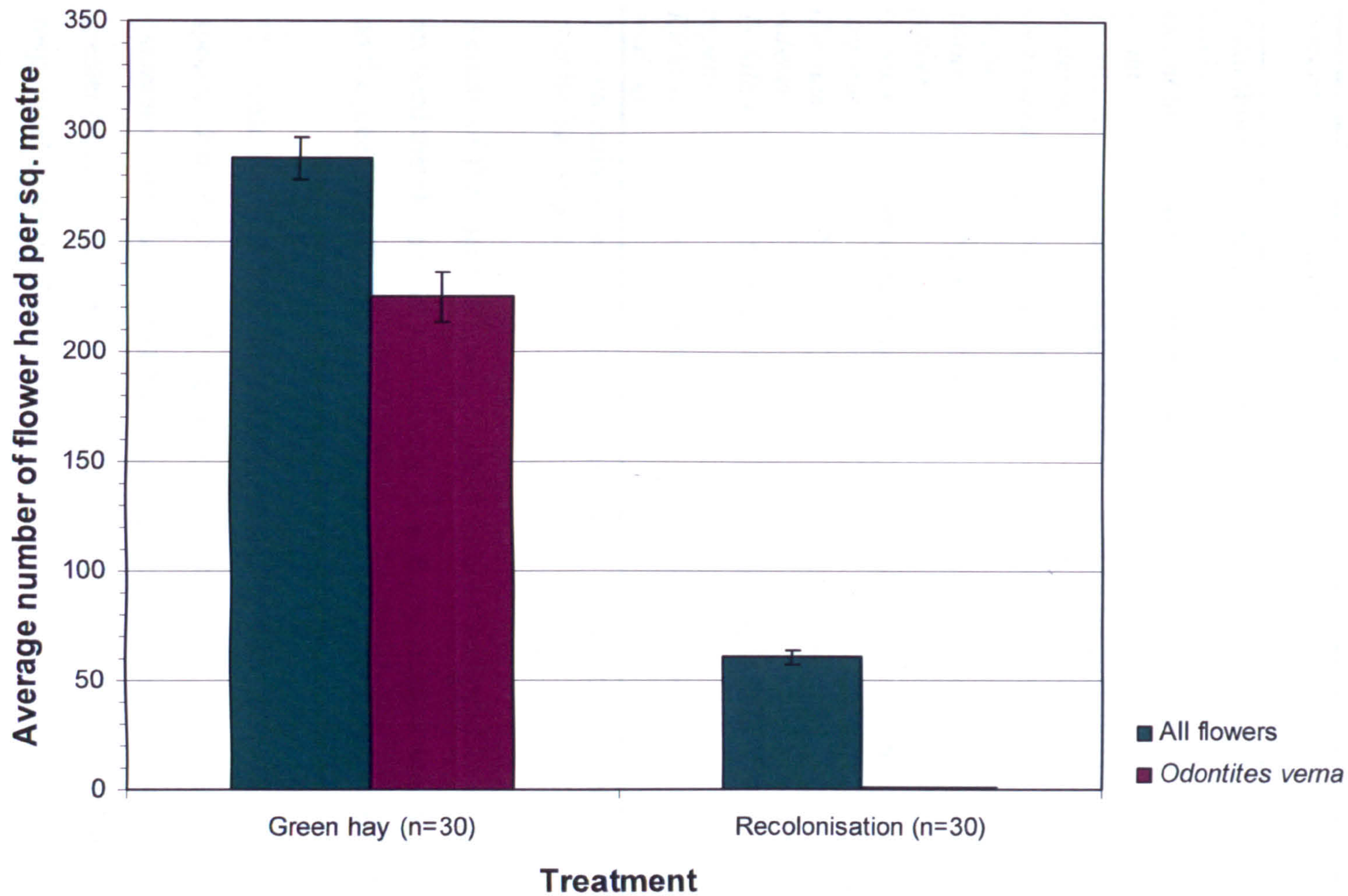


Figure 7.8. Average number of all flower heads and *Odontites verna* flower heads on the Hadleigh Castle Country Park trial plots, August 2007. Values calculated from 1x1m quadrat surveys. [Error bars represent the standard error of the mean]

Table 7.4. Results of Mann-Whitney U exact tests assessing the difference between the mean number of flower heads and percentage cover of plants in each of the treatment areas. Values calculated from 1x1m quadrat surveys, August 2007. Significance level p≤0.05)

Species	Number of flowers	Percentage cover of plants	More abundant treatment
Total flower heads	Significant (p<0.001)	* Significant (p=0.021)	Green hay
Odontites verna	Significant (p<0.001)	Significant (p<0.001)	Green hay
Trifolium pratense	Significant (p<0.001)	Significant (p<0.001)	Green hay
Centaurea nigra	N. S. (p=0.492)	Significant (p=0.011)	Green hay
Lotus glaber	N. S. (p=0.492)	N. S. (p=0.492)	-
Cirsium arvense	Significant (p<0.001)	Significant (p=0.006)	Natural recolonisation
Cirsium vulgare	N. S. (p=0.462)	N. S. (p=0.365)	-
Trifolium repens	N. S. (p=1.00)	N. S. (p=1.00)	-
Kickxia spurina	N. S. (p=0.052)	Significant (p=0.024)	Natural recolonisation

* Comparison of percentage vegetation cover on green hay and natural recolonisation plot.

Results of the Mann-Whitney U exact tests of independent samples (Table 7.4) revealed that there were significantly more flower heads available to foraging bees on the green hay trial plot than on the plot left to recolonise naturally (p<0.001).

The green hay plot also supported significantly more flower heads of target floral species than the naturally recolonised plot (Table 7.4). The mean number of *Odontites verna* and *Trifolium pratense* flowers per quadrat were significantly greater on the green hay plot than the natural recolonisation (p<0.001 and p<0.001 respectively). Mean numbers of *Lotus glaber* flower heads were not significantly different between the two plots (p=0.49), but the average number of these flowers was low in the green hay plot (0.17) and they were completely absent in the naturally recolonised plot. Mean numbers of the other priority target species, *Centaurea nigra*, were not significantly different between the two plots (p=0.49). Whilst few of these plants were present on the natural recolonisation plot, many

were present on the green hay plot but very few flowers were open. This was demonstrated by comparing the percentage cover of these plants on the two plots. A Mann-Whitney U exact test revealed a significantly greater cover of *Centaurea nigra* on the green hay plot (p=0.01).

Results for *Cirsium vulgare* and *Trifolium repens* showed no significant difference between the trial plots in terms of average number of flowers or average percentage cover (p=0.46 and p=1.00 respectively). *Cirsium vulgare* appeared to have a fairly even distribution across the trial plots, whereas *Trifolium repens* was only recorded once during the surveys. One plant was recorded on the green hay trial plot.

Cirsium arvense and *Kickxia spurina* were the only floral species observed during timed bee counts as receiving visits by *B. humilis* or *B. sylvarum* which showed a larger distribution on the naturally recolonized plot. The mean number of *Cirsium arvense* flowers per quadrat was significantly greater on the natural recolonisation plot than the green hay plot (p<0.001). Although the mean number of *Kickxia spurina* flowers per quadrat showed no significant difference between the treatment plots (p=0.05), a Mann-Whitney U exact test on the average percentage cover revealed a significantly greater cover on the naturally recolonised plot (p=0.02). However, neither of these species was recorded as being a priority forage species for *B. humilis* or *B. sylvarum* in the floral behaviour surveys of this study (chapters 3-5).

2007 Timed bee surveys

Average counts of *B. humilis* and *B. sylvarum* per hour were greater on the green hay plot than the naturally colonised plot (Figure 7.9).

Results of a Mann-Whitney U exact test of independent samples revealed that the average number of both *B. humilis* and *B. sylvarum* counted per hour on the green hay plot were significantly greater than on the naturally recolonised plot (p<0.001 and p<0.001 respectively). A Mann-Whitney U exact test also showed no significant difference between numbers on the green hay plot and those recorded

foraging on the good forage patches across the rest of Hadleigh Castle Country Park (*B. humilis* p=0.85, *B. sylvarum* p=0.48). A Mann-Whitney U test between the naturally recolonised plot and the rest of the park revealed that significantly fewer *B. sylvarum* and *B. humilis* were recorded on the naturally recolonised plot (*B. humilis* $p<0.001$, *B. sylvarum* $p<0.001$).

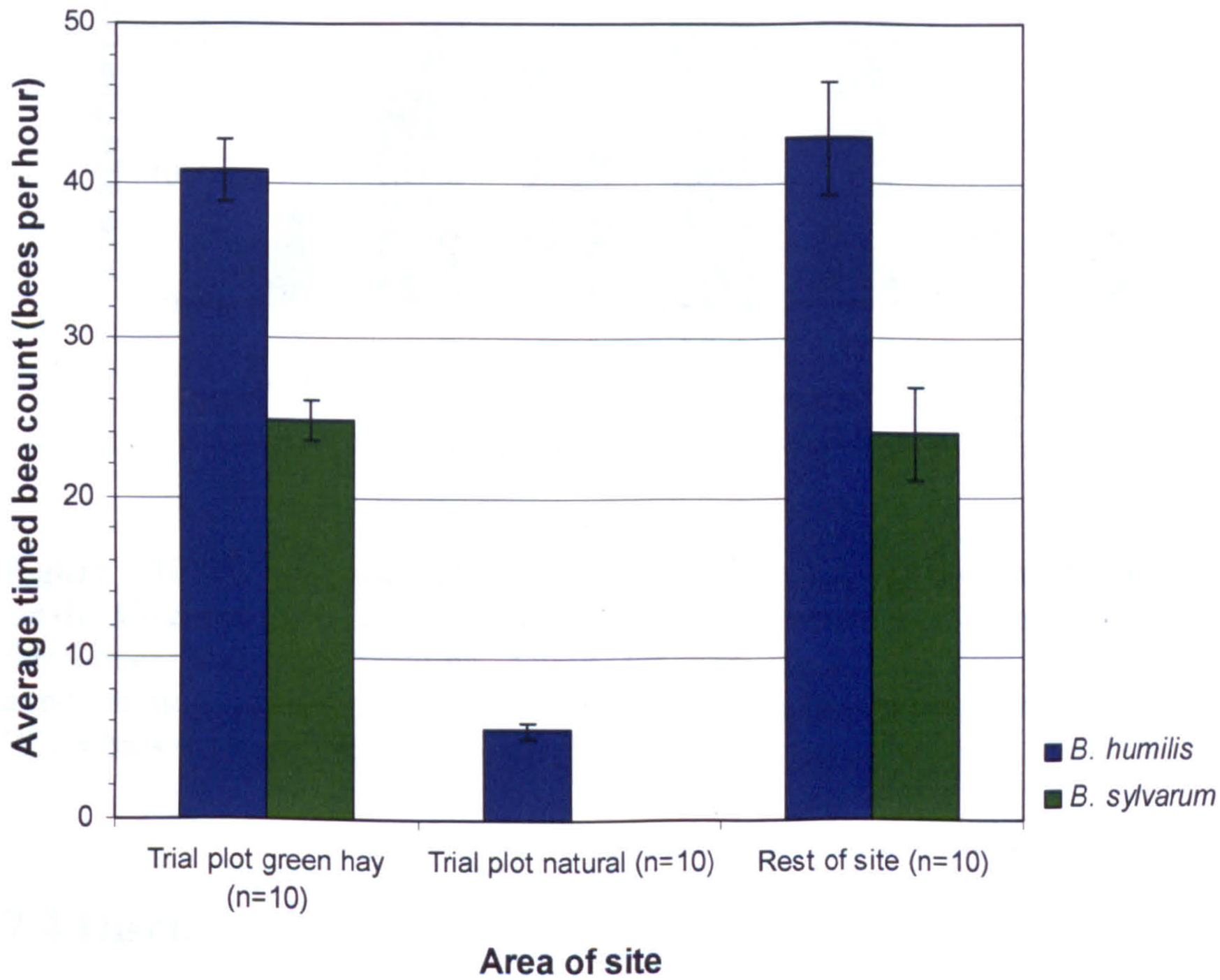


Figure 7.9. Average bee counts per hour for the trial plots and the rest of Hadleigh Castle Country Park forage patches, August 2007. Timed counts comprised of 30 minute surveys within areas of approximately 300m^2 [Error bars represent the standard error of the mean]

Also, a comparison of the annual average scores of timed counts at Hadleigh Castle Country Park was made (Figure 7.10). Average numbers of both species appeared to have increased throughout this study, with the exception of the 2004 *B. sylvarum* count which was as high as in 2007.

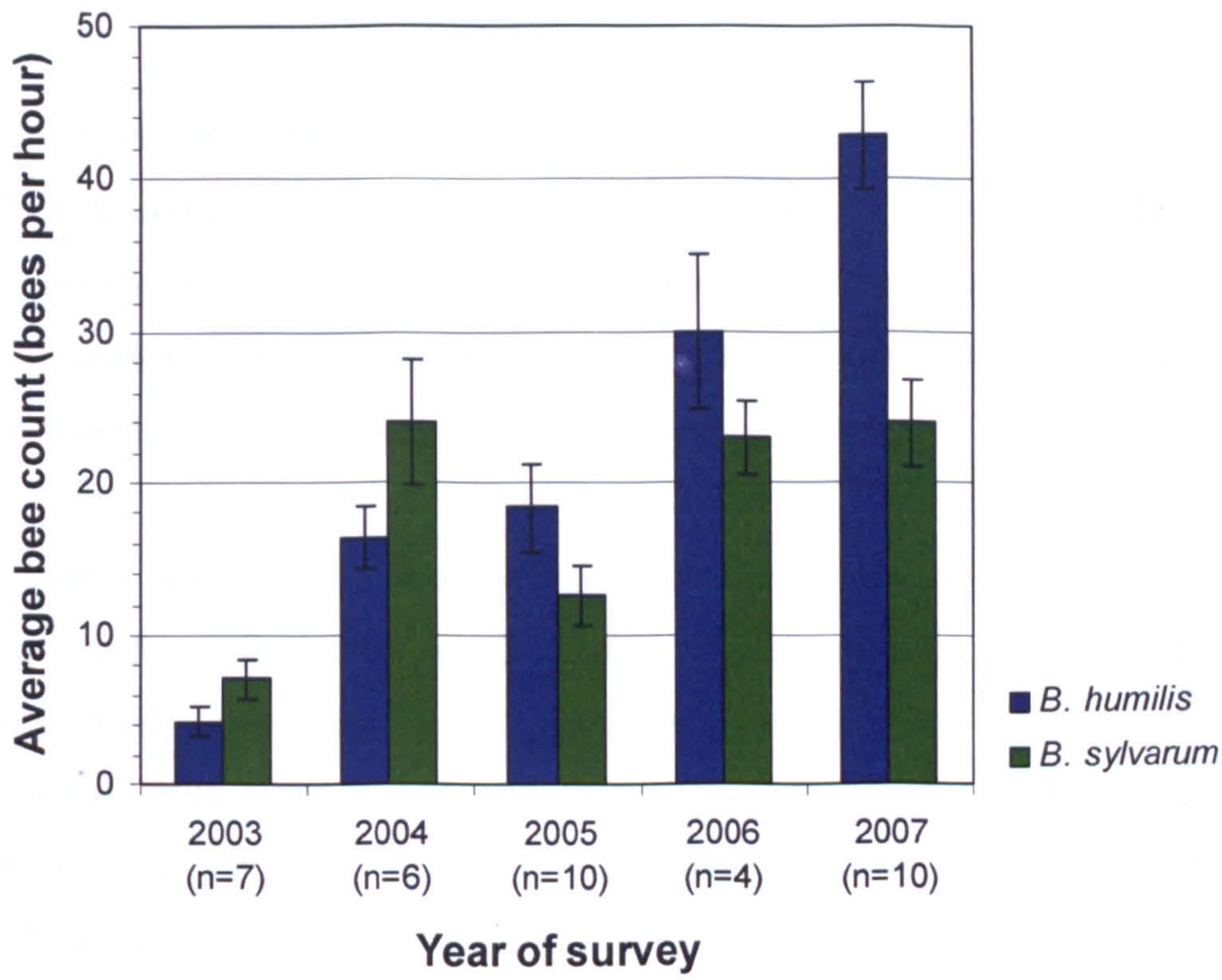


Figure 7.10. Average annual *B. humilis* and *B. sylvarum* counts at Hadleigh Castle Country Park, 2003-2007. Counts were all conducted during August and September, the main foraging period for workers of the two species, over approximately equal areas. n= number of timed surveys carried out each year. [Error bars represent the standard error of the mean].

7.4 Discussion

In accordance with the findings of Trueman and Millet (2003), results indicated that spreading green hay was an effective method for recreating wildflower meadows containing target floral species. *Bombus humilis* and *B. sylvarum* favoured forage plants were significantly more abundant on the green hay plot than through natural recolonisation. Of the target forage species identified earlier in this study, *Odontites verna* and *Trifolium pratense* flowers were found in significantly higher numbers on the green hay area than the naturally colonised area. Whilst the mean number of flowers of another target forage species, *Centaurea nigra,* was not significantly different between the two plots, the mean

percentage cover of the plants was. It was likely that this disparity was due to the surveys being slightly too early in the year to record open flowers of this species.

The only plants which were recorded as being visited by *B. humilis* or *B. sylvarum* that had a significantly greater cover or number of flowers on the natural recolonisation plot were *Cirsium arvense* and *Kickxia spurina*. Neither of these floral species was identified as being a particularly favoured forage source for the bees in this study (section 3.3).

Lotus glaber was the only target forage plant identified earlier in the study from the area cut for green hay which did not perform significantly better on the green hay site. Very few *Lotus glaber* plants were recorded on either plot. Further monitoring would be necessary to assess whether this species colonises either of the trial sites in later years. If this did not occur, seed collection for this species by hand from other areas of the site might be considered to establish the species on newly created forage patches. This method of cultivation was successful on the Pitsea Landfill site (section 6.3) and would therefore also be expected to be successful here. However, hand collection is a more time consuming method and would therefore be less practical for large scale site management.

Spreading green hay from areas of the site rich in *Odontites verna*, *Trifolium pratense* and *Centaurea nigra* following scrub clearance appeared to be an effective technique for establishing these species on newly created forage patches for *B. humilis* and *B. sylvarum*. Further monitoring would be necessary however, to establish whether this technique is beneficial for long term establishment of these species. Cutting or grazing experiments on these trial plots once favoured forage species have established would also be necessary to ensure that the timing of forage availability is synchronised with worker requirements. One observation of note in this study was that when surveys were carried out on the trial plots (August) the flowering of many of the *Odontites verna* plants had finished. On other areas of the site the existing cutting regime ensured that *Odontites verna* flowering was just beginning. *Bombus humilis* and *B. sylvarum* workers require relatively late forage (Harvey, 2000a) and have been recorded foraging in late September and even into early October in this study. Thus, it might be necessary to

cut or graze these trial plots in March, as the other semi-natural grassland areas of the site are, in order to provide forage at an appropriate time for the bees.

Forage on the green hay plot attracted significantly higher numbers of both *B. humilis* and *B. sylvarum* than the naturally recolonised area. In fact, no *B. sylvarum* were recorded foraging on the naturally colonised site during timed bee counts. Numbers of bees recorded foraging on the green hay plot were similar to those foraging on other wildflower rich areas of the site.

Although bee counts are a fairly imprecise method for measuring population size for eusocial insects (Chapman and Bourke, 2001), a more accurate method for analysis was not available prior to this study (see discussion 9.1). Timed bee counts in forage-rich areas of the site were therefore used to assess the number of *B. humilis* and *B. sylvarum* foraging on the site. Counts were replicated within and between years to provide the most accurate assessment possible. Corresponding with data from forage provision studies on more ubiquitous UK *Bombus* species (Kells *et al.*, 2001; Carvell *et al.*, 2004; Pywell *et al.*, 2005), results of these counts indicated that numbers of foraging *B. humilis* and *B. sylvarum* at Hadleigh Castle Country Park were increasing annually. Whilst such field based studies do not permit direct correlations between bee numbers and forage provision to be made, it seemed that increased bee numbers on the site were at least partly a consequence of *B. humilis* and *B. sylvarum* specific habitat management.

With further nesting and foraging habitat provision planned for the site, assessment of nesting requirements, appropriate spatial scale for such habitat elements and a more accurate method for measuring benefit to bumblebee populations was required. This is discussed further in chapters 8 and 9.

Chapter 8

Further species ecology: emergence, artificial forage, thermodynamics and nesting

8.1 Introduction

An understanding of habitat requirements of individual bumblebee species at their various life stages is required for effective bumblebee conservation (Edwards, 1998). Dietary behaviour and habitat creation results from this study have been fed into habitat management plans to conserve *B. humilis* and *B. sylvarum* in their Thames Corridor stronghold (Harvey, 2000b). However, whilst the results generated thus far have helped to further the understanding of the habitat requirements of these two species, additional knowledge is required if habitat management is to effectively support and conserve these populations. This includes *B. humilis* and *B. sylvarum* habitat requirements in terms of nesting habitat, timing of colony development, provision of forage specific to *B. humilis* and *B. sylvarum*, and the spatial dynamics over which populations operate.

To investigate these requirements, the next stage of this study was a series of surveys and experiments further analysing *B. humilis* and *B. sylvarum* species ecology. The aims of these investigations were:

i) To identify the timing of emerging queens in spring so that forage provision could be targeted towards queens as well as workers.

ii) To identify whether artificial forage sources could be provided which were specifically designed for long tongued bees.

iii) To investigate whether more nationally ubiquitous bumblebees have a competitive advantage by arriving at forage earlier than *B. humilis* and *B. sylvarum*.

iv) To identifying a reliable method to locate bumblebee nests and investigate the nesting preferences of *B. humilis* and *B. sylvarum*.

v) To investigate the spatial dynamics of *B. humilis* and *B. sylvarum* using a mark-recapture method from nests to forage.

8.2 Emerging queens

Introduction

Studies have been carried out to investigate the seasonal phenology of UK bumblebees in general (Prŷs-Jones, 1982; Goodwin, 1995) but no study has compared *B. humilis* and *B. sylvarum* queen emergence times with those of nationally ubiquitous species on sites where they are all common. There has also been no study comparing the phenology of the South Essex *B. humilis* and *B. sylvarum* populations with those nationally. The most recent study of bumblebee emergence was carried out by Goodwin (1995). Results for queens in this study are displayed in Table 8.1. Results identified orders of emergence similar to those found in previous studies by Alford (1975) and Prŷs-Jones (1982). Due to the location of the study in West London, *B. humilis* and *B. sylvarum* were not recorded. *Bombus sylvarum* and *B. humilis* were confirmed as late emerging in a study on Salisbury Plains by the Bumblebee Working Group (BWG), with *B. lucorum* being one of the first species recorded emerging (Edwards, 1998). *Bombus pascuorum* and *B. hortorum* were also identified as early emerging species in BWG studies, being recorded emerging during the end of March in South London (Edwards, 2002). In a separate study, Chapman (2004) recorded *B. humilis* queen numbers peaking in mid-June on site in the London area (UK).

The aim of this investigation was to identify the timings of queen emergence on the South Essex sites and to assess any variation caused by the East Thames Corridor's unique warm and dry climate (Harvey, 2000b; DEFRA, 2007).

Table 8.1. Emergence times for British bumblebee queens (Goodwin, 1995). Observation taken in West London.

Bombus species	Earliest date queens observed
B. terrestris	19-Feb
B. lucorum	06-Mar
B. lapidarius	09-Mar
B. pratorum	17-Mar
B. pascuorum	20-Mar
B. hortorum	31-Mar

Methods

Modified bee walk surveys (Banaszak, 1980; Saville *et al.*, 1997) were carried out from January to June 2004 across all of the South Essex sites. Walks followed the same methodology as those used previously in this study (chapter 2, section 2.2), although surveys were carried out in all temperature and weather conditions. Walks were carried out twice a week. The date of the survey, weather conditions, site surveyed, area of the site, species and caste of bumblebee observed were recorded. Identification of the bees followed Prŷs-Jones and Corbet's Naturalist Handbook key (1987). From these surveys it was possible to assess the date of the first observed queen of each *Bombus* species.

Results

Results of the first observed queen of each *Bombus* species across the South Essex sites are displayed in Figure 8.1.

Bombus vestalis and *B. sylvestris* were recorded as the latest emerging queens. Both of these species are cuckoo bees from the subgenus *Psithyrus*. Of the true bumblebees, *B. sylvarum* and *B. humilis* queens were the latest to be observed almost two months later than *B. terrestris*, *B. lucorum* and *B. lapidarius* queens, the more nationally ubiquitous species. *Bombus terrestris*, *B. lucorum* and *B. lapidarius* were the earliest queens observed, all were observed for the first time

during the same visit. *Bombus pascuorum* and *B. pratorum* were the next queens to be observed, almost a month after the first three were observed.

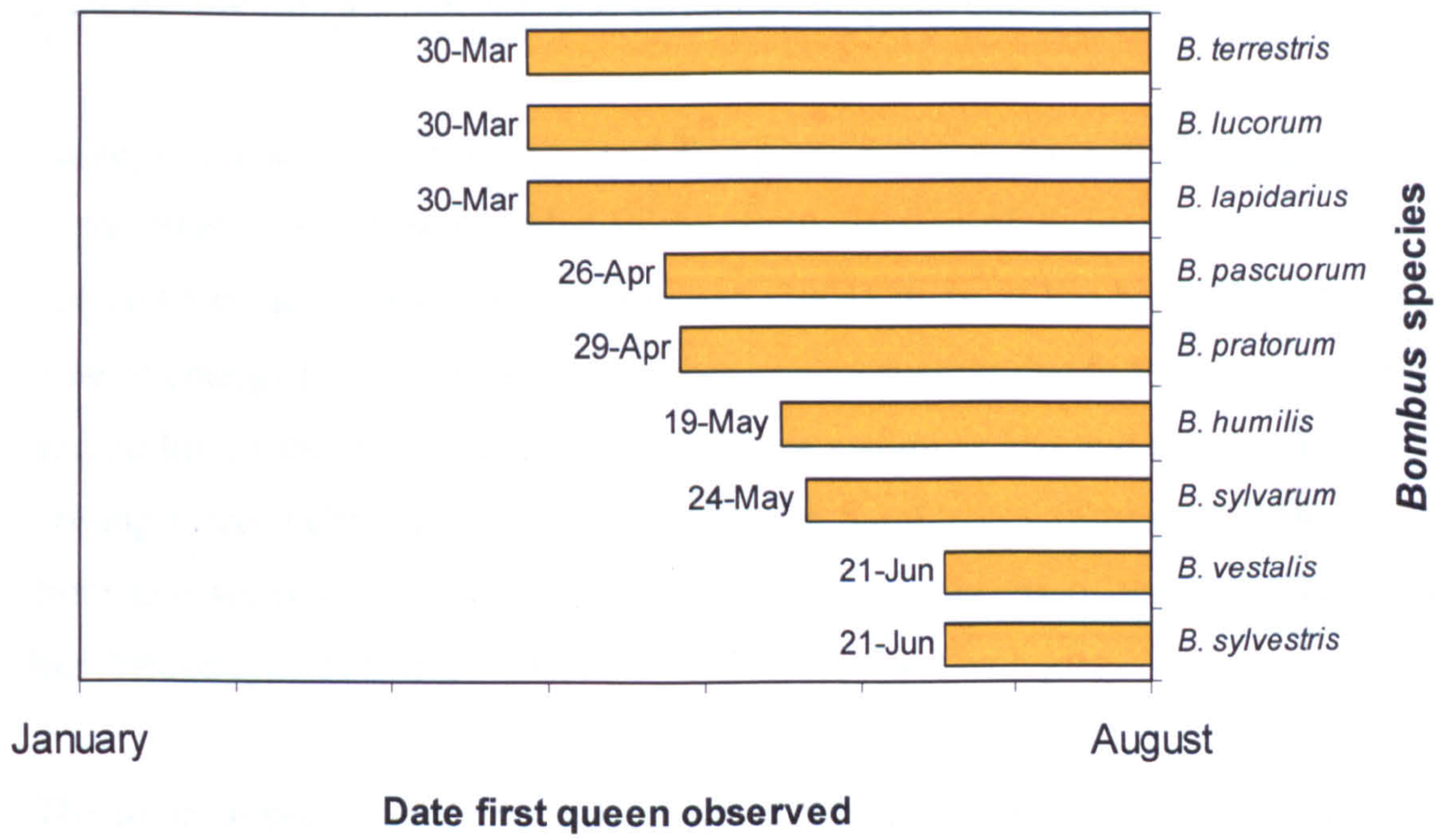


Figure 8.1. Date of first queen of each *Bombus* species observed during surveying, 2004. Dates correspond to the first queen of each *Bombus* species observed during bee walks across all of the South Essex sites, 2004. Walks began in January and were twice weekly.

Discussion

The two latest emerging species were the cuckoo bees *B. vestalis* and *B. sylvestris*. Cuckoo bees are inquilines in the nests of other *Bombus* species. They usurp the nests of other bumblebees using the bumblebee workers of the nest to rear their eggs (Goulson, 2003). This can only be done once their hosts' nests are sufficiently developed, so it is not surprising that queens of *B. vestalis* and *B. sylvestris* were the last to be recorded.

As in the Goodwin study (1995), *B. terrestris*, *B. lucorum* and *B. lapidarius* were the first queens to be recorded with *B. pascuorum* and *B. pratorum* observed later. The dates of first observation were slightly later in the South Essex study, but this

may have been a product of the more infrequent sampling methodology in this study than the West London study or due to variability in prevailing weather conditions between years. A study with replicated sampling methodology would need to be carried out across the two study sites during the same year for a more accurate representation of regional variability in queen emergence.

Surveys revealed that *B. humilis* and *B. sylvarum* queens were the latest emerging of the true bumblebees recorded in the study. Timing of *B. humilis* queens were similar to those recorded in the Chapman (2004) study. It has been theorised that time of emergence can impact bumblebee colony success due to forage availability and ability of the bees to exploit these forage resources and that this is one of the driving forces behind declines in some British bumblebees (Edwards, 1998). Data from this study correlated with this theory, as the queens of the two rarest UK bumblebee species being recorded were also the latest emerging.

The main importance of this data however was in providing a timescale for the provision of forage suitable for queens of *B. humilis* and *B. sylvarum*. The first queens of both of these species in South Essex were observed foraging in late May. Habitat management for the conservation of these bees should therefore be designed to provide the forage species identified in this study (chapters 3 and 5) as being favoured by foraging *B. humilis* and *B. sylvarum* queens (*Lathyrus latifolius*, *Colutea arborescens*, *Vicia sativa*, *Vicia villosa*, *Trifolium pratense* and *Rubus fruticosus*) during May and June each year.

8.3 Artificial forage resource

Introduction

Laboratory based foraging behaviour studies have been carried out on the more ubiquitous British bumblebees (Raine and Chittka, 2005; Raine and Chittka, 2007). Attempts at encouraging *B. humilis* and *B. sylvarum* to use artificial nests have however been unsuccessful (Edwards, 2000; Harvey, 2001; Edwards, 2002) and consequently laboratory studies of foraging behaviour have been impossible. Bumblebees in laboratory and greenhouse studies are commonly fed using artificial nectar and pollen sources (Whittington and Winston, 2003; Wiegmann *et al.*, 2003; Pelletier and McNeil, 2004; Weinberg and Plowright, 2006). The use of artificial forage sources with wild bees does not appear to have been attempted.

This study investigated the potential for attracting wild bees to artificial forage sources. It has been hypothesised in numerous studies that tongue length drives resource partitioning in bumblebees (Inouye, 1980; Ranta and Lundberg, 1980; Prŷs-Jones and Corbet, 1987; Goulson and Darvill, 2004; Goulson *et al.*, 2005). If wild bees could be attracted to artificial feeders, by manipulating the feeder, it would be possible to investigate this theory. If this theory proved to be true, artificial forage could be provided specifically for long tongued bumblebees, such as *B. humilis* and *B. sylvarum*, supplementing natural forage sources.

Methods

An artificial nectar feeder was designed which could be manipulated to reward bees dependent on the tongue length of the bee (Figure 8.2). Artificial flower heads were made from purple/pink drinking straws as this colour has been found to be favoured by foraging bumblebees (Chittka and Walker, 2006) and was the colour of many flowers recorded as being favoured by the bees in this study (*Odontites verna, Ballota nigra, Centaurea nigra, Trifolium pratense*). Thirty feeders of the same design were made and filled with 50% sugar solution as a nectar source (Pouvreau, 1974). Half of the feeders were rubbed with crushed

lavender flowers (*Lavandula* species) and half were left untreated. This was done to investigate the effectiveness of lavender scent in attracting bees to feeders compared with feeders only having the scent of sugar solution. The distance from the artificial flower head to the wick soaked in sugar solution could be adjusted for different bee tongue lengths. For the initial study the wick was level with the flower head so that bees with any tongue length could obtain nectar.

In August 2004, the artificial feeders were placed together on flower beds that the bees were observed visiting in the cottage garden at Wat Tyler Country Park (TQ738861). This site was chosen because *B. humilis*, *B. sylvarum*, *B. pascuorum*, *B. lapidarius* and *B. terrestris/lucorum* workers had all been recorded foraging here in large numbers during the 2003 and 2004 bumblebee surveys (chapters 2 and 3). The feeders were observed on days when the weather was warm and favourable to bumblebee activity (temperatures greater than 15°C). The feeders were observed for 30 minute intervals five times a day. Any bumblebee observed visiting the feeders was recorded.

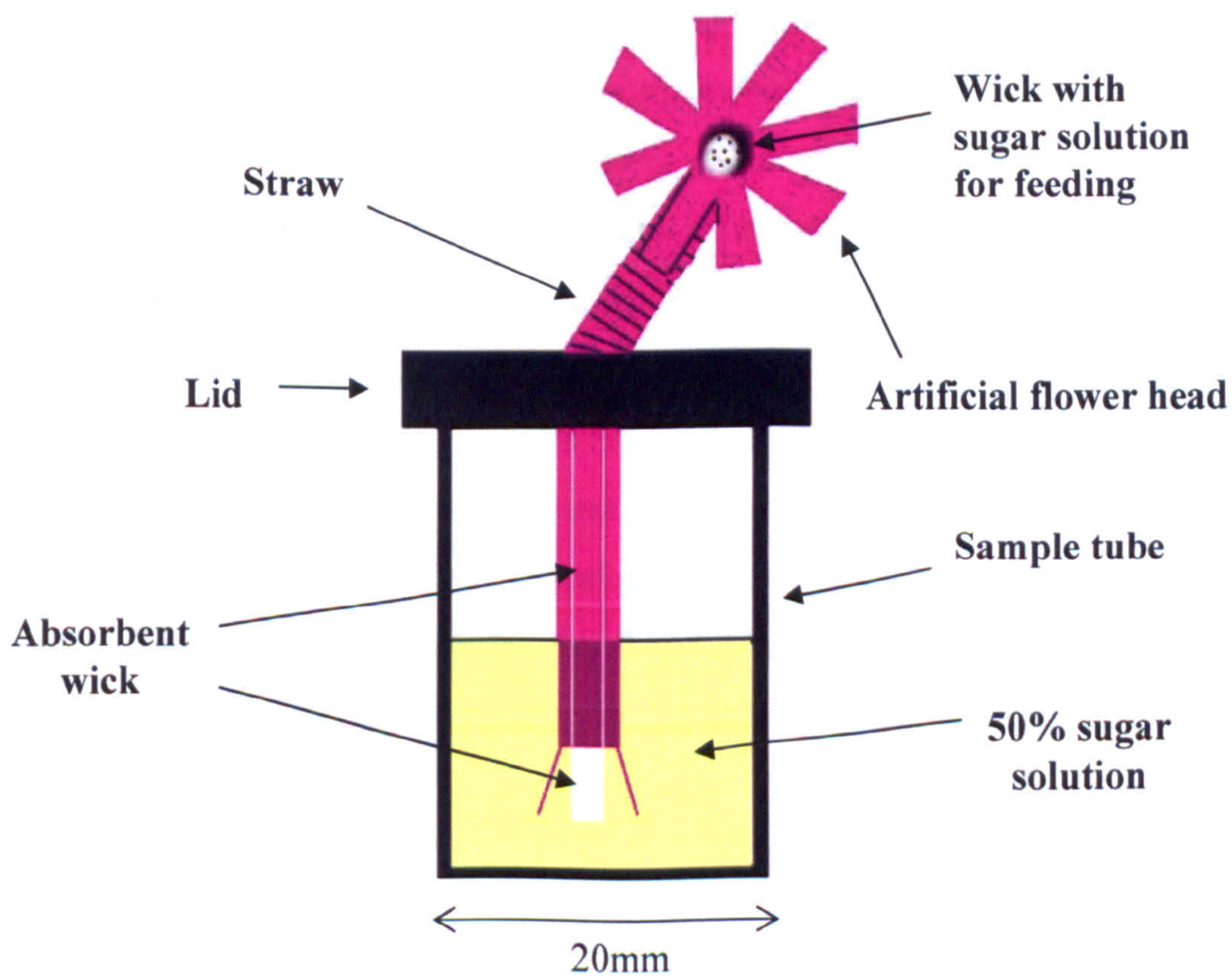


Figure 8.2. Design of artificial bumblebee feeder. Straw acts as artificial flower head. Wick absorbs sugar solution to provide an artificial nectar source at the flower head.

Results

Although the feeders attracted wasps (*Vespula* species), ants (*Lasius niger*) and hoverflies (Syrphidae), no bumblebees were recorded visiting. No difference was observed between the scented and unscented artificial feeders.

Discussion

Attempts at attracting wild *Bombus* species to artificial nectar sources were unsuccessful. Results indicated that this was not a suitable method for providing nectar sources for *B. sylvarum* and *B. humilis* when other forage sources were available. Further research could be done to investigate whether bumblebee pheromones similar to those reported in a Dornhaus and Chittka study (2001) could be use to attract *B. humilis* and *B. sylvarum* workers to artificial nectar sources, but this was beyond the scope of the current project.

With the failure of this study to attract bees to artificial forage and encouraging these two species to use artificial nest boxes proving unsuccessful (Edwards, 2000; Harvey, 2001; Edwards, 2002), the only alternative for study of resource partitioning would be the capture of queens to initiate colonies under laboratory conditions. In bumblebee populations each single fertilised queen founds its own colony (Goulson, 2003). Removing a queen from a population effectively removes an entire colony and thus also removes the potential for production of new queens and males. Such a technique would not be appropriate for the investigation of conservation priority populations. Therefore, unless a technique for attracting wild bees to artificial forage sources can be found, controlled experiments related to tongue length and nectar provision may prove to be impossible for these rare wild bees.

8.4 Arrival at forage

Introduction

Studies have demonstrated that the ability of bumblebee workers to forage is related to the energetics of flight (Heinrich, 1979; Cresswell *et al.*, 2000) and specifically to their ability to warm up and thermoregulate during flight (Heinrich 1975). Worker size is believed to influence their ability to do this (Free and Butler, 1959; Stone and Willmer, 1989). It has been hypothesised that larger workers may have a competitive advantage over smaller workers when foraging due to their ability to become active at lower ambient temperatures but that at higher temperatures larger workers are more prone to overheating and are therefore disadvantaged (Heinrich, 1975; 1979). Size varies considerably both between and within bumblebee species, even within workers from the same colony (Pouvreau, 1989; Ribeiro, 1994). *Bombus sylvarum* and *B. humilis* are, however, considered to be some of the smaller UK bumblebees (Peat *et al.*, 2005). This generally smaller size was confirmed during bee surveys for this study, which observed *B. humilis* and in particular *B. sylvarum* workers as consistently appearing smaller than the more nationally ubiquitous species. No attempt was made in this study to quantify sizes as the Peat *et al.* study (2005) had recorded these.

If theories on foraging energetics and worker size are true it would be expected, that *B. humilis* and *B. sylvarum* workers, tending to be smaller than some of the more ubiquitous UK bumblebee species on the South Essex sites, would be less able to forage at cooler temperatures early in the day than the larger *Bombus* species. An ability to forage earlier might give the larger bumblebees a competitive advantage over *B. humilis* and *B. sylvarum*. Due to the warm climate of the East Thames corridor (Harvey, 2000b; DEFRA, 2007), once ambient temperatures have increased, it might also be expected that smaller bees would have a competitive advantage. This might explain why *B. humilis* and *B. sylvarum* still have strong populations in this area when they are disappearing from the rest of the country.

Methods

This investigation attempted to assess which *Bombus* species were the first to arrive at a forage patch, and at what ambient temperatures. Monitoring involved surveying a forage patch from sunrise for several hours to record *Bombus* species visiting the patch. The cottage garden at Wat Tyler Country Park (TQ738861) was used for this study.

In August 2004, an area of *Lavandula* flowers previously recorded during the 2003 and 2004 bumblebee surveys (chapters 2 and 3) as supporting large numbers of foraging *B. humilis*, *B. sylvarum*, *B. pascuorum*, *B. lapidarius* and *B. terrestris/lucorum* workers was monitored. The time bees reached the patch, ambient temperature at that particular time, *Bombus* species and caste were recorded for each bee observed foraging on the patch. Temperature was measured using a digital thermometer. The thermometer was placed in the shade between flowers in the forage patch. Initial measurements were taken on observation of bees. Once bee numbers increased to being constantly present, temperature measurements were taken every ten minutes. Identification of the bees followed Prŷs-Jones and Corbet's Naturalist Handbook key (1987).

Results

Observations of the time and temperature that each *Bombus* species was observed first arriving at the forage were recorded (Table 8.2).

Bombus pascuorum was earliest species to arrive at the forage (ambient temperature 14.0°C) quickly followed by *B. humilis* five minutes later. The first *B. sylvarum* did not arrive until 48 minutes after *B. pascuorum* workers had arrived at the patch (ambient temperature 16.8°C). Before the first *B. sylvarum* worker arrived, 29 *B. pascuorum* workers and 18 *B. humilis* workers had been recorded foraging at the site. The first *B. lapidarius* was recorded foraging 50 minutes after *B. sylvarum* arrived and *B. terrestris/lucorum* another 30 minutes later.

Table 8.2. Time and ambient temperature of first worker arrivals on a forage patch at Wat Tyler Country Park, August 2004.

Bombus species	Time of first worker at forage patch	Ambient temperature at time (°C)
B. pascuorum	06:17	14.0
B. humilis	06:22	14.0
B. sylvarum	07:10	16.8
B. lapidarius	08:00	22.2
B. terrestris/lucorum	08:30	23.8

Discussion

Unfortunately due to delays in obtaining keys for the survey site, observations were not carried out until late August. At this time of year *B. pascuorum*, *B. humilis* and *B. sylvarum* were the only bees foraging on these sites in substantial numbers. This meant that few *B. terrestris/lucorum* and *B. lapidarius* workers were recorded in the study. (n=2 and n=11 respectively compared to 140 *B. humilis*, 122 *B. pascuorum* and 48 *B. sylvarum*) There was no way of knowing therefore whether their late arrival at the forage patch was an indication of their inability to forage at cool temperatures or merely representative of their rarity on the site. Results for these two species could therefore not be considered as representative of the species' ability to forage during cool temperatures.

Overall, results from the arrival at forage survey were anecdotal at best. Without any controls on the investigation in terms of measuring temperatures at source colonies or quantifying the size of workers and distances travelled, it was impossible to make any definitive conclusions. However, the delayed arrival of *B. sylvarum* compared to *B. humilis* and *B. pascuorum* was interesting when combined with theories on bumblebee size and thermoregulation (Heinrich, 1975). *B. sylvarum* workers are the smallest of the three species based on mean thorax width (Peat *et al.*, 2005) and if thermodynamic theories on forager size are correct it would be expected that these smallest workers would arrive at the forage last. A study by Peat *et al.* (2005), which was carried out concurrently with this South

Essex study, was able to demonstrate that bumblebees from colder climates tended to be larger than those of the same species from warmer climates. Despite investigations however, they were unable to identify any evidence for ambient temperature having a differential effect on the activity of *B. terrestris* workers according to their size. It seems likely therefore that size variation does not directly affect ability to forage with temperature, but is more likely to be an adaptation to another ecological function such as flower handling.

8.5 Nesting preferences

Introduction

Numerous studies have investigated the nesting behaviour and requirements of bumblebees and attempted to identify the habitats which bumblebees favour for nesting (Wojtowski, 1963; Sakagami and Katayama, 1977; Harder, 1986; Fussell and Corbet, 1992a; Svensson *et al.*, 2000; Carvell, 2002; Svensson, 2002; Kells and Goulson, 2003; Osborne *et al.*, 2007). The habitats identified in these studies included many of those which have been altered by agricultural intensification: hedgerows, established grassland, field margins and boundaries (Osborne and Corbet, 1994; Buchmann and Ascher, 2005). It has been proposed that declines in certain bumblebees can be partly explained by differential nesting habitat requirements between species and that loss of these habitats is impacting British bumblebee populations. For a more detail discussion see chapter 1 (section 1.5).

Habitat management for the conservation of *B. humilis* and *B. sylvarum* should include the provision of nesting habitat suitable for these species (Edwards, 1998). However, very little is known about the nesting requirements of *B. humilis* and *B. sylvarum* (for a full description of current knowledge see chapter 1, section 1.5.2). Several surveys have been carried out but *B. humilis* and *B. sylvarum* nests have proved extremely difficult to locate (Edwards, 1999; Harvey, 2000a; Carvell, 2002). Both species are believed to be reliant on undisturbed tall grassland, nesting at or below the soil surface in dense grassy tussocks, but much of the evidence for this has come from observing the nest searching behaviour of queens rather than actually locating nests (Edwards, 1998; Svensson *et al.*, 2000; Kells and Goulson, 2003). It can be argued that this is an unreliable method for assessing bumblebee nesting preferences as, by its very definition, it is the monitoring of queens searching for suitable nesting habitat but being unable to find it. Unless a nest is actually observed the precise requirements for nesting can only be assumed.

The aims of this study were to survey the South Essex sites to locate *B. sylvarum* and *B. humilis* nests and to generate a novel approach for reliably locating nests of

these bees. If nests could be reliably located, mark recapture studies would be carried out on foraging workers to assess the spatial scales over which the bees were operating. Also, estimates of colony density could be made.

Methods

Nest surveys were carried out from May to October, 2003-2005. Surveys comprised of a modified version of the 'bee walk' utilised by Banaszak (1980) and Saville *et al.* (1997). The bee walks comprised of slow systematic walks across the sites scanning for nests or for *B. humilis* and *B. sylvarum* workers emerging from or returning to nests. All searches were conducted between 9:30 and 17:00 BST and during warm weather favourable to bumblebee activity (temperatures greater than 15°C).

Fixed point observation of forager traffic was also trialled across the sites during the same time period. Methodology was based on that of Osborne *et al.* (2007). Twenty minute long fixed point observation was used to monitor areas of grassland approximately 10m x 10m. Observations were made of bees flying into or out of one location in an attempt to identify the presence of a nest. Investigations of other potential techniques investigated during this study are included in the discussion.

Results

During the 2003 to 2005 *B. humilis* and *B. sylvarum* nest surveys only three nests were found. All three were *B. humilis* nests and all were found during nest survey walks. Fixed point observation failed to locate any nests in this study.

Of the three nests found, one was found at Vange Hill (TQ721876) during a survey with Peter Harvey in 2003. The other two were found along the sea wall at Two Tree Island, Essex Wildlife Trust site (TQ825852) in 2005. All three nests had been disturbed, possibly predated on by badgers (*Meles meles*). If such predation is common, it could be an important factor limiting bumblebee populations in the

region and is therefore worthy of further research. The potential impact of predation and parasitism on rare bumblebees is discussed further in section 1.5.1.

The nest at Vange Hill was first observed on the 31st July. It was located in rough grassland on a southwest facing slope at approximately 50m above sea level. The nest was situated in a sward made up of *Anthoxanthum odoratum* (sweet vernal grass) and *Cirsium arvense* (creeping thistle). Within 50cm of the nest was a single *Lathyrus pratensis* (meadow vetchling). The nest was approximately 2-3m away from a footpath along which *Odontites verna* was growing. The surface area of the nest appeared to have been about 10cm x 10cm and comprised of combed-together grasses and mosses at the soil surface level. Images of the nest are included as Figures 8.3 - 8.5.

Figure 8.3. *Bombus humilis* nest found at Vange Hill, July 2003.

Figure 8.4. *Bombus humilis* approaching nest at Vange Hill, July 2003.

Figure 8.5. Vegetation surrounding the *Bombus humilis* nest at Vange Hill, July 2003.

Although the nest had been disturbed, it appeared that the bees were either continuing to use a section of the nest that had survived, or they were rebuilding the nest. Regular visits were made to the nest site throughout August to monitor its development. On each visit *B. humilis* workers were seen entering and leaving the nest. On August 14th however the nest appeared to have been abandoned. It had become very exposed and the dry weather had killed the majority of the surrounding grass tussocks. It is not known whether the cessation of the nest was caused by the natural cycle of a colony or whether the heat and exposure of the site had made it unhabitable. No further *B. humilis* were seen entering or leaving the nest during any of the later visits in August. The site was resurveyed in the following years but no further nests were observed.

The two nests found at Two Tree Island were on the southeast facing slope of the seawall approximately a metre above sea level. These two nests were also located at the base of grass tussocks in an area of rough grassland. *Bombus humilis* activity at the nests ceased shortly after locating them. These two nests were discovered in September, so it was possible that their cessation was due to the completion of colony development. Although in both cases disturbance had occurred and could have ended the colonies prematurely.

During nest surveys and forage surveys (chapters 2 and 3) numerous *B. humilis* and *B. sylvarum* queens were observed exhibiting nest searching behaviour: flying in areas with no obvious forage available and disappearing into the base of grass tussocks then reappearing a few seconds later (Svensson *et al.*, 2000; Kells and Goulson, 2003). All *B. humilis* and *B. sylvarum* queens exhibiting nest searching behaviour were seen in rough grassland often along the side of paths.

Discussion

As the walked surveys and fixed point observation proved to be unreliable techniques for locating *B. humilis* and *B. sylvarum* nests, other techniques were investigated. These included:

i) Harmonic radar – research at IACR-Rothamsted has utilised harmonic radar to track bumblebees in an experimental environment (Osborne *et al.*, 1997; Carreck *et al.*, 1999; Osborne *et al.*, 1999). Bumblebees were fitted with transponders and their movements were tracked during foraging trips. The potential for use of this technique for locating rare bumblebee nests was discussed (personal correspondence). The limited range of the equipment (up to 700m) and the need for flat areas with nothing breaking the radar's local horizon meant that the technique would not be effective in the scrub covered and fragmented landscape of the South Essex sites.

ii) Trace vapour detection using honey bees (*Apis mellifera*) – Inscentinel (Inscentinel, 2007), a company based at Rothamsted Research, Harpenden, UK, has developed the use of honey bees as trace vapour detectors (Pain, 2006). During conversations with Rachael Carson and Mathilde Briens, the potential for using the bees to locate *B. humilis* and *B. sylvarum* nests was discussed. After much discussion, it was decided that this technique would not be effective for locating the nests because the honeybees would need to be so close to the nest to detect the scent that the nest would already be visible to the eye. It was not considered necessary to research this method further.

iii) Thermal imaging – queen bumblebees are known to maintain their nest temperatures at ranges of approximately 30-32°C for brood care (Heinrich, 1972; 1974). Because of this it would be expected that bumblebee colonies would be warmer than surrounding ambient temperatures, particularly early in the morning when the ground is at its coolest. It might be possible to detect this heat differential using thermal imaging equipment. Discussions with Dr Clive Alabaster of Cranfield University Royal Military College of Science, Shrivenham, Oxfordshire, identified that a thermal imager with rotating polarisation filter may be able to detect the disturbed ground at a nest site. Problems with access to such equipment however meant that this method was never trialled. If equipment could be obtained, this line of research seemed fairly promising.

iv) Acoustic detection – Bumblebees emit an audible buzz. In the case of *B. sylvarum* (the shrill carder bee) this buzz is unique amongst bumblebees. So, another possibility for locating *B. humilis* and *B. sylvarum* nests might be to survey grasslands to detect the particular frequencies emitted by the bees. This method was also considered possible for reliable nest location but due to problems with correspondence this method was not researched further.

v) Sniffer dog – Numerous discussions within the Bumblebee Working Group have identified the potential for a sniffer dog to locate bumblebee nests. In July 2006 'Quinn' the Bumblebee Conservation Trust sniffer dog was trialled locating bumblebee nests. Initial results have been promising (personal correspondence with Joe Waters) and there are hopes that Quinn will be brought out to the Essex sites to search for *B. humilis* and *B. sylvarum* nests. Unfortunately, due to Quinn's high work load, this will be impossible until at least June 2008.

Without a reliable technique to locate *B. humilis* and *B. sylvarum* nests, it was impossible to accurately assess the habitat requirements of these two species in terms of nesting sites. However, sites where the three *B. humilis* nests were found and evidence from nest searching behaviour in this study did correspond to observations from a Carvell study (2002). Important characteristics appeared to be relatively undisturbed tall rough grassland, with a substantial layer of leaf litter and moss. Nests in this survey were found on sunny southwest and southeast facing slopes. This necessity for warm sites with sunny exposure was also recognised in the Bumblebee Working Group surveys (Edwards, 1998; 1999; 2000; 2001; 2002).

The lack of nests located in this study meant that mark-recapture studies to assess the spatial dynamics of foraging workers were not possible.

Chapter 9

Microsatellite DNA studies

9.1 Introduction

Knowledge of *B. humilis* and *B. sylvarum* South Essex distributions, foraging preferences and methods for forage patch creation is necessary for designing conservation habitat management plans. Without reliable data on the appropriate spatial scales for the creation of such habitat, conservation management could be ineffective for existing populations (Steffan-Dewenter *et al.*, 2002).

Human-driven habitat loss and fragmentation is a major concern in conservation biology and landscape ecology (Gilpin, 1987; Opdam, 1990; Hanski and Gilpin, 1991; Reed, 2004). Agricultural intensification has resulted in the fragmentation, degradation and loss of semi-natural habitats and is believed to be a major driving force behind the reduction of local biodiversity in temperate regions (Steffan-Dewenter and Tscharntke, 2002; Robinson and Sutherland, 2002; Weibull *et al.*, 2003). Fragmentation of essential bumblebee foraging and nesting habitat has been hypothesised as being one of the driving forces behind declines in the abundance and distribution of a number of British bumblebees (Williams, 2005). If this is the case, to increases local population densities, lost foraging and nesting habitat must be recreated on spatial scales appropriate to target conservation species thereby increasing habitat connectivity and removing the effects of fragmentation (Steffan-Dewenter *et al.*, 2002; Steffan-Dewenter, 2003).

Bumblebees require different habitats for foraging and nesting (Alford, 1975) and their success as pollinators depends upon their ability to exploit these spatially and temporally varied habitats (Bronstein, 1995). Studies on the temporal patterns of bumblebees are numerous and have revealed much interspecies variation (Alford, 1975; Prŷs-Jones, 1982). An investigation in this study demonstrated that emergence times for *B. humilis* and *B. sylvarum* queens were late compared to other true bumblebees on the South Essex sites (section 8.2).

This investigation focused on the spatial dynamics of *B. humilis* and *B. sylvarum* workers in terms of foraging visits and colonization of new forage patches. Past studies on the spatial dynamics of bumblebees have indicated that some *Bombus*

species may differ significantly in terms of their spatial requirements for habitat characteristics (Walther-Hellwig and Frankl, 2000b; Darvill *et al.*, 2004; Knight *et al.*, 2005). If this were the case, it would be expected that habitat fragmentation would differentially affect *Bombus* species, and may in part explain patterns being observed in British bumblebee declines (Williams, 1982). Unfortunately, due to traditional methods for studying animal movements being impractical for use with bumblebees, spatial studies have been limited both in terms of number of species investigated and precision of results.

Initial plans for the investigation of the spatial dynamics of *B. humilis* and *B. sylvarum* worker foraging in this study were to use mark-recapture techniques (Kwak, 1987; Kearns and Inouye, 1993; Walther-Hellwig and Frankl, 2000b; Kreyer *et al.*, 2003). Bumblebee workers tend to be forage patch constant (Plowright and Laverty, 1984; Osborne *et al.*, 1999; Osborne and Williams, 2001). This means that if bumblebees are marked whilst foraging, it is highly probable that on subsequent occasions they will be re-recorded in or around the position that they were originally marked. Mark-recapture of workers whilst foraging is therefore not a suitable technique to assess their spatial movements as it merely measures movements within the foraging landscape rather than distances travelled from nests to forage. For this distance to be measured workers would need to be marked as they leave the nest and surveyed whilst foraging.

As discussed previously (section 8.5), locating bumblebee nests is a notoriously difficult task however, and cannot be done with any reliability (Fussell and Corbet, 1992a). Attempts in both this study and in other studies (correspondence with the Bumblebee Working Group) have been unsuccessful in identifying a technique to reliably locate *B. humilis* and *B. sylvarum* nests. Without such a technique mark-recapture methods cannot be used to study the spatial movements of bumblebees in a natural landscape.

The inability to use standard research techniques to analyse landscape scale bumblebee movements has forced researchers to experiment with new methods to investigate the localised spatial patterns of bumblebees. Harmonic radar has been used with great success to track bumblebees in an experimental environment

(Osborne *et al.*, 1997; Carreck *et al.*, 1999; Osborne *et al.*, 1999). Using this technique workers of *B. terrestris* have been recorded flying over 700 m to forage in an arable landscape contradicting theories on optimal foraging theory proposed by Heinrich (1979). The technique is limited in range and requires flat open study areas with nothing breaking the radar's local horizon making it impractical for the assessment of *B. sylvarum* and *B. humilis* foraging ranges within the scrub covered and fragmented landscape of the South Essex sites.

The other emerging technique for measuring foraging range and foraging patterns in bumblebees is the use of microsatellite genetic analysis (Chapman *et al.*, 2003; Darvill *et al.*, 2004; Knight *et al.*, 2005). In these studies DNA was sampled from bees at known forage patches and microsatellite loci were used to infer relationships between the sampled bees. Chapman *et al.* (2003) sampled bees from habitat 'islands' within an urban landscape whereas Darvill *et al.* (2004) and Knight *et al.* (2005) sampled bumblebees at known distances within a continuous habitat. The studies used the proportion of sister pairs (bees from the same colony) at different distances to estimate foraging distances. Whilst these studies demonstrated significant differences between *Bombus* species they were only able to do this for the more nationally ubiquitous species. All of these studies were restricted by their sample sites only supporting populations of nationally ubiquitous species or, when rarer species were present, they were sparsely distributed across relatively diffuse forage sources and so sample sizes were low. No studies of *B. humilis* or *B. sylvarum* foraging movements within a landscape have been attempted.

Molecular techniques have been used with *B. humilis* and *B. sylvarum* in a study by Ellis *et al.* (2007) which looked at migratory distances and genetic isolation of populations. Gilpin (1987) and Opdam (1990) proposed that if habitat fragmentation occurred to such an extent that inter-patch distances were no longer bridgeable, population isolation could occur. This could lead to a loss of genetic diversity and decreased effective population size eventually leading to extinction. In haplodiploid hymenoptera such as bumblebees, loss of genetic diversity can cause the additional cost of diploid male production instead of workers (Cook and Crozier, 1995), causing a loss of approximately half of the workers contributing to

a colonies' resources (Duchateau and Mariën, 1995). Ellis *et al.* (2007) confirmed that effective population sizes of remaining UK populations of *B. humilis* and *B. sylvarum* were low and that genetic structuring (genetic differentiation) was occurring between the populations indicating their isolation from each other.

Bumblebees are a group of eusocial insects in which most individuals don't reproduce (Goulson, 2003). Reproduction is performed by just one or a few related queens mated to one or a few males and populations are comprised of a series of colonies where the number of colonies rather than number of individuals are a measure of the effective population size (Chapman and Bourke, 2001). The inability to reliably locate rare bumblebee nests (Fussell and Corbet, 1992a) therefore makes estimations of colony density and population estimates almost impossible. Without these estimates it is difficult to accurately assess the conservation status of populations or to monitor the efficacy of habitat management. Various studies have used microsatellite DNA analysis techniques to look at colony number (the number of bee nests) and, combined with data from forage distance estimates, made estimates of colony densities for the more ubiquitous UK bumblebees (Chapman *et al.*, 2003; Darvill *et al.*, 2004; Knight *et al.*, 2005). With colony density estimates ranging from 26 to 117 km^{-2} in semi-natural area and counts of 66 to 96 colonies found foraging on areas ≈ 1ha in urban areas, these studies demonstrated great variability between species.

This study attempted to further develop the use of molecular genetic techniques to assess *B. humilis* and *B. sylvarum* movements within the landscape and to estimate population size. In previous studies bees have been surveyed in designated patches within larger diffuse areas of suitable habitat (Darvill *et al.*, 2004; Knight *et al.*, 2005). This study provided the opportunity to survey discrete forage patches surrounded by areas of scrub and semi-natural grassland not supporting favoured forage species. Following several years of surveying, the most important forage patches for the bees at each site had been identified (chapters 3-5). The combination of these two factors meant that a large number and proportion of the foraging *B. humilis* and *B. sylvarum* workers would be sampled and an accurate picture of foraging patterns of the bees could be obtained.

The aims of this investigation were threefold:

i) To assess the foraging distances of *B. humilis* and *B. sylvarum* workers – the spatial dynamics of the bees were investigated by taking DNA samples from workers at discrete forage patches at known distances apart. Microsatellite analysis was used to identify sister bees. From these data, estimates of the minimum distance the bees must have travelled from a theoretical mid-point nest were calculated providing information on the spatial dynamics of the bees. Data generated can be fed into habitat design for the provision of forage and nesting habitat.

ii) To assess colony number – the identification of sister bees was also used to identify the colonies of the bees sampled. From these data, the number of colonies at each site which were not sampled and the population size (the number of breeding individuals) were estimated. This data was used to assess the relative importance of the South Essex sites in terms of number of colonies supported. The data could also be use and as a baseline to monitor the effects of habitat management at the sites in terms of change in *B. humilis* and *B. sylvarum* colony number.

Microsatellite DNA data generated in this study was also used to supplement the Ellis *et al.* (2007) dataset on migratory distances and heterozygosity (frequency of heterozygotes, a measure of genetic variation) of remaining *B. humilis* and *B. sylvarum* populations. By assessing population sizes and heterozygosity within populations it is possible to identify which remnant populations are most isolated and most at risk of extinction. Such information could be used to target conservation management towards the most isolated populations, with an aim of reducing inter-patch distances between populations.

iii) To assess the spatial scales over which *B. humilis* and *B. sylvarum* are able to colonise new forage patches - Hardy-Weinberg equilibrium theorem states that in the absence of other influences (such as mutation and inbreeding), allele and genotype frequencies are in equilibrium and do not change over generations (Frankham *et al.*, 2002). The basis of this theory was used to determine the

distances over which the bees were able to colonise a newly created forage patch. DNA samples were taken from *B. humilis* and *B. sylvarum* workers on a forage patch created at the Cleanaway Pitsea Landfill site. Microsatellite analysis was performed on the samples and the resulting allele frequencies were compared to those at each of the surrounding sites sampled in the previous year to distinguish the subpopulation from which the bees had colonised. Distances of the surrounding sites from the trial plot varied between sites.

A population is defined as a set of individuals belonging to a single species and forming a functional demographic unit (Burel and Baudry, 2004). For the purpose of this study, the demographic unit was considered to be a distinct group of inter-breeding individuals. Darvill *et al.* (2006) have demonstrated that 10km is enough distance to genetically separate populations of the declining bumblebee species *B. muscorum*. In the absence of more accurate data for *B. humilis* and *B. sylvarum* and due to their ecological similarities to *B. muscorum*, assumptions of similar spatial dynamics were made for the South Essex populations. Based on these assumptions, none of the sites surveyed in this study should be genetically isolated and thus workers of bees sampled could be assumed to be operating within the same populations.

9.2 Methods

9.2.1 DNA sample collection and processing

2005 Sample collection

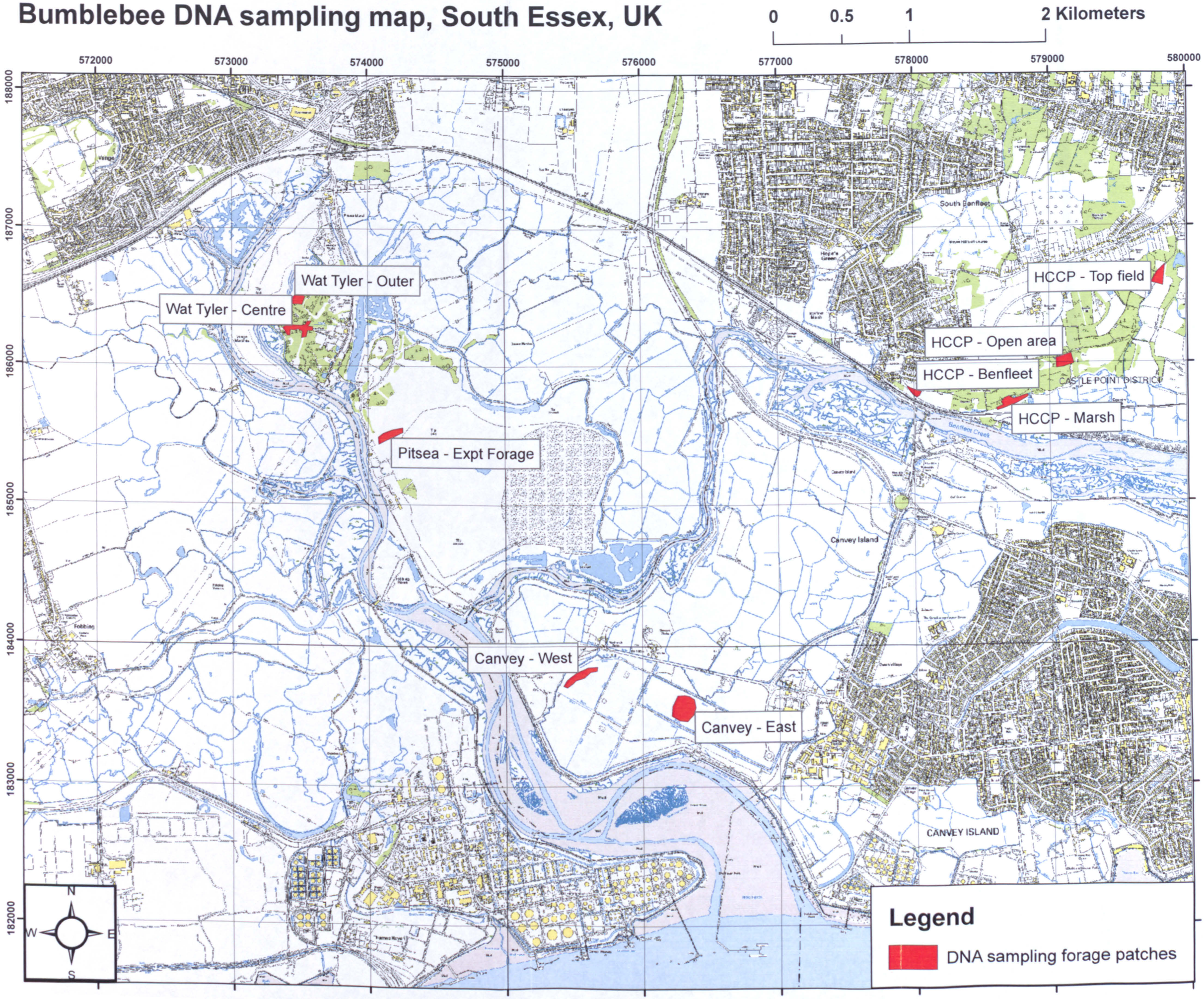
The main forage patches visited by *B. humilis* and *B. sylvarum* workers at Wat Tyler Country Park, Hadleigh Castle Country Park, and the Canvey Northwick site (see map Figure 2.1) were identified during bee walk surveys in 2003, 2004 and 2005 (chapter 3). The forage patches identified during these surveys were mapped (Figure 9.1). The patches were then surveyed regularly from August to September 2005. Any *B. humilis* and *B. sylvarum* workers observed foraging on these areas

were caught in Thorne Supplies (Thorne, 2007) queen bee marking plunger cages (Kwak, 1987). Due to the conservation status of the two species under investigation non-lethal DNA mid-tarsal clip sampling (sampling the terminal portion of the tarsus of a mid-leg) was carried out on the captured bees. The bees were released immediately afterwards. Sampling methodology followed that recommended by Holehouse *et al.* (2003) and samples were preserved in 100% ethanol straight after collection for later DNA extraction. This technique was used as it was considered to have the least impact on worker foraging ability of the DNA sampling techniques available at the time of the study (Holehouse *et al.*, 2003).

Thirty *B. humilis* workers were sampled from each of the following forage patches: Wat Tyler Country Park (centre and outer patches), Canvey Wick (west and east patches) and Hadleigh Castle Country Park (benfleet, marsh, open area and top field patches). Thirty workers from each patch was considered to be a sufficiently large sample size to demonstrate enough genetic variability for microsatellite analysis whilst being a realistic sample size from rare bumblebee populations (personal correspondence with Jon Ellis and Ben Darvill). Due to *B. sylvarum* workers being rarer than *B. humilis* on the South Essex sites, sufficient *B. sylvarum* workers were only observed on some of the forage patches. Thirty *B. sylvarum* workers were sampled from Wat Tyler (centre patch), Canvey Wick (west and east patches) and Hadleigh Castle Country Park (benfleet and marsh patches). For locations of these forage sites see sampling map (Figure 9.1).

Figure 9.1. Map of the South Essex DNA sampling sites. (overleaf) Sites are those from which thirty *B. sylvarum* and/or *B. humilis* mid-tarsal clips were taken during the 2005 and 2006 DNA sampling. [Pitsea Expt Forage = the trial plot at Pitsea Landfill site; Canvey = the Canvey Wick site; HCCP = Hadleigh Castle Country Park; Wat Tyler = Wat Tyler Country Park]

Bumblebee DNA sampling map, South Essex, UK



2006 sample collection

In 2006, 30 *B. humilis* and 30 *B. sylvarum* workers were sampled on the experimental forage plot created on the Cleanaway Pitsea Landfill site (see map Figure 9.1). The same sample sizes and techniques were used as in the 2005 sample collection.

Microsatellite genotyping

Prior to DNA extraction, tarsal segments were cut into smaller segments to improve the quality of the DNA obtained. DNA was extracted using the HotSHOT protocol (Truett *et al.*, 2000). The samples were genotyped at 11 of the microsatellite loci tested by Estoup *et al.* (1995 and 1996): B10, B96, B101, B116, B118, B119, B121, B124, B126, B131 and B132. To increase efficiency, reactions were multiplexed so that more than one microsatellite locus could be run at once. Polymerase chain reaction (PCR) conditions were optimised by experimenting with varying PCR reaction temperatures, times and reagent concentrations for each multiplex reaction. The PCR reactions were then run on a 40 well pre-cast 4% 3:1 NuSieve agarose gel in 1 x TBE buffer with Ethidium Bromide dye and electrophoresed at 180v. On completion the gels were photographed under ultraviolet light, and the fragment sizes determined using a 50bp marker (Promega, Southampton, UK). For an example of these gels see Figure 9.2.

PCRs were carried out on a RoboCycler® gradient 96 (Stratagene, La Jolla, CA). Reactions were performed using 25 µl reactions containing; 1.0 µl DNA, 0.2 µl HotStart Taq (Abgene Ltd, Epsom UK), 2.5 µl Thermostart buffer (containing 1.5 mM Mg), 10 µl primer mix [each primer 0.5 µM], 0.2 mM d'NTP and 15.8 µl DI. Reactions were multiplexed with up to 3 loci per reaction. Primers were fluorescently labelled with FAM and HEX (Eurogentec, Southampton, UK). The reaction cycle was: 95°C for 15 minutes; then 35 cycles with three steps 95°C for 30 seconds, 52-63°C for 45 seconds, and 72°C for 40 seconds, followed by an extension period of 72°C for 10 minutes. Optimised reaction conditions for each multiplex reaction are listed in Table 9.1.

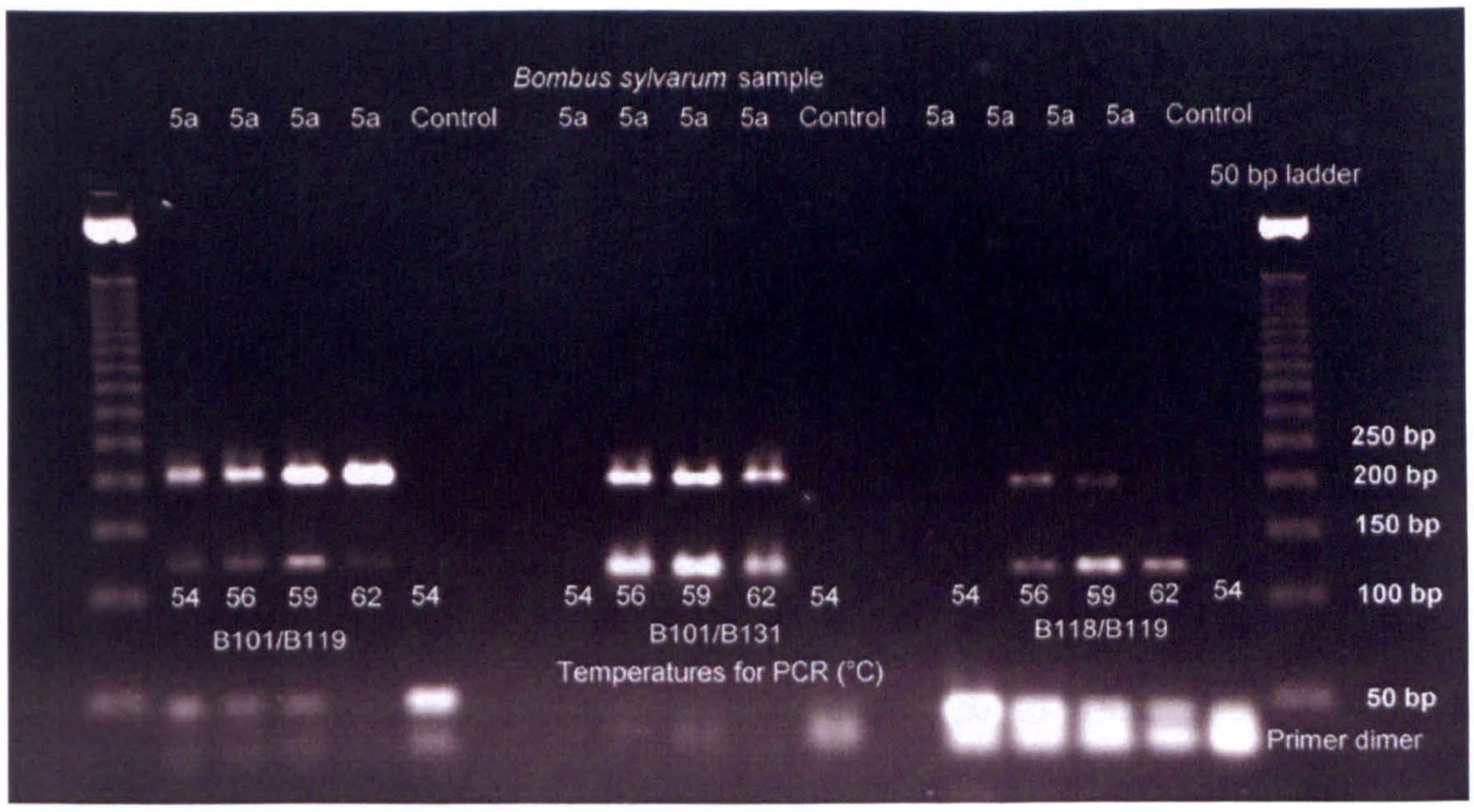


Figure 9.2. Gel of B101, B118, B119 and B131 microsatellite loci multiplexed PCR products of a *Bombus sylvarum* worker run against a 50bp standard under varied PCR temperatures. All PCRs on the gel were run for the same *B. sylvarum* individual (coded bee '5a') against a control with DI water.

Table 9.1. Table of optimised reaction conditions for PCR multiplexes. Includes multiplex combinations, approximate PCR product lengths, optimum temperature of annealing step for multiplex and the fluorophore tag used with each primer.

Primer combinations	PCR product base pairs	Optimal temperature	Fluorophore tag
B10	~170	56°C	HEX
B119	~130	56°C	FAM
B96	~250	56°C	FAM
B121	~160	56°C	HEX
B118	~200	56°C	FAM
B132	~150	56°C	HEX
B124	~250	59°C	HEX
B126	~140	59°C	FAM
B101	~220	58°C	HEX
B116	~180	58°C	FAM
B131	~130	58°C	HEX

PCR products were run on a BaseStation 51 DNA fragment analyser (Roach *et al.*, 2003) using BasePack-20 Polyacrylamide Mix gel packs and KBB buffer (MJ Research/Bio-Rad, Hemel Hempstead, UK). Base pair lengths were calibrated with internal size standards (ROX400, Applied Biosystems), and allele scores were assigned using Cartographer™ (version 1.0) software (MJ Research Inc., San Francisco, CA). Identical sample controls were used throughout for each species.

Any cases of scoring ambiguity or non-amplification (Murray *et al.*, 1993; Queller *et al.*, 1993; Magnuson *et al.*, 1996; Wang, 2004) were reprocessed until allele sizes could be confirmed. Across both species, 270 of 4050 genotypes were retyped following the same PCR conditions to assess an error rate for allele scoring.

9.2.2 Data analysis

Hardy-Weinberg and Linkage Disequilibrium

Tests for linkage disequilibrium (non-random association of alleles) and departure from Hardy-Weinberg equilibrium were carried out on subsamples of workers from each of the sites using GENEPOP version 3.1 (Raymond and Rousset, 1995). Twenty workers were randomly selected ten times from each site using a random number generator. This subsampling procedure was carried out to reduce comparisons of non-independent genotypes due to the presence of sisters in samples from the same site (Chapman *et al.*, 2003). Sequential Bonferroni corrections (Rice, 1989) were applied to minimise type I errors for multiple tests.

Estimating foraging range from kinship analysis

Microsatellite Analyser (MSA) was used to check datasets for typographical errors (Dieringer and Schlötterer, 2003). Polymorphic allele scores for each individual bee and allelic frequencies within a population can be used to calculate kinship (relationships between individual bees from the population). In this study the kinship analysis program Kinship 1.3.1 (Goodnight and Queller, 1999) was used for kinship calculations. In studies of monoandry and polyandry in European

bumblebees only *B. hypnorum* was shown to exhibit polyandry (Estoup *et al.*, 1995; Schmid-Hempel and Schmid-Hempel, 2000). For this study it was therefore assumed that *B. humilis* and *B. sylvarum* queens were once-mated and that, due to their haplodiploidy, the expected coefficient of relationship between sister bees was 0.75. Kinship used this correlation coefficient, with individual worker microsatellite scores and population allelic frequencies to calculate the likelihood of sibships, the null hypothesis being zero relatedness (Goodnight and Queller, 1999). Likelihood tests were performed between all sampled individuals of the population at the forage patch, site and entire South Essex population levels. The Kinship program calculates results at the p=0.05, 0.01 and 0.001 significance levels. In this study, the occurrence of sisters in each sample was calculated applying the $p \leq 0.01$ significance level.

Foraging ranges were estimated for each species by identifying sister pairs foraging at separate forage patches. The distance between forage patches was known from mapping data, so a theoretical mid-point nest could be calculated. Microsatellite techniques for analysing foraging range give no specific nest location, so a maximum distance the nest could have been located from the forage patches could not be estimated. This lack of specific location for the nest meant that the distance calculated from the hypothetical mid-point nest to forage patch was a minimum estimate of foraging distance for each species. In other words, a minimum distance that one of the two sister bees must have travelled for both to be present at the two different sites on which they were recorded. The largest distance recorded between two sister bees therefore gave a minimum estimate of maximum foraging distance. Relative frequencies of sister pairs within a forage patch and between forage patches was also calculated.

Estimating colony number

Estimates of the number of *B. humilis* and *B. sylvarum* colonies utilising each forage patch and in the population as a whole were carried out using the same methods as Darvill *et al.* (2004). Utilising the same methods meant that results were directly comparable between the studies. Kinship analysis was used to identify bumblebee colonies utilising each forage patch. The number of sister bees

from each identified colony varied between colonies. Some colonies had only 1 bee identified as coming from them whilst others had 2 bees, 3 bees, 4 bees and so on. Using the distribution of sister bees from each colony it was possible to estimate the number of colonies utilising each patch from which no workers were sampled. The distribution of foraging bumblebees was assumed to be random and the number of nests with no workers sampled was extrapolated after fitting the data to a truncated Poisson distribution using the program FITTING (Abramson and Gahlinger, 2001). Values were also calculated using the non-parametric ecological diversity measure Chao I (Chao, 1984) to assess whether values varied greatly between methodologies (Hill, *et al.*, 2003). Estimates were made per site and for the population as a whole.

Measures of genetic variation were also calculated for each site: allelic richness (number of alleles per locus) and heterozygosity (frequency of heterozygotes). Allelic richness was calculated using Fstat 2.9.3.2 (Goudet, 2001) and heterozygosity was calculated using MSA (Dieringer and Schlötterer, 2003).

Ability to colonise new forage patch

Bombus humilis and *B. sylvarum* workers sampled on the Pitsea Landfill trial plot were tested for linkage disequilibrium and departure from Hardy-Weinberg equilibrium using GENEPOP version 3.1 (Raymond and Rousset, 1995) using the same methodology as previously. Results were also checked with MSA for typographical errors (Dieringer and Schlötterer, 2003). Kinship analysis was carried out on the workers from the trial plot to assess sibships between the bees and estimates of colony number were calculated as previously.

Bombus humilis and *B. sylvarum* workers sampled on the newly created trial plot were used to identify from which South Essex site or sites the bees had colonised. These sites were located at varying distances from the trial plot (870m, 2220m and 3870m respectively). Due to time restraints on the project, it was not possible to sample all of the south Essex forage patches and the experimental forage plot in the same year. This meant that sibships comparisons using kinship analyses between all sites were not possible. Therefore, to identify which original forage

site(s) workers were colonising from, a factor analysis comparison of allelic frequencies of each subpopulation was carried out to look for correlations between the trial plot and one of the other forage sites (Hedrick *et al.*, 1997). Also, probability that the allele frequencies recorded at the trial plot came from each of the Hadleigh Castle Country Park, Canvey and Wat Tyler subpopulations was calculated for each microsatellite locus. Probabilities were calculated using a multinomial likelihood distribution (Evans *et al.*, 2000).

9.3 Results

Survivorship

Two or three weeks after DNA sampling, *B. humilis* and *B. sylvarum* workers with tarsal clips were re-observed foraging on the same forage patches.

Amplification of loci

Microsatellite locus B10 could not be amplified reliably and locus B119 was monomorphic for both *Bombus* species so both loci were removed from further analysis. Locus B101 was found to be monomorphic for *B. sylvarum.* This locus was therefore removed from further analysis for *B. sylvarum* but was retained for analysis of *B. humilis.* Error rates for other loci were calculated as 2.5% for *B. humilis* homozygotes and 3.1% for *B. humilis* heterozygotes and 2.2% for *B. sylvarum* homozygotes and 4.4% for *B. sylvarum* heterozygotes. These low error rates combined with the high rate of reprocessing of ambiguous allele scores meant that errors in scoring were unlikely to have biased results.

Hardy-Weinberg and Linkage Disequilibrium

Small genetically isolated populations are prone to inbreeding and genetic drift, (Frankham, 2005; Ellis *et al.*, 2007). Such populations are no longer considered to be mating randomly and therefore allelic frequencies would no longer be expected

to meet Hardy-Weinberg assumptions. Prior to this study it was not know whether the South Essex *B. humilis* and *B. sylvarum* populations would have sufficient genetic variability for Hardy-Weinberg assumptions to be met. If this were the case, Kinship analysis of the data would not be possible as it assumes no linkage disequilibrium, no inbreeding and no mutation (Queller and Goodnight, 1989). However, after correcting for multiple tests, no *B. humilis* locus deviated significantly from Hardy-Weinberg equilibrium and only one locus of *B. sylvarum* was found not to meet Hardy-Weinberg assumptions (B118). Analysis with MICROCHECKER (van Oosterhout *et al.*, 2004) revealed a lack of heterozygosity and evidence of null alleles for *B. sylvarum* at locus B118. There was also no evidence of significant linkage disequilibrium between loci in *B. humilis* or *B. sylvarum*.

The fact that all loci for *B. humilis* and all but one for *B. sylvarum* met Hardy-Weinberg assumptions indicated that the South Essex populations had not reduced to such an extent that the bees were no longer mating randomly. Prior to Kinship analysis locus B118 was removed from the *B. sylvarum* dataset but all other loci were included. This enabled analyses of foraging range and colony number to be carried out.

Estimating foraging range

In total 240 *B. humilis* and 150 *B. sylvarum* workers were genotyped from the 2005 sampling across Hadleigh Castle Country Park, Canvey Wick and Wat Tyler Country Park. Kinship analysis sorted sister bees into colonies at a $p \leq 0.01$ significance level. Circular nest were found at a low frequency for both species (i.e. incidences when bee X was found to be related to bee Y, bee Y was found to be related to bee Z, but bee Z was not found to be related to bee X). When circular nests were found, data was re-examined and results at the more stringent level of $p \leq 0.001$ were accepted as sisters. When no such relationship was found between the individuals, the most parsimonious method for dividing the sisters was taken (Knight *et al.*, 2005). For the purpose of this study comparisons at the forage patch, site and whole population were made to resolve these circular colony issues. If the issue was still not resolved, the bee collected from the furthest site was

omitted so that any bias caused to foraging range results would be in terms of underestimations of foraging distance. Use of this technique was very infrequent (1.7% of *B. humilis* colonies and 2.4% of *B. sylvarum*) and would therefore have had little effect on the results of the study. In the majority of cases circular nests were resolved by comparisons at different spatial scales.

Type II error rates generated from the Kinship program for these analyses were calculated as 0.101 for *B. humilis* sisters (n=240, 9 loci, type II error when $p \leq 0.01$) and 0.046 for *B. sylvarum* sisters (n=150, 8 loci, type II error when $p \leq 0.01$). In total 202 *B. humilis* and 135 *B. sylvarum* sister pairs were identified. At these error rates it would be expected that 20 *B. humilis* sister pairs and 6 *B. sylvarum* sister pairs would be falsely rejected. These frequencies of falsely rejected pairs were low and being randomly distributed throughout the study area, they would not be expected to impact the overall patterns of forage visitation in this study.

A list of all of the colonies identified at each patch is included in the appendix (section 12.6). A summary of the total number of colonies and the number of sister bees found in each colony are shown in Figure 9.3.

The forage patches sampled in this survey were located at a range of distances apart. A list of the distances calculated using ArcGIS 9.0 (ESRI, 2007) is recorded in Table 9.2.

For comparisons of foraging distances between *B. humilis* and *B. sylvarum* only forage patches at which DNA samples for 30 workers from both species were collected were included in the analyses. This removed any bias which may have been created by a greater sampling effort for *B. humilis* than *B. sylvarum*. An estimation of the maximum distance apart that *B. humilis* sisters were recorded was calculated using the data from all forage patches samples, but this was not compared to the data for *B. sylvarum* (Table 9.3).

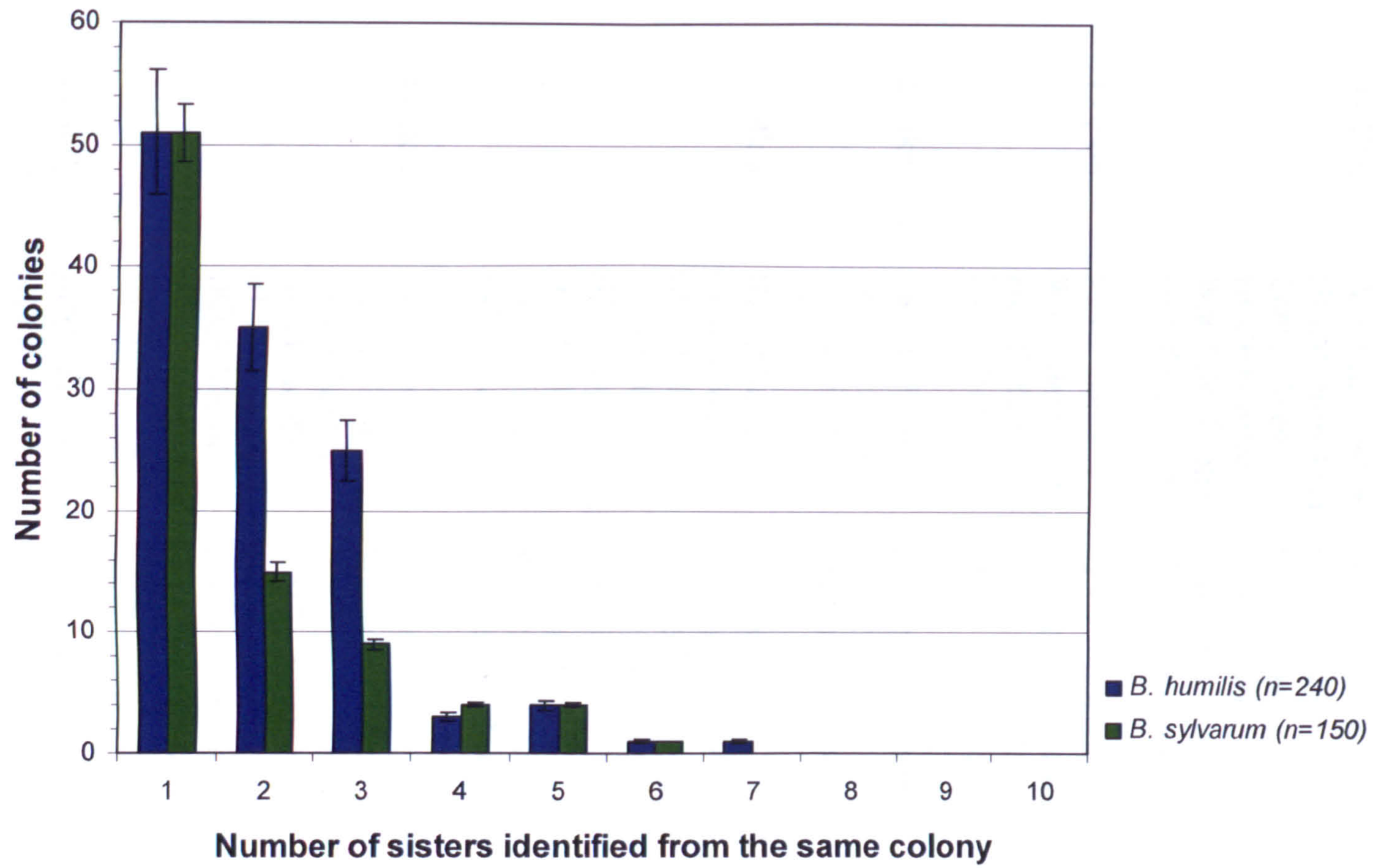


Figure 9.3. Summary of predicted colonies from Kinship analysis for *B. humilis* and *B. sylvarum* across all sites, 2005.
Sister bees calculated from kinship likelihood analysis ($p \leq 0.01$). DNA samples taken from a series of forage patches across Canvey Wick, Hadleigh Castle Country Park and Wat Tyler Country Park, South Essex. [Error bars represent the calculated type II error rates in kinship analysis, 10.1% for *B. humilis* and 4.6% for *B. sylvarum*]

Table 9.2. Distance between DNA sampling sites. Forage patches at which *B. humilis* and *B. sylvarum* DNA sampled were plotted using ArcGIS 9.0 (ESRI, 2007) and distances between plots were calculated.

Distance group	Sample site 1	Sample site 2	Distance between patches (m)
0 - 1km	Wat Tyler Centre	Wat Tyler Outer	170
	HCCP Open Area	HCCP Marsh	380
	Canvey West	Canvey East	690
	HCCP Benfleet	HCCP Marsh	530
	Wat Tyler Centre	Expt patch (Pitsea)	890
	HCCP Open Area	HCCP Top Field	700
1 - 2km	HCCP Benfleet	HCCP Open Area	1100
	Wat Tyler Outer	Expt. patch (Pitsea)	1130
	HCCP Marsh	HCCP Top Field	1220
	HCCP Benfleet	HCCP Top Field	1860
2 - 3km	Canvey West	Expt. patch (Pitsea)	2200
	HCCP Benfleet	Canvey East	2810
	Canvey East	Expt. patch (Pitsea)	2850
3 - 4km	HCCP Benfleet	Canvey West	3130
	Canvey West	Wat Tyler Centre	3150
	HCCP Marsh	Canvey East	3030
	Canvey West	Wat Tyler Outer	3380
	HCCP Open Area	Canvey East	3630
	HCCP Marsh	Canvey West	3490
	Canvey East	Wat Tyler Centre	3800
	HCCP Benfleet	Expt. patch (Pitsea)	3870
4 - 5km	HCCP Open Area	Canvey West	4120
	Canvey East	Wat Tyler Outer	4010
	HCCP Benfleet	Wat Tyler Centre	4560
	HCCP Benfleet	Wat Tyler Outer	4630
	HCCP Top Field	Canvey East	4480
	HCCP Marsh	Expt. patch (Pitsea)	4380
	HCCP Open Area	Expt. patch (Pitsea)	4950
	HCCP Top Field	Canvey West	4950
5 - 6km	HCCP Marsh	Wat Tyler Centre	5060
	HCCP Marsh	Wat Tyler Outer	5140
	HCCP Open Area	Wat Tyler Centre	5580
	HCCP Open Area	Wat Tyler Outer	5660
	HCCP Top Field	Expt. patch (Pitsea)	5640
6 - 7km	HCCP Top Field	Wat Tyler Centre	6200
	HCCP Top Field	Wat Tyler Outer	6240

Sites where DNA was sampled were grouped by their relative distances apart for subsequent frequency analysis:

1 – same forage patch
2 – sites between 0 and 1km apart
3 – sites between 2 and 3km apart
4 – sites between 3 and 4km apart
5 – sites between 4 and 5km apart
6 – sites between 5 and 6km apart

These categories were selected to correspond with sampling distances and forage distance results of other microsatellite studies (Chapman *et al.*, 2003; Darvill *et al.*, 2004; Knight *et al.*, 2005; Darvill *et al.*, 2006). Only *B. humilis* were collected from sites between 1 and 2km apart and between 6 and 7km apart, so these were not included in relative distance comparisons.

A comparison of the frequencies of *B. humilis* and *B. sylvarum* sister pairs identified at each relative distance are shown in Figure 9.4. The majority of *B. sylvarum* sister pairs were recorded at the same forage patch (relative distance 1). *B. humilis* sister pairs were more evenly distributed across the relative distances. These results demonstrated divergence between the distances that *B. humilis* and *B. sylvarum* workers were found apart.

Calculation of mean distance apart that *B. humilis* and *B. sylvarum* workers were sampled also demonstrated a large difference between the two species, 464m (±47m S.E.) for *B. sylvarum* sisters and 1512m (±86m S.E.) for *B. humilis* sisters (Table 9.3). A Mann-Whitney U exact test of independent samples revealed a significant difference between the average relative distances apart that *B. humilis* and *B. sylvarum* sisters were recorded ($p < 0.001$). This indicated that *B. humilis* sisters were more likely to be found foraging at greater distances apart than *B. sylvarum* sisters. A summary of all of forage distance data is shown in Table 9.3.

Table 9.3. Summary table of distance data. Comparison of the mean distances apart that sister bees were recorded whilst foraging. For comparison data, only forage patches at which both *B. humilis* and *B. sylvarum* DNA was sampled were included. Values were also calculated for *B. humilis* for all patches (including patches on which *B. sylvarum* were not sampled). Bee sister pairs were identified using kinship likelihood analysis ($p \leq 0.01$). Average distances are given ± S.E. * A Mann-Whitney U exact test on independent samples was used to compare the distances apart that *B. humilis* and *B. sylvarum* sisters were recorded.

	B. humilis (metres)	*B. sylvarum* (metres)
Average distance of sister pairs (patches both species sampled)	1512 ± 86	464 ± 47
Average distance of sister pairs (all patches)	2138 ± 74	N.A.
Maximum distance apart sister bees recorded (patches both species sampled)	5140	3030
Maximum distance apart *B. humilis* sisters recorded (all patches)	6240	N.A.
* Difference between relative distances of bumblebee sisters	$p < 0.001$	$p < 0.001$

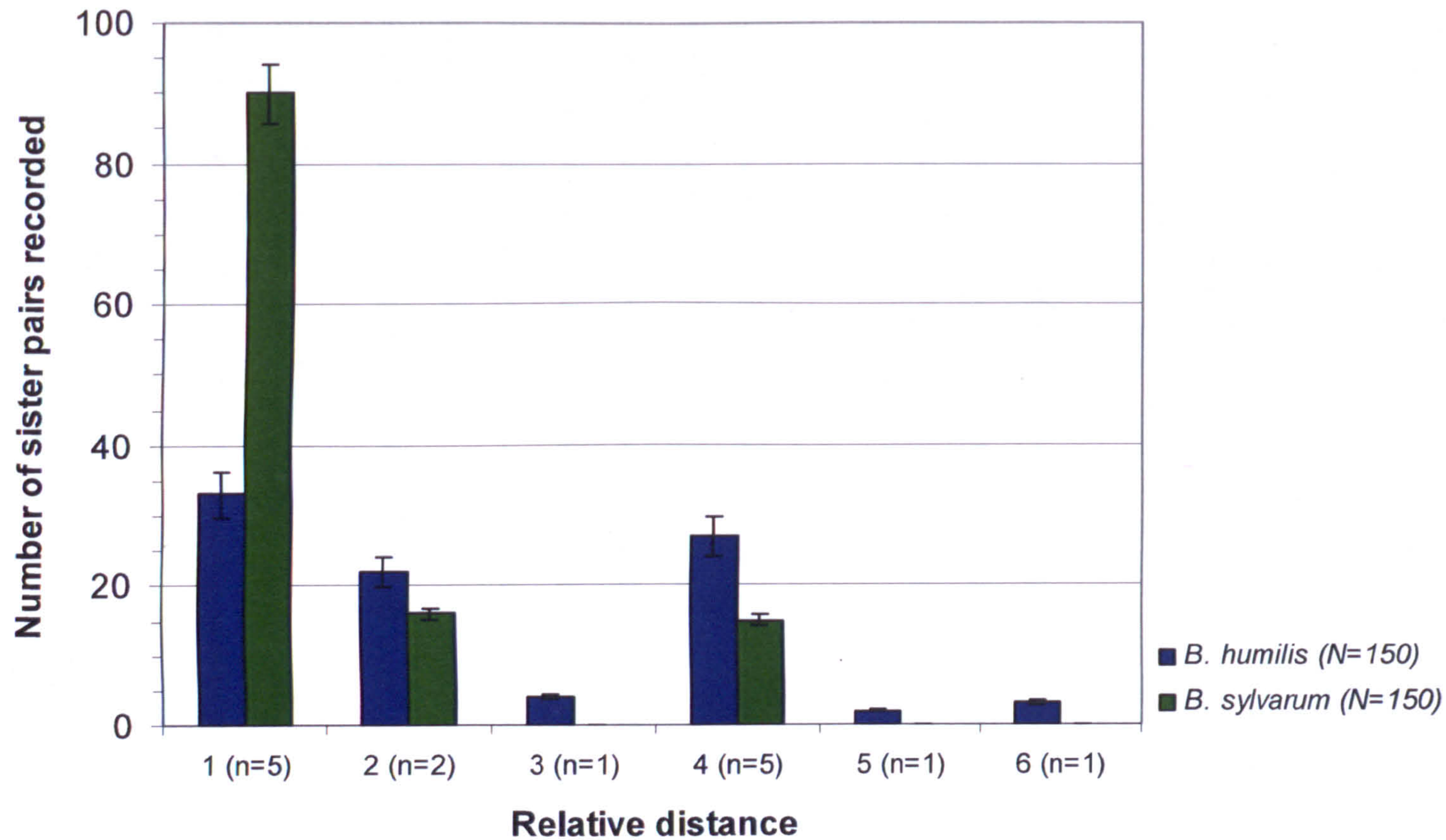


Figure 9.4. Frequency of *B. humilis* and *B. sylvarum* sister pairs across all patch distances. Relative distances represent: 1 - same forage patch, 2 - sites between 0 and 1km apart, 3 - sites between 2 and 3km apart, 4 - sites between 3 and 4km apart, 5 - sites between 4 and 5km apart and 6 - sites between 5 and 6km apart. N= number of bees sampled. n= number of pairs of sites at each relative distance (or number of patches for category 1, same forage patch. [Error bars represent the calculated type II error rates in kinship analysis, 10.1% for *B. humilis* and 4.6% for *B. sylvarum*]

Estimating colony number

Estimates of the number of nests not sampled at each forage patch from the program FITTING calculations are shown in Tables 9.4 and 9.5. The frequency distributions of all but two of the nest ratios detected (1 bee, 2 bees, 3 bees, etc) conformed to Poisson distributions (p>0.05) (Tables 9.4 and 9.5). Nest ratios for *B. humilis* HCCP marsh and *B. sylvarum* were less than p=0.05 (p=0.04 and 0.01 respectively) but were not significant after a Bonferroni correction (Rice, 1989) for multiple test was applied to the data (p=0.007). Estimates for these two nest ratios were therefore still made using Poisson distributions.

A comparison of the total colony estimates for *B. humilis* and *B. sylvarum* on patches where both species were sampled is represented in Figure 9.5. Estimates of colony number were higher for *B. humilis* at all patches except the Pitsea landfill trial plot.

An analysis of the distribution of sister bees from the same colony was made at the patch and site spatial scales. Results of this are recorded in Figures 9.6. and 9.7 respectively. *B. sylvarum* sisters were more frequently recorded at the same patch and site than at different patches or sites. The opposite was true for *B. humilis* sisters at the patch scale with sisters being more frequently recorded at different patches. At the site scale *B. humilis* sisters were found at different sites approximately as frequently as at the same site.

A table of the mean number of patches that sister bees from the same colony were recorded on for colonies with 2 bees, 3 bees and so on was compiled for *B. humilis* and *B. sylvarum* (Tables 9.6 and 9.7). Results indicated that for all numbers of sister bees from the same colony the mean number of patches and sites the sisters were recorded on was greater for *B. humilis* than *B. sylvarum*. Whilst sample sizes for colonies with 4, 5 and 6 sister bees from the same colony were too small for meaningful comparison, a Mann-Whitney U exact test of independent samples for colonies with two sisters confirmed this difference. Results showed a significant difference between *B. humilis* and *B. sylvarum* workers in terms of the number of patches and sites on which two sisters from the same colony would be expected to

be found (p=0.026 and p=0.017 respectively). For 3 sisters, results of the Mann-Whitney U exact tests were low for patch scale analysis (p=0.094) but not for site scale (p=0.489). Neither spatial scale (number of patches or sites) was significant at the $p \leq 0.05$ significance level, despite the mean number of patches and sites of *B. sylvarum* being substantially lower.

Summary tables of genetic diversity were produced for the *B. humilis* and *B. sylvarum* workers sampled at a forage patch, site and entire South Essex population scale. Results are displayed in Tables 9.8 and 9.9. This data indicated that *B. sylvarum* workers demonstrated greater heterozygosity and allelic richness than *B. humilis* workers, despite having a smaller population size.

Table 9.4. Estimates of the number of *B. humilis* nests not sampled at each forage patch. Values for the number of sister bees from the same colony represent the number of sister bees identified by kinship analysis (Goodnight and Queller, 1999); total colonies recorded is the sum of these values; Poisson estimates (FITTING) and Chao I are estimates of the number of colonies that were not sampled at each forage patch; Chi sq, d.f. and *p*-values are measurements of the goodness of fit of the Poisson distribution.

Patch	Number of bees from the same colony							Total colonies recorded	Poisson estimate for zero	Chao I	Chi sq	d.f.	$p =$
	1 Bee	2 Bees	3 Bees	4 Bees	5 Bees	6 Bees	7 Bees						
HCCP Benfleet	22	4	0	0	0	0	0	26	69	87	0.47	1	0.49
HCCP Open	22	4	0	0	0	0	0	26	69	87	0.47	1	0.49
HCCP Marsh	20	1	1	0	1	0	0	23	31	223	4.38	1	0.04
HCCP Top Field	14	3	2	1	0	0	0	20	14	53	1.76	1	0.18
Canvey West	20	5	0	0	0	0	0	25	48	65	0.80	1	0.37
Canvey East	22	4	0	0	0	0	0	26	69	87	0.47	1	0.49
Wat Centre	19	4	1	0	0	0	0	24	39	69	0.10	1	0.75
Wat Outer	26	2	0	0	0	0	0	28	178	197	0.10	1	0.75
Experimental patch	16	5	0	1	0	0	0	22	27	48	0.02	1	0.88
All Sites	51	35	25	3	4	1	1	120	31	157	0.48	1	0.49

Table 9.5. Estimates of the number of *B. sylvarum* nests not sampled at each forage patch. Values for the number of sister bees from the same colony represent the number of sister bees identified by kinship analysis (Goodnight and Queller, 1999); total colonies recorded is the sum of these values; Poisson estimates (FITTING) and Chao I are estimates of the number of colonies that were not sampled at each forage patch; Chi sq, d.f. and *p*-values are measurements of the goodness of fit of the Poisson distribution.

Patch	Number of bees from the same colony							Total colonies recorded	Poisson estimate for zero	Chao I estimate for zero	Chi sq	d.f.	*p* =
	1 Bee	2 Bees	3 Bees	4 Bees	5 Bees	6 Bees	7 Bees						
HCCP Benfleet	13	4	0	1	1	0	0	19	16	40	0.30	1	0.59
HCCP Marsh	10	3	1	0	1	1	0	16	8	33	1.02	1	0.31
Canvey West	12	5	1	0	1	0	0	19	14	33	0.01	1	0.91
Canvey East	16	4	2	0	0	0	0	22	22	77	0.54	1	0.46
Wat Tyler	12	6	3	0	0	0	0	21	11	32	0.01	1	0.91
Experimental patch	20	5	0	0	0	0	0	25	47	80	0.80	1	0.37
All sites	51	15	9	4	4	1	0	84	37	171	6.82	1	0.01

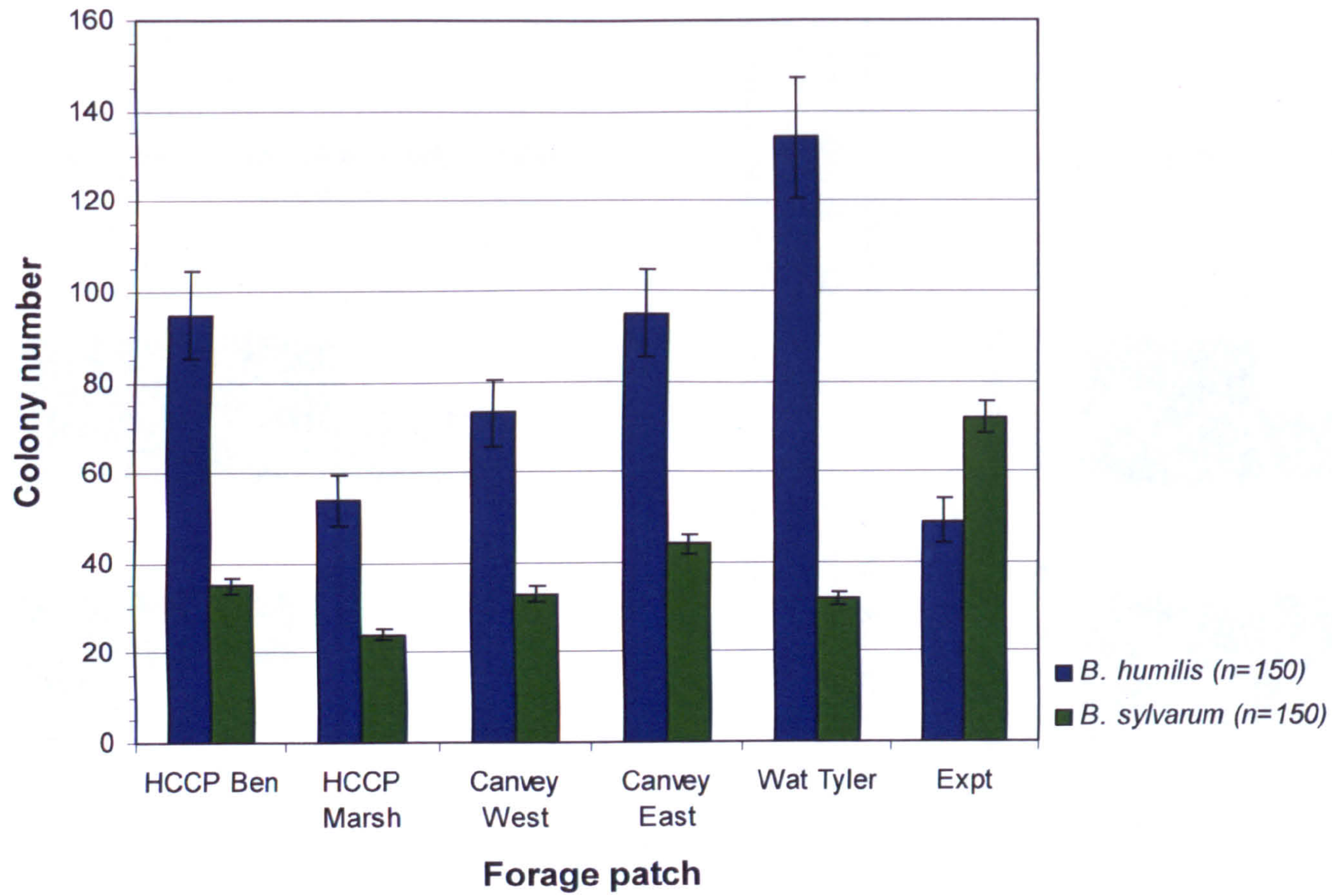


Figure 9.5. Total estimated colony number at each forage patch for *B. humilis* and *B. sylvarum* in South Essex. Sister bees calculated from kinship likelihood analysis ($p \leq 0.01$). Totals calculated by combining the number of colonies identified with an estimate of those not sampled at each site calculated by Poisson distribution (FITTING). [Error bars represent the type II error rates from kinship analysis, 10.1% for *B. humilis* and 4.6% for *B. sylvarum*]

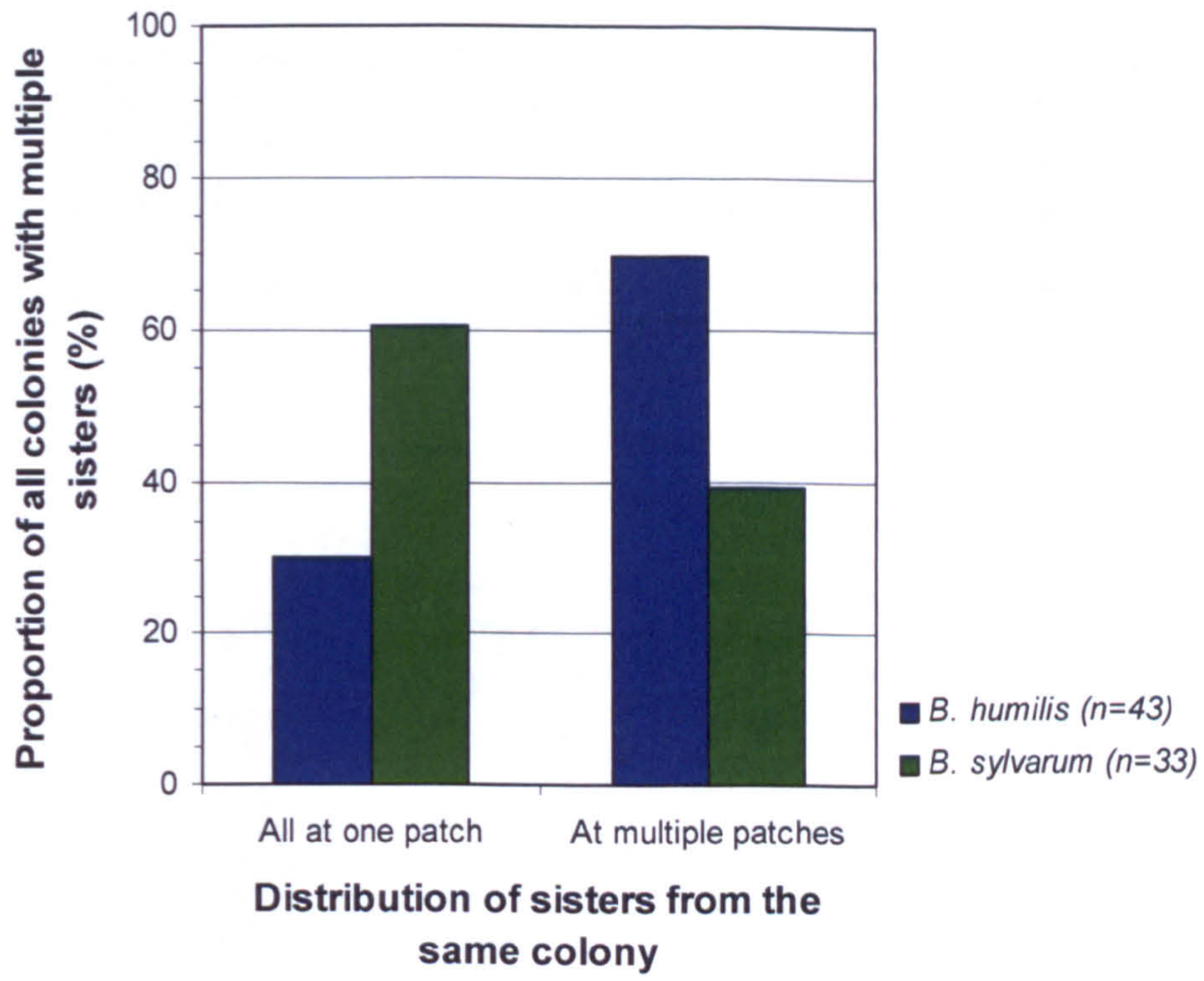


Figure 9.6. Distribution of bees from the same colony at the patch spatial scale. Sister bees calculated from kinship likelihood analysis ($p \leq 0.01$). Number of colonies (n) calculated from the same number of *B. humilis* and *B. sylvarum* workers sampled from the same forage patches.

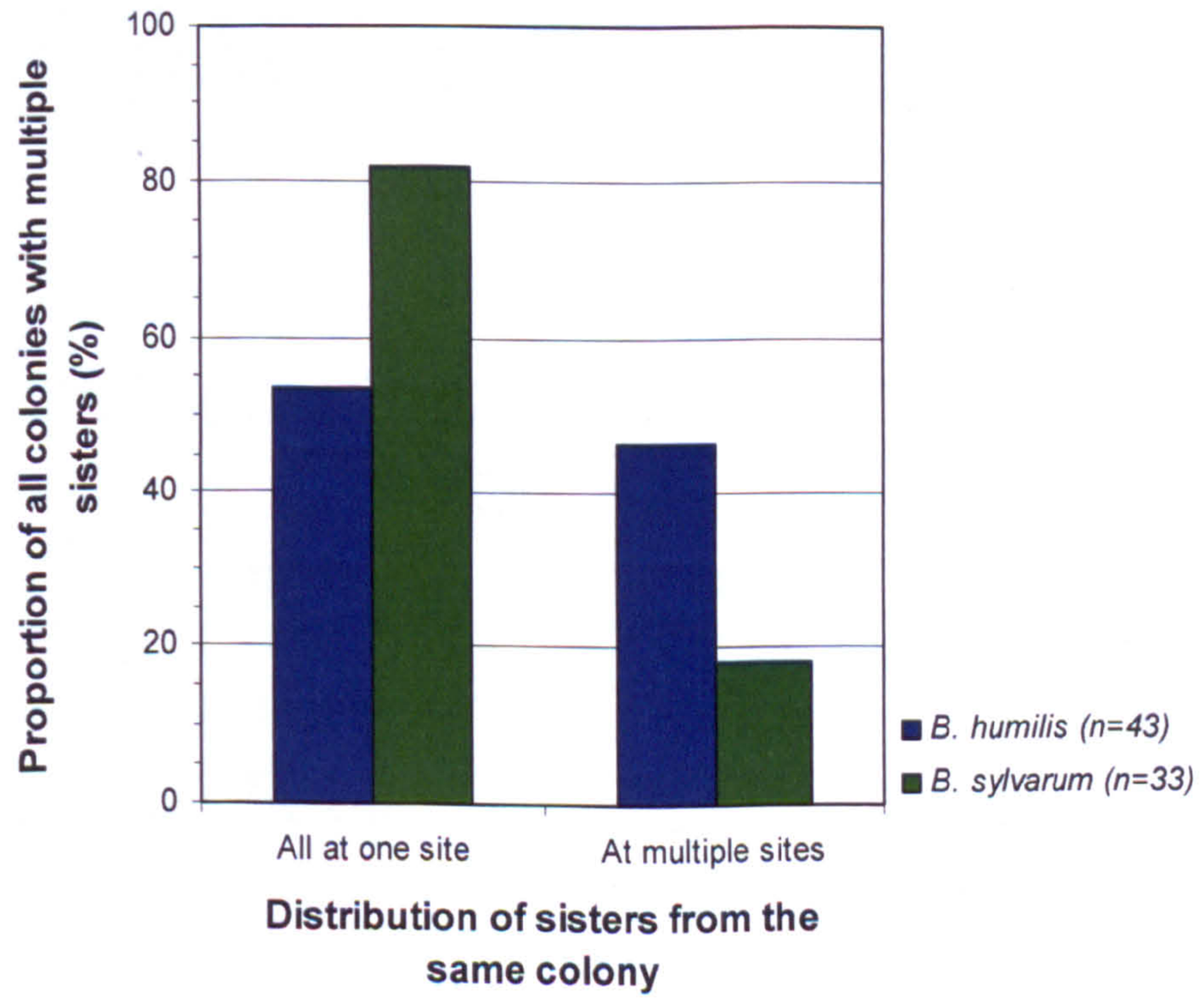


Figure 9.7. Distribution of bees from the same colony at the site spatial scale. Sister bees calculated from kinship likelihood analysis ($p \leq 0.01$). Number of colonies (n) calculated from the same number of *B. humilis* and *B. sylvarum* workers sampled from the same forage patches.

Table 9.6. Mean number of patches on which sister bees from the same colony were found for *B. humilis* and *B. sylvarum* workers. Sister bees calculated from kinship likelihood analysis ($p \leq 0.01$). Colonies calculated from the same number of *B. humilis* and *B. sylvarum* workers sampled from the same forage patches.

No. of sisters identified from the same colony	Mean number of patches sisters sampled on				Mann-Whitney U exact test
	B. humilis	Sample size	*B. sylvarum*	Sample size	*p*-value
2	1.58	31	1.20	15	0.026
3	2.22	9	1.67	9	0.094
4	3.00	2	1.75	4	N/A
5	N/A	0	1.25	4	N/A
6	2.00	1	1.00	1	N/A

Table 9.7. Mean number of sites on which sister bees from the same colony were found for *B. humilis* and *B. sylvarum* workers. Sister bees calculated from kinship likelihood analysis ($p \leq 0.01$). Colonies calculated from the same number of *B. humilis* and *B. sylvarum* workers sampled from the same forage patches.

No. of sisters identified from the same colony	Mean number of patches sisters sampled on				Mann-Whitney U exact test
	B. humilis	Sample size	*B. sylvarum*	Sample size	*p*-value
2	1.45	31	1.07	15	0.017
3	1.78	9	1.44	9	0.489
4	1.50	2	1.00	4	N/A
5	N/A	0	1.25	4	N/A
6	1.00	1	1.00	1	N/A

Table 9.8. Measures of genetic diversity for the South Essex populations of *B. humilis* for nine microsatellite loci. Allelic richness and H_E were calculated using all loci. Population size was calculated by combining the number of colonies identified with an estimate of those not sampled at each site calculated by Poisson distribution (FITTING). * 'One sister from each colony' calculated to avoid any bias caused by the high proportion of related individuals in the samples. [n = sample size, ns = sample size of workers after sister pairs removed, H_E = expected heterozygosity, N.A. = no available data]

Sample site	n	ns	H_E (± S.E.)	Allelic Richness (± S.E.)	Population size (± S.E.)
HCCP Benfleet	30	25	0.43 ± 0.04	N.A.	95
HCCP Open	30	19	0.46 ± 0.03	N.A.	95
HCCP Marsh	30	17	0.47 ± 0.04	N.A.	54
HCCP Top	30	9	0.46 ± 0.03	N.A.	34
HCCP site (average)	120	70	0.46 ± 0.02	4.49 ± 0.45	69.5 ± 7.64
Canvey West	30	13	0.44 ± 0.03	N.A.	73
Canvey East	30	9	0.47 ± 0.03	N.A.	95
Canvey site (average)	60	22	0.45 ± 0.01	3.15 ± 0.26	84 ± 5.50
Wat Tyler Outer	30	12	0.47 ± 0.03	N.A.	63
Wat Tyler Centre	30	16	0.43 ± 0.03	N.A.	206
Wat Tyler site (average)	60	28	0.45 ± 0.03	3.91 ± 0.42	134 ± 35.75
Experimental patch	30	11	0.43 ± 0.03	3.00 ± 0.33	49
Whole population	270	N.A.	0.46 ± 0.03	4.12 ± 0.37	N.A.
Whole population except experimental patch	240	120	0.46 ± 0.03	N.A.	151
* One sister from each colony	N.A.	131	0.48 ± 0.03	3.55 ± 0.28	N.A.

Table 9.9. Measures of genetic diversity for the South Essex populations of *B. sylvarum* for eight microsatellite loci. Allelic richness and H_E were calculated using all loci except B101 and B118. Population size was calculated by combining the number of colonies identified with an estimate of those not sampled at each site calculated by Poisson distribution (FITTING). * 'One sister from each colony' calculated to avoid any bias caused by the high proportion of related individuals in the samples. [n = sample size, ns = sample size of workers after sister pairs removed, H_E = expected heterozygosity, N.A. = no available data]

Sample site	n	ns	H_E (± S.E.)	Allelic Richness (± S.E.)	Population size (± S.E.)
HCCP Benfleet	30	18	0.52 ± 0.05	N.A.	35
HCCP Marsh	30	12	0.54 ± 0.05	N.A.	24
HCCP site (average)	60	30	0.54 ± 0.05	5.45 ± 1.49	29.5 ± 2.75
Canvey West	30	18	0.47 ± 0.07	N.A.	33
Canvey East	30	19	0.53 ± 0.06	N.A.	44
Canvey site (average)	60	37	0.51 ± 0.06	4.98 ± 1.40	38.5 ± 2.75
Wat Tyler site	30	17	0.45 ± 0.06	4.29 ± 1.19	32
Experimental patch	30	20	0.48 ± 0.06	4.86 ± 1.37	72
Whole population	180	N.A.	0.52 ± 0.06	5.57 ± 1.57	N.A.
Whole population except experimental patch	150	84	0.52 ± 0.06	N.A.	121
* One sister from each colony	N.A.	104	0.52 ± 0.06	5.14 ± 1.38	N.A.

Ability to colonise new forage patch

Factor analysis comparing allelic frequencies for each allele for *B. humilis* and *B. sylvarum* at the four sampled sites; Hadleigh Castle Country Park, Canvey Wick, Wat Tyler and the trial forage patch on Pitsea Landfill was carried out using SPSS version 10.1 (SPSS Inc, Chicago, Illinois, USA). This method was used to identify whether the allelic frequencies of the bees sampled at the trial forage patch were more similar to the nearest site than the other more distant sites. The surrounding sites were located at varying distances away from the trial plot (see map, Figure 9.1). Therefore, if frequencies from the nearest forage site were more closely correlated with those at the trial plot than the other forage sites, it could be concluded that the ability of *B. humilis* or *B. sylvarum* to colonise new forage patches was limited by such distances.

Any allele which was only found on one of the four sample sites could not be used for factor analysis and was therefore removed from the study. Such alleles could not provide information on the source subpopulation of the bees and were only found at low frequencies so their removal from the analysis did not affect the results. For both *B. humilis* and *B. sylvarum* 100% of the variability of allelic frequency was summarised in 3 component factors. Each of the 3 components was relatively equal for both *B. humilis* and *B. sylvarum* (48%, 27% and 24% for *B. humilis* and 45%, 35% and 20% for *B. sylvarum*). Graphical representations of the 3 component factors from the analyses are presented in Figures 9.8 and 9.9. These Figures demonstrated that none of the allelic frequencies of the surrounding sites appeared to be correlated with the trial plot on Pitsea Landfill.

Multinomial function probabilities calculated for each microsatellite locus at each site to assess the probability that the allelic frequencies at the trial plot came from each of the subpopulations (Hadleigh Castle Country Park, Canvey and Wat Tyler) are represented in Tables 9.10 and 9.11. A lower value indicated higher likelihood that the allele frequencies of each locus at the trial plot could have come from each surrounding site. Results demonstrated a pattern of increasing likelihood with proximity for *B. humilis* at locus B101 and for *B. sylvarum* at locus B116 and B126, but values could not be calculated for the majority of loci.

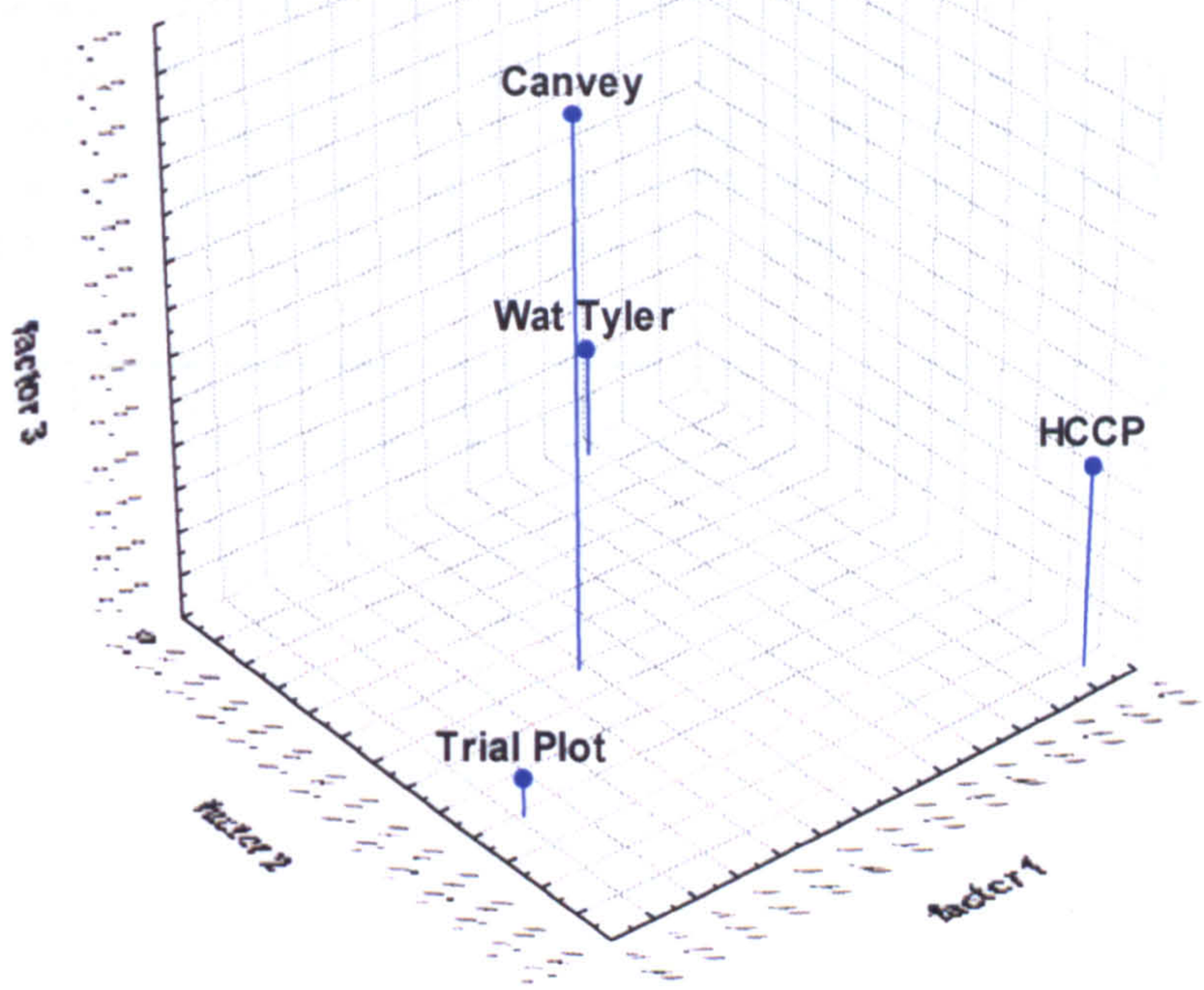


Figure 9.8. Factor analysis of allelic variability between nine *B. humilis* microsatellite loci at three semi-natural forage sites and the Pitsea Landfill trial plot. The 3 component factors summarised 100% of the variability between allelic frequencies (48%, 27% and 24% respectively). HCCP – Hadleigh Castle Country Park.

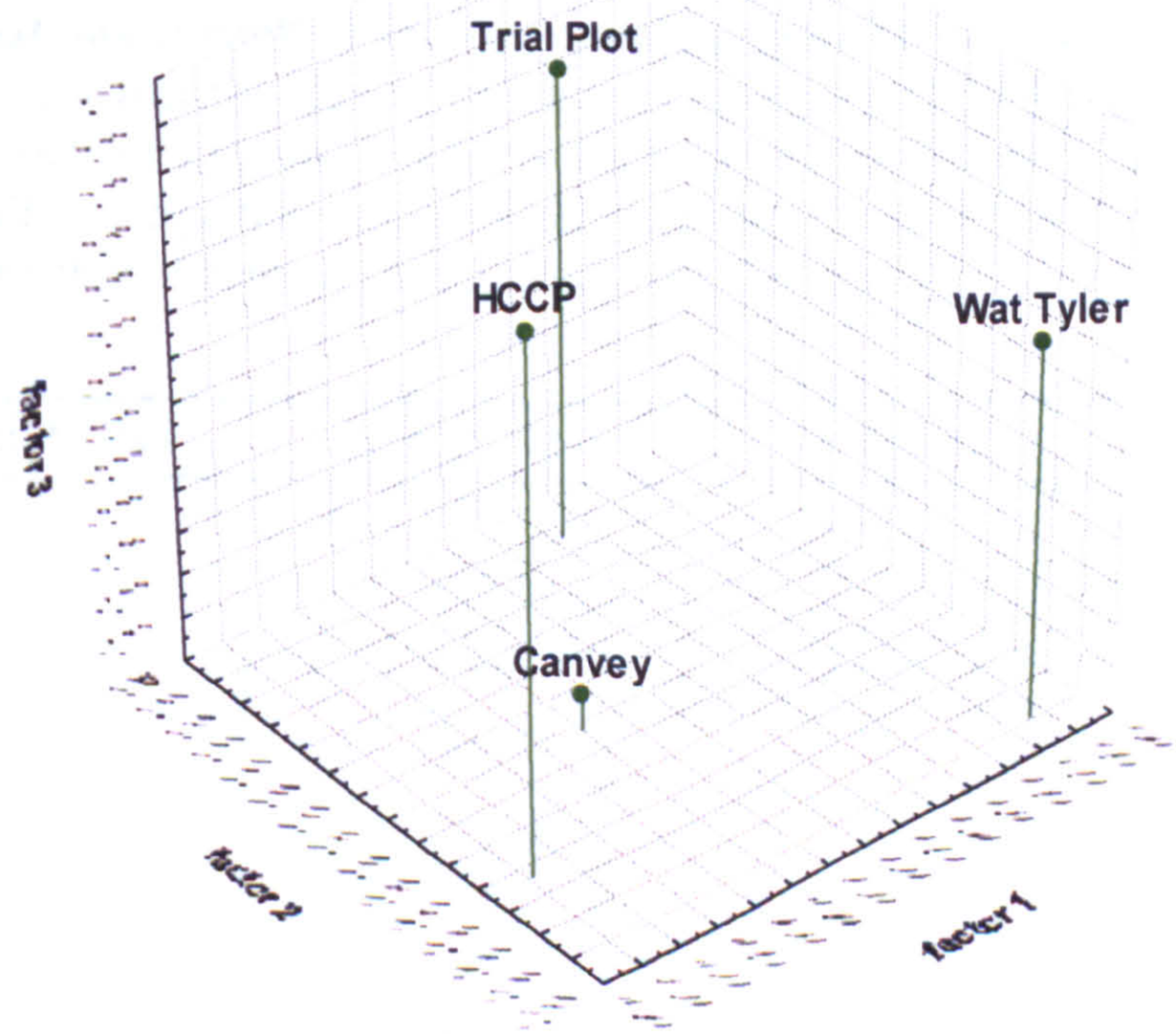


Figure 9.9. Factor analysis of allelic variability between nine *B. sylvarum* microsatellite loci at three semi-natural forage sites and the Pitsea Landfill trial plot. The 3 component factors summarised 100% of the variability between allelic frequencies (45%, 35% and 20% respectively). HCCP – Hadleigh Castle Country Park.

Table 9.10. *Bombus humilis* multinomial likelihood calculations comparing the probability that allele frequencies at the trial plot could have come from subpopulations at each of the surrounding sites. The lower the value the higher the probability that the allele frequency at the trial plot could have come from the particular surrounding site. HCCP – Hadleigh Castle Country Park. [∞ – if locus featured allele frequency values of zero at either the trial patch or colonising site a likelihood value of zero was calculated. –ln of zero is ∞]

Microsatellite loci	HCCP (-ln)	Canvey (-ln)	Wat Tyler (-ln)
B124	∞	∞	∞
B126	∞	∞	∞
B118	∞	∞	∞
B132	∞	∞	∞
B96	7.95	∞	∞
B121	10.84	∞	∞
B101	4.20	2.24	1.90
B116	6.17	∞	4.53
B131	7.11	∞	∞

Table 9.11. *Bombus sylvarum* multinomial likelihood calculations comparing the probability that allele frequencies at the trial plot could have come from subpopulations at each of the surrounding sites. The lower the value the higher the probability that the allele frequency at the trial plot could have come from the particular surrounding site. HCCP – Hadleigh Castle Country Park. [∞ – if locus featured allele frequency values of zero at either the trial patch or colonising site a likelihood value of zero was calculated. –ln of zero is ∞]

Microsatellite loci	HCCP (-ln)	Canvey (-ln)	Wat Tyler (-ln)
B124	5.86	∞	∞
B126	9.07	1.47	1.47
B118	70.44	13.09	37.20
B132	∞	∞	∞
B96	∞	∞	∞
B121	∞	∞	∞
B101	0	0	0
B116	18.73	9.01	1.60
B131	7.46	12.12	∞

9.4 Discussion

Following DNA sampling, mid-tarsal clipped *B. humilis* and *B. sylvarum* workers were re-observed foraging on the same forage patches 2 or 3 weeks after the original sampling. These observations were consistent with results from the Holehouse *et al.* (2003) study on survivorship which indicated that mid-tarsal clips did not significantly affect survivorship of sampled workers.

Microsatellite DNA analysis proved to be an effective technique for identifying relationships between *B. humilis* and *B. sylvarum* workers. Despite both species being nationally rare and the South Essex population being genetically isolated from other UK populations (Ellis *et al.*, 2007) there was sufficient genetic variability within the populations to identify sibships between the bees using kinship analysis (Goodnight and Queller, 1999).

Estimating foraging range

Mean minimum foraging ranges and maximum distances recorded between sisters varied considerably between *B. sylvarum* and *B. humilis* workers. As the location of nests was unknown in this study, an estimate of foraging range was made by halving the distance between the two identified sister bees. This provided an estimate of the minimum distance one of the sister bees must have travelled for the sisters to have been sampled at the separate forage patches Results for mean minimum foraging distance for *B. humilis* and *B. sylvarum* were 756m and 232m respectively. This difference between species was demonstrated as being significantly different (Table 9.3). When sites that *B. sylvarum* sisters were not sampled from were included in this analysis, results for *B. humilis* were 1176m. Standard errors of the mean distance that sister bees were recorded apart were relatively small (±86m for *B. humilis* and ±47m for *B. sylvarum*) indicating that the difference between the means of the two *Bombus* species was relatively accurate.

Estimated maximum foraging distances recorded for each species were calculated using the same method. Results for *B. humilis* and *B. sylvarum* were ≥2570m and ≥1515m respectively. When sites were included in the study where only *B. humilis* DNA samples were taken because *B. sylvarum* workers were too few in number, the value for *B. humilis* became ≥3120m.

These estimates of foraging range compared well with those estimated for the more ubiquitous species investigated in the Chapman *et al.* (2003), Darvill *et al.* (2004) and Knight *et al.* (2005) studies (Table 9.12). The *B. sylvarum* mean foraging range of 232m was the lowest mean foraging range compared to those recorded in the Chapman *et al.* (2003) study, whilst 756-1176m for *B. humilis* was similar to results for *B. pascuorum* and *B. terrestris*. Maximum foraging distances recorded in the South Essex study also compared well with those calculated in Chapman *et al.* (2003). Chapman *et al.* calculated maximum forage distances for *B. terrestris* and *B. pascuorum* as 3900m for 3200m, corresponding to the ≥3120m maximum recorded for South Essex *B. humilis* sisters. By comparison, *B. sylvarum* sisters were only recorded at a maximum distance of 3030m with sisters therefore travelling ≥1515m. No *B. sylvarum* sisters were recorded at patches further apart than this indicating that their maximum foraging distance was substantially less than that of the more nationally ubiquitous species.

Maximum estimates of foraging distance of both *Bombus* species in this study were substantially larger than those recorded by Knight *et al.* (2005) and Darvill *et al.* (2004) for *B. terrestris*, *B. pascuorum*, *B. lapidarius* and *B. pratorum*. Darvill *et al.* and Knight *et al.* investigated bumblebee movements at fixed points within areas of continuous suitable habitat whereas Chapman *et al.* (2003), similarly to the South Essex study, investigated bumblebee foraging behaviour in fragmented habitat. The differing results between the studies could indicate a necessity for bees to forage over greater distances in fragmented landscapes, or could represent a greater likelihood of identifying sister bees on islands of suitable habitat within landscapes featuring large unsuitable areas for the bees.

Table 9.12. Forage distances and forage ranges identified in previous bumblebee microsatellite DNA studies. Knight *et al.* (2005) and Darvill *et al.* (2004) calculated the forage distances as a minimum estimated maximum foraging range. Chapman *et al.* (2003) calculated a foraging range based on colony number and nest density.

Bombus species	Knight *et al.* (2005)	Darvill *et al.* (2004)	Chapman *et al.* (2003)	South Essex study
B. terrestris	758m	625m	620-2800m	N.A.
B. pascuorum	449m	312m	510-2300m	N.A.
B. lapidarius	450m	N.A.	N.A.	N.A.
B. pratorum	674m	N.A.	N.A.	N.A.
B. humilis	N.A.	N.A.	N.A.	Mean-756m max.-2570m
B. sylvarum	N. A.	N.A.	N.A.	Mean-232m max.-1515m

However, differences between the methods used for assessing foraging dynamics in this study and those previous (Chapman *et al.*, 2003; Darvill *et al.*, 2004; Knight *et al.*, 2005) made it difficult to draw direct comparisons between the studies. A further study using the same sampling and microsatellite techniques as this study but for *B. humilis*, *B. sylvarum*, *B. terrestris/lucorum*, *B. pascuorum* and *B. lapidarius* foraging on the same forage sites would have to be carried out for more definitive conclusions to be drawn. The most important conclusions that could be made from this study alone were by directly comparing results recorded for *B. humilis* and *B. sylvarum* workers.

There was a difference between the relative distances over which *B. humilis* and *B. sylvarum* sisters were recorded foraging (Figure 9.4). Statistical analyses showed that the difference between the foraging distances of *B. humilis* and *B. sylvarum* sisters was significant. *Bombus sylvarum* sisters were frequently recorded foraging at the same forage patch, whereas *B. humilis* sisters were more evenly distributed throughout the forage distances. Overall the results demonstrated that *B. sylvarum* workers were more restricted in the distances they covered when foraging than *B. humilis*. This supported evidence provided in previous studies (Walther-Hellwig

and Frankl, 2000b; Darvill *et al.*, 2004; Knight *et al.*, 2005) which demonstrated that species may differ in their foraging ranges. If this is the case, it would be expected that *B. sylvarum* would be impacted by habitat fragmentation at smaller spatial scales than other species and might explain in part why this *Bombus* species in particular has become so rare in the UK. Such findings are consistent with Bumblebee Working Group investigations which indicated that *B. humilis* was better able to survive in fragmented smaller patches of habitat than *B. sylvarum* which seemed to have a requirement for $10km^2$ of continuous suitable habitat (Edwards 1998; 1999; 2000; 2001; 2002).

These differing results in terms of foraging behaviour between *B. humilis* and *B. sylvarum* have implications for habitat management with *B. sylvarum* requiring suitable and substantial areas of foraging and nesting habitat over smaller spatial scales than for *B. humilis*.

Estimating colony number

As can be seen from tables 9.4 and 9.5, Chao I estimates tended to be higher than those calculated in FITTING. Estimates from FITTING were used for estimates of colony number as they were the more conservative estimates and were therefore less likely to be overestimating the true number. Also the FITTING estimation technique was used in studies by Darvill *et al.* (2004), Knight *et al.* (2005) and Ellis *et al.* (2007) so results from this study should be directly comparable.

The number of colonies rather than number of individuals are a measure of the effective population size of eusocial insects (Chapman and Bourke, 2001). Therefore, by calculating an estimate of the number of colonies at each patch, site and for the population as a whole, it was possible to make an estimate of effective population size of *B. humilis* and *B. sylvarum* on the South Essex sites and to identify the relative importance of each site in supporting the metapopulation.

As in Chapman *et al.* (2003), no attempts to estimate nest density were made in this study. It was considered that such estimates would be inaccurate due to the unsuitable nature of much of the surrounding habitat for nesting of *B. humilis* and

B. sylvarum and the fragmented nature of suitable nesting areas (section 8.5). Instead, estimates were made of the number of colonies supported by each sampled forage patch.

Population estimates in Tables 9.8 and 9.9 indicated that population sizes for *B. humilis* were greater than those for *B. sylvarum*. In fact, the summary graph in Figure 9.5 showed that this was the case for all sites surveyed except for the experimental forage patch on Pitsea Landfill site. This opposite trend on the experimental forage patch indicated that more *B. sylvarum* colonies were using the newly created forage than *B. humilis* and may have indicated that *B. sylvarum* colonies were more efficient at locating and utilising new forage patches if provided over the appropriate spatial scales. Alternatively, the design of the patch may have been more suitable/attractive for *B. sylvarum* foragers than *B. humilis*. However, numbers of *B. humilis* during timed counts of bees on the trial forage patch were higher than those of *B. sylvarum* (Figure 6.9), indicating that, despite fewer *B. sylvarum* workers being recorded on the trial plot, those that were came from more individual nests than the *B. humilis* workers.

Landfill trial plot results also contradicted data on the likelihood of sisters being located on the same patch. With 30 *B. sylvarum* bees being recorded from 25 colonies compared to only 22 colonies for *B. humilis*. This maybe a factor influenced by the generally larger colony size of *B. humilis* meaning that more workers are available to exploit new forage resources, but again, no evidence for such a theory could be supported by this study's results. Further research would be required to substantiate whether *B. sylvarum* colonies are more efficient at locating new forage patches or whether colonisation is dependent upon colony size.

The estimated number of *B. humilis* colonies supported by forage patches ranged from 34 to 206 with an overall South Essex population estimated at 151 colonies. Numbers for *B. sylvarum* at patches ranged from 24 to 44 (with 72 recorded from the experimental forage patch) with an overall South Essex population of 121 colonies. These results indicated that the population size of *B. sylvarum* was lower than that for *B. humilis* in South Essex. This evidence was supported by the timed

bee counts (chapter 2) which consistently recorded greater numbers of *B. humilis* than *B. sylvarum* foraging on the sites.

Using the same methodologies as this study, Ellis *et al.* (2007) calculated colony number, expected heterozygosity and allelic richness for *B. humilis* and *B. sylvarum* populations nationally. The national average colony number per patch for *B. humilis* was 109.6, similar to the numbers recorded in South Essex. For *B. sylvarum* the national average colony number was recorded as 26.6 per patch sampled. This was lower than numbers recorded for *B. sylvarum* on all but one of the South Essex sites. Results from Ellis *et al.* (2007) therefore showed that the South Essex populations were relatively abundant when compared to other populations nationally. The two studies also consistently demonstrated that, when comparing the two species on the same sites, *B. humilis* tended to be more abundant than *B. sylvarum*.

Expected heterozygosity and allelic richness are measures of genetic diversity. Genetic diversity is considered to be a measure of population health which is impacted by population isolation and habitat fragmentation (Frankham, 2005; Ellis *et al.*, 2007). Ellis *et al.* (2007) identified genetic isolation in remaining populations of *B. humilis* and *B. sylvarum* in the UK and evidence for reduced genetic diversity. Ellis *et al.* (2007) recorded a UK average expected heterozygosity as 0.38 ± 0.01 for *B. sylvarum* and 0.44 ± 0.02 for *B. humilis*. This study recorded expected heterozygosity as 0.52 ± 0.06 for *B. sylvarum* and 0.48 ± 0.03 for *B. humilis* (Tables 9.8 and 9.9), both above the national averages recorded by the Ellis *et al.* (2007) study.

The Ellis *et al.* (2007) study recorded a UK average allelic richness for *B. sylvarum* as 2.36 ± 0.06 and 3.19 ± 0.11 for *B. humilis*. Results from this Essex study were 5.14 ± 1.38 for *B. sylvarum* and 3.55 ± 0.28 for *B. humilis*, again both being above the national averages recorded in the Ellis study. Care must be taken however with such direct comparisons of allelic richness and heterozygosity between studies when different microsatellite loci are used.

Regardless of the reliability of genetic diversity comparisons between data sets, these results demonstrated the importance of the South Essex populations of *B. sylvarum* and *B. humilis* as one of the largest in the UK. This highlighted the need for the protection of these populations to preserve genetic diversity nationally and to conserve these two UK BAP species. As part of such conservation, baseline microsatellite data on colony number produced in this study could be used to monitor the effects of habitat improvements in the area, particularly those on Hadleigh Castle Country Park.

Frequency of sister pairs recorded on the same and different patches and sites was also calculated from the colony data (Figures 9.6 and 9.7). Results showed that *B. sylvarum* workers from the same nest were more likely to all be recorded on the same patch than on different patches. This trend was even stronger at the site scale, indicating that the spatial scale that *B. sylvarum* workers tended to operate over was at the patch and site level with sisters from the same colony rarely being found at different sites. By comparison, *B. humilis* workers from the same nests were much more likely to be recorded at different forage patches and approximately equally likely to be found at the same or different sites. Statistical analyses of the mean number of patches and sites *B. humilis* and *B. sylvarum* workers from the same colony would be expected to be recorded on showed a significant difference between the two species with two *B. humilis* sisters from the same nest being expected to be found on a significantly greater mean number of forage patches and sites than two *B. sylvarum* sisters (Tables 9.6 and 9.7).

Although the mean number of patches and sites 3 sister bees from the same site were recorded visiting was lower for *B. sylvarum* than *B. humilis*, the difference was not found to be significant (Tables 9.6 and 9.7). This was most likely due to the low numbers of colonies recorded with 3 sister bees sampled (n=9 for both *B. sylvarum* and *B. humilis*). Alternatively, it could indicate that the more individuals found from a single colony the greater the probability of finding them at the same patch. Either way, these results supported the theory that *Bombus* species operate on different spatial scales with *B. sylvarum* tending to operate within patches and particularly within sites, whereas *B. humilis* appeared to be operating more on a

site and landscape scale. Such differences might in part explain the declines that are being seen in some UK bumblebee species.

Whilst this study cannot provide evidence for the reasons behind such foraging range differences, it might be hypothesised that differences could be due to differences in worker size limiting their ability to forage (Steffan-Dewenter *et al.*, 2002; Peat *et al.*, 2005) or differences in colony size (Prŷs-Jones, 1982) or colony density (Knight *et al.*, 2005) requiring further foraging trips for nest mate avoidance to reduce intra-colony competition. Further studies investigating forager and colony size and distance of forage visits would be required to assess these theories. However, management guidelines from this study must be designed to address inter-species variation in terms of the spatial dynamics of foraging visits recorded in this study.

Ability to colonise new forage patch

Results of factor analysis on the variability of allele frequencies between workers foraging on the experimental plot at the Cleanaway Pitsea Landfill site and those from Hadleigh Castle Country Park, Canvey Wick and Wat Tyler Country Park sites showed no obvious correlation with any of the surrounding plots for *B. humilis* or *B. sylvarum* workers. When the factor analysis results of each site were plotted no grouping occurred and each point appeared to be approximately equidistant from the others (Figures 9.8 and 9.9). This indicated that bees on the experimental plot had colonised from 2 or more of the surrounding sites and could therefore colonise over distances greater than 1km (the approximate distance of the nearest site). This corresponded to estimates of foraging range in this study and Chapman *et al.* (2003) which recorded worker bumblebees regularly travelling distances greater than 1km to forage.

Results of multinomial likelihood tests of the probability that allele frequencies at the experimental plot could have come from each of the surrounding sites were incomplete (Tables 9.10 and 9.11). This was largely due to the fact that if an allele was present at the experimental plot which had not been recorded at a surrounding plot (or vice versa) the resulting probability was 0. This occurred for several

microsatellite loci, but such alleles were only ever recorded very infrequently. This meant that conclusions as to the source site of the allele could not be made as their absence at a site could merely have been due to sampling from a subpopulation not being sufficient to record them. Of the alleles that results were obtained for, a general trend appeared to be that bees from the furthest site (Hadleigh Castle Country Park) had the highest –ln values and were therefore the least likely to have been the source of the bees colonising the experimental plot. Bees from the Wat Tyler and Canvey Wick sites alternated in being most likely to be the colonising subpopulation dependent upon locus.

Thus, from this investigation it could be concluded that the Canvey Wick and Wat Tyler sites were most likely to have provided the source of the *B. humilis* and *B. sylvarum* colonies which were using the experimental patch in 2006. This would indicate that 1–2km might be an appropriate maximum distance for forage patch creation for existing populations. This distance corresponded with a study of population genetic isolation in the ecologically similar species *B. muscorum* (Darvill *et al.*, 2006). Darvill *et al.* (2006) found populations of *B. muscorum* to be significantly genetically differentiated at distances as little as 3km apart.

Methods of analysis in this study were however fairly inconclusive and a further study would be required to substantiate these findings. A study creating and monitoring forage patches at increasing distances away from the South Essex population and monitoring colonisation might provide greater insight.

Chapter 10

Conclusions

10.1 Project summary

Evidence from the South Essex site surveys and timed count data demonstrated the importance of the network of South Essex sites. Mean bee counts at each site varied annually and the years featuring the highest counts varied between sites. This highlighted the value of a range of sites for providing an abundance of forage in the region under annually fluctuating environmental conditions.

The Canvey Wick site, Wat Tyler Country Park, Hadleigh Castle Country Park and Two Tree Island were identified as key sites in the region, but several smaller sites were also recorded supporting *B. humilis* and *B. sylvarum*. Absence of the bees on sites which appeared to have suitable habitat at increasing distances from the key sites may have been indicative of fragmentation distances between sites being so great that they restricted the bees' movements to a centrally strong population (Fig 2.1). The prevention of further landscape fragmentation should be targeted for conservation efforts and the production of 'stepping stone' sites with suitable habitat should be developed if attempts are to be made to consolidate and expand the South Essex population.

The importance of each site in terms of foraging bee numbers was shown to vary throughout the foraging period demonstrating an increased need for adequate forage throughout the whole colony cycle (May to October). This was particularly demonstrated by the apparent shift of *B. humilis* and *B. sylvarum* workers and males from the Canvey Wick site, Wat Tyler Country Park and Hadleigh Castle Country Park in August to the neighbouring sites of the Old County Council Landfill and Two Tree Island in September. Forage surveys revealed *Ballota nigra* to be the preferred forage source on the sites in September and this species was largely absent from the Canvey Wick, Wat Tyler and Hadleigh Castle sites. Habitat management is required on the key sites to ensure forage is available throughout *B. humilis* and *B. sylvarum* colony cycles (May – October).

The predominately coastal nature of the distributions of both species was similar to that found in other UK studies. Possible reasons for such a distribution were discussed in chapter two (section 2.4). Regardless of reason however, the location of these Essex sites with respect to the Thames Corridor and the results of geographical surveys indicated that areas within 10km of the Thames would be the most suitable sites in the region for conservation efforts. This does not mean however, that efforts should not be made to expand these populations further 'inland', merely that likelihood of success is greater on sites situated alongside the River Thames. Due to its central position within the South Essex populations, the RSPB managed West Canvey Marshes site was recognised as being an ideal location to develop further *B. humilis* and *B. sylvarum* forage and nesting habitat.

Forage behaviour studies investigated the general foraging patterns of *B. humilis* and *B. sylvarum* queens, workers and males to identify the most frequently visited forage sources for each species and caste. Although no precise assessment of floral abundance was made on the sites, observation and records of floral visits corresponded with previous studies indicating that bees make active foraging choices dependent upon available flora.

Surveys identified mid to late May as the emergence time of the earliest *B. humilis* and *B. sylvarum* queens. This was much later in the year than queens of the more ubiquitous UK species which were recorded on the sites as early as March. Corresponding with Edwards' (1998) theory on emergence and rarity in bumblebees, *B. humilis* and *B. sylvarum* were the latest recorded queens in the study and were also the rarest nationally. The carder bee species *B. pascuorum*, considered to be the most similar to *B. humilis* and *B. sylvarum* in terms of ecological requirements and the most nationally abundant species of the carder bees, was recorded foraging in April, a month earlier than the two study species. It was concluded that forage creation targeted specifically for *B. humilis* and *B. sylvarum* queens should be provided throughout May and June.

During floral surveys the majority of *B. humilis* and *B. sylvarum* queen foraging visits were recorded on *Lathyrus latifolius*, *Colutea arborescens*, *Vicia sativa* and *Vicia villosa*. *Bombus humilis* queens were also regularly recorded visiting

Trifolium pratense flowers but this was not found to be the case for *B. sylvarum* queens.

Pollen analysis provided further information on the specific foraging behaviour of the two *Bombus* species and differentiated between forage plants visited for pollen and those for nectar. For *B. sylvarum* queens Fabaceae was recorded as being the most important pollen forage source. Sample sizes were small to avoid impacting foraging queens but species of importance appeared to be *Colutea arborescens*, *Lathyrus latifolius* and *Trifolium pratense*. *Bombus humilis* queens foraged on Fabaceae and Rosaceae pollen, with species of importance being *Lathyrus latifolius*, *Vicia* species, *Trifolium pratense*, *Colutea arborescens* and *Rubus fruticosus*.

Plant families Fabaceae, Scrophulariaceae and Lamiaceae were recorded as receiving the majority of visits from foraging *B. humilis* and *B. sylvarum* workers. Of particular importance appeared to be *Ballota nigra*, *Lotus glaber* and *Odontites verna*. *Trifolium pratense* was also identified as being a preferred forage species, but more so for *B. humilis* workers than *B. sylvarum*.

Pollen analysis studies revealed that *B. sylvarum* workers predominantly collected from both Fabaceae and Scrophulariaceae flowers. *Odontites verna* was the major pollen source with *Lotus glaber*, *Rubus fruticosus*, *Galega officinalis*, *Trifolium pratense* and *Lamium* species also being collected. *Bombus humilis* however appeared to collect particularly from Fabaceae and it was hypothesised that this might provide evidence of differentiation between the two *Bombus* species. *Bombus humilis* was found to favour the Fabaceae plants *Lotus glaber*, *Trifolium pratense*, *Galega officinalis*, *Vicia* species, *Trifolium repens* and *Lotus corniculatus*. *Odontites verna*, *Centaurea nigra* and *Rubus fruticosus* were also recorded as being visited for pollen by *B. humilis* workers.

Of particular interest was the lack of pollen from Lamiaceae flowers compared to the number of visits the bees were recorded making to plants of this plant family, particularly *Ballota nigra* flowers. *Ballota nigra* was therefore concluded as being of particular importance as a nectar resource rather than for pollen, but a pollen

survey later in the field season when *Ballota nigra* is one of very few available forage resources remaining, would need to be carried out to confirm this. Regardless of this, *Ballota nigra* should be considered an important nectar forage resource for *B. humilis* and *B. sylvarum* workers and males.

Comparison of pollen foraging behaviour over two survey years indicated that *B. sylvarum* worker pollen collection was fairly consistent. Results for *B. humilis* workers were quite varied with a smaller proportion of Fabaceae pollen in the second year samples. It was hypothesised that this may have been a reaction to differing floral availability between the years, and if so could have demonstrated an increased ability by *B. humilis* to react to forage availability. No analysis of forage availability was made in this study however, so this theory could not be proved.

The pollen analysis study also highlighted the value of *Rubus fruticosus* as a pollen source for *B. humilis* and *B. sylvarum* workers and queens. The presence of this species on sites supporting *B. humilis* and *B. sylvarum* had previously been recognised in a study by Harvey (1999), but its importance was identified as habitat suitable for nesting rather than a forage source.

Comparing *B. humilis* and *B. sylvarum* pollen collecting behaviour with that of *B. terrestris/lucorum, B. pascuorum* and *B. lapidarius* revealed that *B. humilis* and *B. sylvarum* collected pollen from Fabaceae and Scrophulariaceae flowers to a greater extent than the more ubiquitous UK bumblebees. Results also indicated that *B. humilis* and *B. sylvarum* workers appeared more specialised in their pollen collecting behaviour than their most ecologically similar UK species, *B. pascuorum*.

These findings were confirmed by dietary preference analysis. *Bombus sylvarum* workers consistently had a narrower dietary breadth in terms of plant species and plant families visited than *B. humilis* and the more nationally ubiquitous species investigated. *Bombus terrestris/lucorum* recorded the broadest. Due to small sample sizes few statistical tests proved to be significant, but *B. sylvarum* was demonstrated as having a significantly narrower dietary breadth in terms of floral

species than *B. humilis* and *B. pascuorum*, the two most ecologically similar *Bombus* species.

Results of dietary preference analysis failed to identify substantial differences in forage species by any *Bombus* species with respect to the others. The nationally ubiquitous species *B. terrestris/lucorum* was however recorded with the highest number of positive floral species correlations (visiting a greater number of floral species more frequently than other *Bombus* species). *Bombus sylvarum* was recorded as having the lowest number of positive floral species correlations. Analysis of dietary preference at the floral family level identified positive preferences for *B. sylvarum* on Scrophulariaceae flowers and for *B. humilis* on Fabaceae flowers when compared to foraging visits for all bees recorded. These results correlated with previous studies suggesting that these were preferred forage sources for these bees.

Results for *B. sylvarum* from this investigation corresponded with theories on dietary preference with tongue length, timing of colony initiation and colony length. However, *B. humilis* was recorded as having a similar dietary breadth to *Bombus* species with different colony timings which remain nationally ubiquitous. This evidence therefore questions the theory that *Bombus* species with shorter and/or later colony timings are more specialised in their forage requirements, although a thorough investigation of dietary behaviour throughout rare and ubiquitous species colony cycles would be required to substantiate this.

One aspect that was highlighted by the differences in the results between the two species is the importance of targeting conservation habitat management efforts to the specific ecological requirements of individual bumblebee species. This necessitates further studies such as this one to identify individual species requirements for all bumblebee species.

Bombus humilis and *B. sylvarum* males were found to be fairly general in their forage choices in terms of available flowers. Lamiaceae flowers were recorded as receiving the majority of visits. Fabaceae, Scrophulariaceae and Asteraceae flowers were also recorded as being regularly visited. *Ballota nigra* received a

high proportion of visits later in the foraging season across several of the surveyed sites. This may have demonstrated a forage preference for *Ballota nigra* but also may have been a result of the limited availability of other forage resources during September and October. These results again highlighted the need for site managers to ensure adequate availability of forage sources throughout the bumblebee colony cycle. Overall, the results from this study indicated that provision of forage for *B. humilis* and *B. sylvarum* workers would be adequate to also target males of these two species.

Results from foraging surveys were fed into a series of forage provision experiments. Under greenhouse conditions *Lotus glaber*, *Lotus corniculatus* and *Ballota nigra* were all successfully cultivated. Growth trials of *Odontites verna* under greenhouse conditions were not successful.

A growth trial on newly capped areas of the landfill site was also successful with the bare topsoil proving to be an ideal substrate for forage patch production. Target forage species *Ballota nigra*, *Odontites verna*, *Lotus glaber*, *Lotus corniculatus*, *Trifolium pratense* and *Lathyrus latifolius* were all successfully cultivated on the Cleanaway Pitsea Landfill site.

Surveys of foraging *B. humilis* and *B. sylvarum* workers demonstrated that both could be attracted to new areas of forage in substantial numbers if provided within the range of existing populations. Significantly greater numbers of foraging *B. humilis* and *B. sylvarum* were attracted to the areas sown with wildflowers known to be preferred by the bees than the surrounding areas which were sown with *Lolium perenne*, the standard topsoil treatment following landfill capping.

Seeding on the site proved an effective method for generating patches of target floral species. Target forage was produced both by sowing continuous areas of mixed species and discrete single species plots. Further experimentation on a less constrained site would be required to assess whether one technique was a more effective method for providing suitable forage at an appropriate time for foraging workers of *B. humilis* and *B. sylvarum*.

A habitat manipulation experiment at Hadleigh Castle Country Park demonstrated that spreading green hay was an effective method for creating forage patches containing target forage species on areas of bare topsoil following scrub clearance. Green haying produced a significantly higher percentage vegetation cover than natural recolonisation a year after treatment. Green haying also produced significantly greater numbers of flower heads available to the bees than natural recolonisation. This included significantly greater numbers of *B. humilis* and *B. sylvarum* preferred floral forage species *Odontites verna* and *Trifolium pratense*.

Bombus humilis and *B. sylvarum* workers were able to locate and utilise this forage patch and numbers of both *Bombus* species foraging on the patch were significantly greater than those on the natural recolonisation trial. Timed counts of the bees revealed that numbers on the green hay trial were similar to those on other suitable forage areas of the site.

It was concluded that further experimentation would be required to assess best practice for timing the production of suitable forage with the peak foraging periods of the worker bees, and monitoring would be required to assess whether the 10 year habitat improvement program at Hadleigh Castle Country Park was having a beneficial effect on colony number of the bees as well as their distribution within the site. In addition to timed bee counts, using microsatellite DNA analysis to estimate colony numbers at forage patches would be an effective method to assess whether habitat improvement was having a beneficial effect on the number of *B. humilis* and *B. sylvarum* colonies utilising the site.

Attempts at utilising artificial nectar sources to examine resource partitioning in bumblebees were unsuccessful. No bumblebees were recorded visiting the artificial nectar sources. With no other method currently available to study the effect of tongue length on foraging ability and resource partitioning in these wild bees however, investigation of the use of pheromones to attract wild bees to artificial forage sources may prove beneficial.

A further study investigating the daily time of arrival of the first workers at a forage patch revealed *B. sylvarum* workers arriving almost an hour later than *B.*

humilis and *B. pascuorum*. Due to an inability to control many of the variables associated with the study, results on whether larger worker bees could arrive at forage earlier gaining a competitive advantage over smaller workers were considered to be inconclusive. A study by Peat *et al.* (2005) carried out at the same time was unable to demonstrate a relationship between size of worker and their ability to forage at a range of temperatures.

Nest searches and methods to reliably locate nests were fairly unsuccessful, although the Bumblebee Conservation Trust's trained sniffer dog has demonstrated some success and might be useful for locating nests on the sites in future. In the absence of such data however, information on nesting requirements came from comparisons of the three *B. humilis* nests located during the surveys and records from previous studies investigating *B. humilis* and *B. sylvarum* nesting requirements. Characteristics of the three *B. humilis* nests located in this study corresponded with those previously reported (Edwards, 1999; Carvell, 2002): areas of relatively undisturbed tall rough grassland with substantial layers of leaf litter and moss. Nests were situated on southwest/southeast facing slopes with a sunny exposure.

In the absence of a reliable technique to locate rare bumblebee nests, microsatellite DNA analysis was used to investigate forage distances and colony numbers of the bees. Microsatellite analysis of sibship relationships between bees revealed significant differences in the spatial scales over which *B. humilis* and *B. sylvarum* operated. Mean foraging distances estimated from distances separating sister bees were calculated as 756m for *B. humilis* and 232m for *B. sylvarum*. Whilst these two figures can only be taken as rough minimum estimates for mean foraging distances, they demonstrated a difference in spatial scales over which the two species operated. *Bombus humilis* appeared to operate over a landscape scale with sisters being identified both within and between sites. In contrast to this, *B. sylvarum* appeared to operate over a site scale, with sisters rarely being identified between sites and more commonly being recorded at patches within the same site.

Bombus sylvarum workers were also much more likely to be found foraging on the same forage patch than *B. humilis* workers. This result highlighted the importance

of forage patch continuity to *B. sylvarum* workers. A greater proportion of *B. sylvarum* workers from a single colony would be foraging on a solitary forage patch than from a *B. humilis* colony, therefore loss of such a patch would be expected to have a greater impact on *B. sylvarum* colonies. These shorter spatial scales over which *B. sylvarum* workers travel from nest to forage require that nesting habitat be provided in the proximity of foraging sources for them to be effective for *B. sylvarum* colonies.

Estimates of colony number utilising each forage patch were higher for *B. humilis* than *B. sylvarum* for all but the trial forage patch on the Cleanaway Pitsea Landfill site. This indicated that the *B. sylvarum* population in the area is scarcer than *B. humilis* and therefore maybe at more immediate risk of extinction. Annual or biannual population estimates are required to monitor population levels to assess whether populations of *B. humilis* and *B. sylvarum* are stable in the region or whether they are in decline.

Measures of population heterozygosity and allelic richness of the South Essex populations of both *Bombus* species were above national averages for each species. These results highlighted the importance of conserving and consolidation the South Essex populations if *B. humilis* and *B. sylvarum* are to be conserved in the UK.

Evidence for the ability of the bumblebees to colonise new forage patches was not conclusive but indicated that both species could cover distances of at least 1-2km to locate new forage patches. The high numbers of *B. sylvarum* colonies estimated to be foraging on the experimental patch compared to those of *B. humilis* suggested that *B. sylvarum* may be more able to locate and utilise new forage patches if created on the appropriate spatial scales. Further investigation of each species' ability to colonise new forage patched would be required to substantiate this.

Overall, the study highlighted that despite the similarity in distribution between *B. sylvarum* and *B. humilis* across the South Essex sites, synchronicity of emergence times and frequency of foraging visits made by the two species to Fabaceae,

Scrophulariaceae and Lamiaceae flowers, numerous dissimilarities occurred between the ecology of the two species. Of particular note, were the narrower dietary breadth and shorter foraging distances of *B. sylvarum*. There also appeared to be a divergence between the two species in terms of pollen collecting behaviour with *B. sylvarum* more frequently collecting Scrophulariaceae pollen and *B. humilis* more frequently Fabaceae. Whilst these results have major implications for the management requirements of each species individually, habitat management designed towards the seemingly more specific requirements of *B. sylvarum* should also be appropriate for *B. humilis* conservation.

10.2 Management guidelines

Results from the study highlighted the need for the following management strategies for Hadleigh Castle Country Park, Wat Tyler Country Park and other semi-natural grassland areas:

General management of the sites should follow the principles of grassland habitat management for invertebrates described by Kirby (1992). The aim of which being to create a mosaic of habitats from bare ground to patchy scrub with a variety of topography and vegetation structures suitable for a broad range of invertebrates.

Specific management for foraging queens should target the production of grassland areas rich in *Vicia* species and *Trifolium pratense*. *Colutea arborescens* and other early flowering Fabaceae should also be encouraged where they occur. For these early flowering Fabaceae species, grassland areas of a site should be cut or grazed in late October/November of the previous year. This is to encourage early suitable forage timed specifically for *B. humilis* and *B. sylvarum* queens. Due to *Lathyrus latifolius* not being native to the British Isles, it is not an appropriate species to be introduced on the sites. However, its importance to foraging *B. humilis* and *B. sylvarum* queens means that on brownfield sites where it already occurs, it should perhaps be encouraged.

Development of *Rubus fruticosus* in areas surrounding grassland habitat should also be encouraged. This study has demonstrated that *Rubus fruticosus* acts as a source of pollen and nectar for foraging queens and workers of both *B. sylvarum* and *B. humilis*. Also, the grass swards that tend to develop at the base of such areas of scrub may act as potential nesting habitat for the bees.

Areas of sites with southern facing slopes and sunny aspects should be selected for nesting habitat. These areas should not be cut or grazed between May and late September to avoid nest disturbance. If possible these areas should be cut no more frequently than bi-annually or managed with very low grazing pressure to allow grass swards and litter and moss layers to develop. On some areas of the site a longer 3 or 4 year rotation on cutting or grazing should be included with management only to control scrub encroachment.

Forage patches must be provided over the appropriate spatial scales for connectivity of nesting and foraging habitats (see chapter 9). Provision of forage throughout the *B. humilis* and *B. sylvarum* colony cycle (May-October) is a key management aim if colonies are to successfully develop and produce sexuals at the end of the season. Such forage patches must contain abundant areas of the floral species identified in this study as being favoured by the bees: *Lotus glaber*, *Odontites verna*, *Ballota nigra*, *Trifolium pratense* and *Centaurea nigra*. Flailing and grazing have both proved effective techniques for producing the target forage species at Hadleigh Castle Country park although low level grazing has perhaps been the most effective. The edges of paths which have been cut and remain short due to rabbit grazing and visitor pressure have also produced some of the best areas of *Odontites verna* and *Lotus glaber* on the site.

This study has demonstrated that covering areas of bare topsoil with green hay or seed collected from within the site are effective ways to produce wildflower areas of target forage species. Once these areas are established annual cutting or grazing should encourage the development of suitable Fabaceae, Scrophulariaceae and Lamiaceae flowers to develop. Further experimentation is planned for Hadleigh Castle Country Park to assess best practice for timing flowering on newly created forage patches created following scrub clearance with peak *B. humilis* and *B.*

sylvarum foraging periods. Results from these studies will be published at a later date.

Cutting on established forage areas should be avoided between May and September to avoid removing forage and disturbing nests. However, it is planned that small areas of Hadleigh Castle Country Park will be cut in late June in 2008 to assess whether foraging availability on the site can be extended to late September and early October. A previous trial of this method successfully produced *Odontites verna* flowers later than the rest of the site, but as demonstrated by the results of this study, such cutting of forage areas should only be carried out on a very small scale on extensive sites to avoid removing entire forage sources particularly for *B. sylvarum* colonies.

Brownfield sites in the region such as the Canvey Wick site (SSSI) do not tend to scrub over at the same rate as the semi-natural parkland sites due to their poor substrate and tendency to drought (Harvey, 2000a), thus these sites require little in the way of cutting or grazing. Scrub removal over longer periods may be adequate management on such sites, although observations made at Canvey Wick during this study have indicated that experimenting with disturbance management on small areas of such sites might be beneficial to bumblebee forage availability and to a variety of other invertebrates on the sites.

Above all however, sites within and surrounding the South Essex populations of *B. humilis* and *B. sylvarum* must be protected from development and managed sympathetically to avoid further habitat fragmentation which could threaten the populations of the bees in the region and nationally.

10.3 Further research

Site surveys

Investigation of migration distances of *B. humilis* and *B. sylvarum*. Although Ellis *et al.* (2007) identified the genetic distances between fragmented *B. sylvarum* and *B. humilis* populations, they were unable to identify a specific threshold distance at which populations became too fragmented for genetic exchange. An investigation creating and monitoring forage patches at increasing distances from the known South Essex distribution might provide information on the distances both species are able to travel to colonise new forage patches.

Forage preferences

It was concluded that a comparison of forage choices by the bees with forage availability might have provided valuable data on forage preferences of the bees. However, without specific knowledge of the spatial scales the bees operate over when foraging it is difficult to assess how the bees are making their forage choices and over what scales comparison of available forage should be made.

Dietary specialisation

A further year's study of dietary breadth and preference would be beneficial to increase the sample size for statistical analysis of forage breadth data.

Pollen analysis

As pollen sampling was carried out in July and August, a later pollen sampling survey in September would be useful to assess whether the importance of *Ballota nigra* is as a nectar source, or whether it is also an important pollen source later in the season when other forage sources have ceased flowering.

Forage patch design

Following the successful forage patch creation, a study of the bees' ability to locate new forage patches at increasing distance from the existing population would provide valuable information for the design of effective habitat management plans.

Habitat manipulation

Further experimental management is required to ensure that forage provided on areas cleared of scrub is timed to coincide with peak worker foraging periods. Continued monitoring of the trial plots is also required to assess the most effective management techniques for the continued annual provision of forage on these plots.

An assessment of the effects of the forage provision on *B. humilis* and *B. sylvarum* populations at Hadleigh Castle Country Park is also required. Knowledge of whether forage provision at the site is benefiting the bees could be measured by a continuation of the timed bee counts and the use of microsatellite DNA analysis (annually or biannually) to monitor the estimated number of colonies of each *Bombus* species at the site compared to the baseline data generated in chapter 9 of this study.

Further species ecology: emergence, artificial forage, thermodynamics and nesting

The use of pheromones to attract wild bees to artificial forage sources could be trialled to continue the investigation of tongue length and resource partitioning in wild bees and to assess whether the provision of artificial forage sources could be utilized in addition to the creation of new forage patches to supplement existing forage resources in the area.

A reliable technique to locate rare bumblebee nests is required. At the time of writing, 'Quinn' the Bumblebee Working Group sniffer dog appeared to be the

most effective method for locating nests and it was hoped that he could be brought to South Essex to carry out nest searches in future. If Quinn could successfully locate nests, more accurate assessments of nesting requirements could be made, mark recapture trials could be carried out to assess forage distances and colony density estimates could be calculated. Results could be compared to estimates generated from the microsatellite DNA investigation in chapter 9 to assess the precision of the technique.

Microsatellite DNA studies

An investigation of the spatial dynamics of the five most abundant *Bombus* species on the South Essex sites could generate further knowledge of forage ranges. Using the same microsatellite techniques as in this study, the foraging distances of *B. humilis* and *B. sylvarum* workers could be compared with those of the more nationally ubiquitous species. This could give greater insight into individual species ecology and the factors driving differential population declines between species. If significant differences were recorded between species, investigation of the effects of forager size and nest size on foraging distance could be used to assess what drives such inter-species variation.

As mentioned previously, colony number estimates generated in this study could be used as baseline data to monitor the effects of habitat improvement on the South Essex sites. Increase in colony number is a more accurate measure of population size than timed counts of bumblebee workers (Chapman and Bourke, 2001).

Chapter 11

References

Abramson, J.H. and Gahlinger, P.M. (2001) *Computer programs for epidemiologists: PEPI version 4.0.* Sagebrush Press, Salt Lake City, USA.

Alford, D.V. (1969) A Study of the Hibernation of Bumblebees (Hymenoptera: Bombidae) in Southern England. The Journal of Animal Ecology 38(1), pp. 149-170.

Alford, D.V. (1975) Bumblebees. Davis-Poynter Ltd, London UK.

Alford, D.V. (ed.) (1980) *Atlas of the bumblebees of the British Isles.* Institute of Terrestrial Ecology publication, No. 30, Cambridge.

Ali, J. (2005) Canadian study suggests natural pesticide impairs bumblebee foraging ability. Bee World 86(2), pp. 50.

Banaszak, J. (1980) Studies on methods of censusing the number of bees (Hymenoptera, Apoidea). Polish Ecological Studies 6(2), pp. 355-365.

Banaszak, J. (1983) Ecology of bees (Apoidea) of agricultural landscapes. Polish Ecological Studies 9, pp. 421-505.

BBCT (2007) Information about the bumblebee conservation trust. www.bumblebeeconservationtrust.co.uk, accessed September 2007.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. and Kunin, W.E. (2006) Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science 313(5785), pp. 351 – 354.

Birkett, N.L. (1956) Some observations on a flight of *Bombus lucorum* L. (Hym., Bombidae). The Entomologist's Monthly Magazine 92, pp. 333.

Blake, C. (2007) Potential cause of honeybee colony collapse disorder. Western Farm Press 29(15), pp 1-11.

Bourn, N.A.D. and Thomas, J.A. (2002) The challenge of conserving grassland insects at the margins of their range in Europe. Biological Conservation 104, pp. 285-292.

Bowers, M.A. (1985a) Experimental analyses of competition between two species of bumblebees (Hymenoptera: Apidea). Oecologia (Berlin) 67, pp. 224-230.

Bowers, M.A. (1985b) Bumblebee colonization, extinction, and reproduction in subalpine meadows in Northeastern Utah. Ecology 66(3), pp. 914-927.

Bronstein, J.L. (1995) The plant-pollinator landscape. In Hansson, L., Fahrig, L., Merrian, G. (eds) *Mosaic Landscapes and Ecological Processes.* Chapman & Hall, London, pp. 256-288.

Buchmann, S. and Ascher, J.S. (2005) The plight of pollinating bees. Bee World 86(3), pp. 71 – 74.

Burel, F. and Baudry, J. (2004) *Landscape Ecology: Concepts, Methods and Applications*. Science Publishers, Inc., Plymouth, UK.

BWARS (2007) Recent distribution maps of *Bombus hypnorum* in the UK. http://www.bwars.com/bombus_hypnorum_map.htm, accessed September 2007.

Carreck, N.L., Osborne, J.L., Capaldi, E.A. and Riley, J.R. (1999) Tracking bees with radar. Bee World 80(3), pp. 124-131.

Carvell, C. (2002) Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. Biological Conservation 103(1), pp. 33-49.

Carvell, C., Meek, W.R., Pywell, R.F. and Nowakowski, M. (2004) The response of foraging bumblebees to successional change in newly created arable field margins. Biological Conservation 118, pp. 327-339.

Carvell, C., Roy, D.B., Smart, S.M., Pywell, R.F., Preston, C.D. and Goulson, D. (2006) Declines in forage availability for bumblebees at a national scale. Biological Conservation 132, pp. 481-489.

Chao, A. (1984). Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11, pp. 265-270.

Chapman, R.E. (2004) PhD Thesis. Submitted to the University of London.

Chapman, R.E. and Bourke, A.F.G. (2001) The influence of sociality on the conservation biology of social insects. Ecology Letters 4, pp. 650-662.

Chapman, R.E., Wang, J. and Bourke, A.F.G. (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumblebee pollinators. Molecular Ecology 12, pp. 2801-2808.

Chittka, L. and Walker, J. (2006) Do bees like Van Gogh's Sunflowers? Optics and Laser Technology 38, pp. 323-332.

Connop, S. (2007) information on bumblebee conservation and links to research institutes. www.bumblebeeresearch.co.uk, accessed September 2007.

Cook, J.M. and Crozier, R.H. (1995) Sex determination and population biology in the hymenoptera. Trends in Ecology and Evolution 10, pp. 281-286.

Corbet, S.A., Williams, I.H. and Osborne, J.L. (1991) Bees and the pollination of crops and wild flowers in the European Community. Bee World 72(2), pp. 47-58.

Cresswell, J.E., Osborne, J.L. and Goulson, D. (2000) An economic model of the limits to foraging range in central place foragers with numerical solutions for bumblebees. Ecological Entomology 25, pp. 249-255.

Croxton, P.J., Carvell, C., Mountford, J.O. and Sparks, T.H. (2002) A comparison of green lanes and field margins as bumblebee habitat in an arable landscape. Biological Conservation 107, pp. 365-374.

Dafni, A. and Shimda, A. (1996) The possible ecological implications of the invasion of *Bombus terrestris* (L.) (Apidae) at Mt Carmel, Israel. In Matheson, A. (ed.) *The conservation of bees*. The Linnean Society of London and The International Bee Research Association, London, pp. 183-200.

Darvill, B., Ellis, J.S., Lye, G.C. and Goulson, D. (2006) Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum*. Molecular Ecology 15, pp. 601-611.

Darvill, B., Knight, M.E. and Goulson, D. (2004) Use of genetic markers to quantify bumblebee foraging range and nest density. OIKOS 107, pp. 471-478.

Darwin, C. (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, Albemarle Street, London, pp. 73-74.

DEFRA (2005a) *Environmental Stewardship*. London: DEFRA publications.

DEFRA (2005b) *Environmental Stewardship: Entry Level Stewardship Handbook*. London: DEFRA publications.

DEFRA (2007) England Rural Development Program 2000-2006 Executive Summary: Appendix A6 East of England Regional Chapter. www.defra.gov.uk/erdp/docs/eastchapter/default.htm, accessed September 2007.

Dennis, B.A. and Haas, H. (1967) Pollination and seed-setting in diploid and tetraploid red clover (*Trifolium pratense* L.) under Danish conditions: II. Studies of floret morphology in relation to the working speed of honey and bumble-bees (Hymenoptera: Apoidea). Royal Veterinary and Agricultural College, Copenhagen, Yearbook 1967, pp. 118-133.

Diekötter, T., Walther-Hellwig, K., Conradi, M., Suter, M. and Frankl, R. (2006) Effects of landscape elements on the distribution of the rare bumblebee species *Bombus muscorum* in an agricultural landscape. In Hawksworth, D.L. and Bull, A.T. (eds) *Topics in Biodiversity and Conservation 1: Arthropod Diversity and Conservation*, pp. 43-54.

Dieringer, D. and Schlötterer, C. (2003) MICORSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3, pp. 167-169.

Doebler S.A. (2000) The Rise and Fall of the Honeybee. BioScience 50(9), pp. 738-742.

Dornhaus, A. and Chittka, L. (2001) Food alert in bumblebees (*Bombus terrestris*): possible mechanisms and evolutionary implication. Behavioral Ecology and Sociobiology 50(6), pp. 570-576.

Dramstad, W. and Fry, G. (1995) Foraging activity of bumblebees (*Bombus*) in relation to flower resources on arable land. Agriculture, Ecosystems and Environment 53, pp. 123-135.

Dramstad, W.E., Fry, G.L.A. and Schaeffer, M.J. (2003) Bumblebee foraging – is closer really better? Agriculture, Ecosystems and Environment 95, pp. 349-357.

Duchateau, M.J. and Mariën, J. (1995) Sexual biology of haploid and diploid males in the bumble bee *Bombus terrestris*. Insectes Sociaux 42(3), pp. 255-266.

Ecoschemes Ltd (2003) *Green Roofs: their existing status and potential for conserving biodiversity in urban areas*. English Nature Research Report No, 498, English Nature, Peterborough.

EDINA (2007) Historical land use maps. L. Dudley Stamp/Geographical Publications Ltd, Audrey N. Clark, Environment Agency/DEFRA and Great Britain Historical GIS.

Edwards, M. (1997) UK Biodiversity Action Plan Bumblebee Working Group Report 1997. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. (1998) UK Biodiversity Action Plan Bumblebee Working Group Report 1998. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. (1999) UK Biodiversity Action Plan Bumblebee Working Group Report 1999. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. (2000) UK Biodiversity Action Plan Bumblebee Working Group Report 2000. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. (2001) UK Biodiversity Action Plan Bumblebee Working Group Report 2001. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. (2002) UK Biodiversity Action Plan Bumblebee Working Group Report 2002. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. (2004) UK Biodiversity Action Plan Bumblebee Working Group Report 2004. Unpublished report for the UK BAP bumblebee working group, Midhurst, UK.

Edwards, M. and Jenner, M. (2006) *Field Guide to the Bumblebees of Great Britain and Ireland.* Ocelli Limited, UK.

Edwards, R. and Broad, G.R. (eds) (2005) *Provisional atlas of the aculeate Hymenoptera of Britain and Ireland. Part 5.* Biological Records Centre, Huntingdon.

Ellis, J. (2006) PhD Thesis. Submitted to University of Southampton.

Ellis, J.S., Knight, M.E., Darvill, B. and Goulson, D. (2007) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae). Molecular Ecology (In Review).

English Nature (2005) Canvey Wick SSSI, Essex: Notification under section 28 of the Wildlife and Countryside Act 1981. English Nature's Essex, Hertfordshire and London team, English Nature Research Report, Peterborough.

English Partnerships (2005) *Tackling brownfield land in Britain.* English Partnerships Publishing, Warrington UK.

ESRI (2007) about ArcGIS. www.esri.com, accessed September 2007.

Estoup, A., Scholl, A., Pouvreau, A. and Solignac, M. (1995) Monoandry and Polyandry in Bumblebees (Hymenoptera, Bombinae) as evidenced by highly variable microsatellites. Molecular Ecology 4, pp. 89-93.

Estoup, A., Solignac, M., Cornuet, J-M., Goudet, J. and Scholl, A. (1996) Genetic differentiation of continental and island populations of *B. terrestris* (Hymenoptera: Apidae) in Europe. Molecular Ecology 5, pp 19-31.

European Pollinator Initiative (2007) Information about the initiative. http://europeanpollinatorinitiative.org/, accessed September 2007.

Evans, M., Hastings, N. and Peacock, N. (2000) *Statistical Distributions.* Wiley, New York, pp. 134-136.

Fitzpatrick, Ú., Murray, T.E., Paxton, R.J., Breen, J., Cotton, D., Santorum, V. and Brown, M.J.F. (2007) Rarity and decline in bumblebees – A test of causes and correlates in the Irish fauna. Biological Conservation 136(2), pp. 185-194.

Frankham, R. (2005) Genetics and extinction. Biological Conservation 126, pp. 131-140.

Frankham, R., Ballou, J.D. and Briscoe, D.A. (2002) *Introduction to conservation genetics.* Cambridge University Press, UK.

Free, J.B. and Butler, C.G. (1959) *Bumblebees*. New Naturalist Series, Collins, London.

Fussell, M. and Corbet, S.A. (1991) Forage for bumblebees and honey-bees in farmland – A case study. Journal of Apicultural Research 30(2), pp. 87-97.

Fussell, M. and Corbet, S.A. (1992a) The nesting places of some British bumblebees. Journal of Apicultural Research 31(1), pp. 32-41.

Fussell, M. and Corbet, S.A. (1992b) Flower usage by bumblebees – a basis for forage plant management. Journal of Applied Ecology 29(2), pp. 451-465.

Gels, J.A., Held, D.W. and Potter, D.A. (2002) Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. Journal of Economic Entomology 95(4), pp. 722-728.

Ghazoul, J. (2005) Buzziness as usual? Questioning the global pollination crisis. TRENDS in Ecology and Evolution 20(7), pp. 367-373.

Gilpin, M.E. (1987) Spatial structure and population vulnerability. In: Soule, M.E. (ed.) *Viable populations for conservation*, pp. 125-140, Cambridge University Press.

Goodnight, K.F. and Queller, D.C. (1999) Computer software for performing likelihood tests of pedigree relationships using genetic markers. Molecular Ecology 8, pp. 1231-1234.

Goodwin, S.G. (1995) Seasonal phenology and abundance of early-, mid-, and long- season bumblebees in Southern England 1985-1989. Journal of Apicultural Research 34, pp. 79-87.

Gotelli, N.J. and Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4, pp. 379-391.

Goudet, J. (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html

Goulson, D. (2003) *Bumblebees: Behaviour and Ecology*. Oxford University Press.

Goulson, D. and Darvill, B. (2003) Distribution and floral preferences of the rare bumblebees *Bombus humilis* and *B. soroeensis* (Hymenoptera: Apidae) on Salisbury Plain. British Journal of Entomology and Natural History 16(2), pp. 95-102.

Goulson, D. and Darvill, B. (2004) Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? Apidologie 35, pp. 55-63.

Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S. and Knight, M.E. (2005) Causes of rarity in bumblebees. Biological Conservation 122, pp. 1-8.

Goulson, D. and Williams, P. (2001) *Bombus hypnorum* (Hymenoptera: Apidae), a new British Bumblebee? British Journal of Entomology and Natural History 14, pp. 129–131.

Govier, R.N., Brown, J.G.S. and Pate, J.S. (1968) Hemiparasitic Nutrition in Angiosperms. II. Root Haustoria and Leaf Glands of *Odontites verna* (Bell.) Dum. and their Relevance to the Abstraction of Solutes from the Host. New Phytologist 67(4), pp. 963-972.

Hallet, H.M. (1928) The Hymenoptera Aculeata of Glamorgan. Transactions of the Cardiff. Naturalists' Society 60, pp. 33-67.

Hanski, I. and Gilpin, M. (1991) Metapopulation dynamics: brief history and conceptual domain. Biological Journal of the Linnean Society 42, pp. 3-16.

Harder, L.D. (1983) Functional differences of the proboscides of the short- and long-tongued bees (Hymenoptera, Apoidea). Canadian Journal of Zoology 61, pp. 1580-1586.

Harder, L.D. (1986) Influences on the density and dispersion of bumblebee nests (hymenoptera: Apidae). Holarctic Ecology 9, pp. 99-103.

Harvey, P.R. (1998) Biodiversity Action Plan Bumblebees, Essex. Privately distributed, Grays, Essex.

Harvey, P.R. (1999) A report on the status of the Shrill carder bee *Bombus sylvarum* in Essex. Essex Naturalist (New Series) 16, pp. 79-82.

Harvey, P.R. (2000a) Shrill Carder Bee: Essex 2000. Report produced for English Nature, Colchester Essex.

Harvey, P.R. (2000b) The East Thames Corridor: a nationally important invertebrate fauna under threat. British Wildlife 12(2), pp. 91-98.

Harvey, P.R. (2001) Shrill Carder Bee at Canvey Northwick, Essex. Report produced for English Nature, Colchester Office.

Harvey, P.R. (2004) Brown roofs for invertebrates. Essex Naturalist (New Series) 21, pp. 79-88.

Heck, K.L., Belle, G.v. and Simberloff, D. (1975) Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. Ecology 56, pp. 1459-1461.

Hedrick, P.W., Miller, P.S., Geffen, E. and Wayne, R. (1997) Genetic evaluation of the three captive mexican wolf lineages. Zoo Biology 16(1), pp. 47-69.

Heinrich, B. (1972) Patterns of endothermy in bumblebee queens, drones and workers. Journal of Comparative Physiology 77, pp. 65-79.

Heinrich, B. (1974) Thermoregulation in bumblebees. I. Brood incubation by *Bombus vosnesenskii* queens. Journal of Comparative Physiology 88(2), pp. 129-140.

Heinrich, B. (1975) Thermoregulation in bumblebees. II. Energetics of warm-up and free flight. Journal of Comparative Physiology 96(2), pp. 155-166.

Heinrich, B. (1976) Resource partitioning among some eusocial insects: Bumblebees. Ecology 57, pp. 874-889.

Heinrich, B. (1979) *Bumblebee Economics*. Harvard University Press, Cambridge, Massachusetts.

Hill, T.C.J., Walsh, K.A., Harris, J.A. and Moffett, B.F. (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiology Ecology 43, pp. 1-11.

Hirsch, M., Pfaff, S. and Wolters, V. (2003) The influence of matrix type on flower visitors of *Centaurea jacea* L.. Agriculture, Ecosystems and Environment 98(1-3), pp. 331-337.

Holehouse, K.A., Hammond, R.L. and Bourke, A.F.G. (2003) Non-lethal sampling of DNA from bumblebees for conservation genetics. Insectes Sociaux 50, pp. 277-285.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., and Johnson, C.A. (eds) (2001) *Climate Change 2001 - the scientific basis*. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Incerti, F., Bortolotti, L., Porrini, C., Micciarelli Sbrenna, A. and Sbrenna, G. (2003) An extended laboratory test to evaluate the effects of pesticides on bumblebees. Preliminary results. Bulletin of Insectology 56(1), pp. 159-164.

Inouye, D.W. (1980) The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebee. Oecologia 45, pp. 197-201.

Inscentinel (2007) Information about the company. www.inscentinel.com, accessed September 2007.

JNCC (1981) Wildlife and Countryside Act 1981. Online only.

Kearns, C.A. and Inouye, D.W. (1993) *Techniques for Pollination Biologists*. University Press, Colorado.

Kells, A.R. and Goulson, D. (2003) Preferred nesting sites of bumblebee queens (Hymenoptera: Apidae) in agroecosystems in the UK. Biological Conservation 109, pp. 165-174.

Kells, A.R., Holland, J.M. and Goulson, D. (2001) The value of uncropped field margins for foraging bumblebees. Journal of Insect Conservation 5, pp. 283-291.

Kirby, P. (1992) *Habitat management for Invertebrates: a practical handbook.* JNCC, RSPB publishing, Sandy, Bedfordshire.

Klein, A-M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S., Kremen, C. and Tscharntke, T. (2007) Importance of Pollinators in Changing Landscapes for World Crops. Proceedings of The Royal Society B: Biological Sciences 274, pp1608.

Knight, M.E., Martin, A.P., Bishop, S., Osborne, J.L., Hale, R.J., Sanderson, R.A. and Goulson, D. (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Molecular Ecology 14, pp. 1811-1820.

Kremen, C., Williams, N.M. and Thorpe, R.W. (2002) Crop pollination from native bees at risk from agricultural intensification. Proceeding of National Academy of Science 99(26), pp. 812-816.

Kreyer, D., Oed, A., Walther-Hellwig, K. and Frankl, R. (2003) Are forests potential landscape barriers for foraging bumblebees? Landscape experiments with *Bombus terrestris* agg. and *Bombus pascuorum* (Hymenoptera, Apidea). Biological Conservation 116(1), pp. 111-118.

Kwak, M.M. (1987) Marking bees without anaesthesia. Bee World 68, pp. 180-181.

Luckham, A.M. (1906) Great migration of "Bumble bees". The Entomologist 39, pp. 65.

Lytzau Forup, M. and Memmott, J. (2005) The relationship between the abundances of bumblebees and honeybees in a native habitat. Ecological Entomology 30, pp. 47-57.

MacDonald, M.A. (2001) The colonisation of northern Scotland by *Bombus terrestris* (L.) and *B. lapidarius* (L.) (Hym., Apidae) and the possible role of climate change. Entomologist's Monthly Magazine 137, pp. 1-13.

MacDonald, M.A. (2006) Highland bumblebees: Distribution, Ecology and Conservation. Highland Biological Recording Group, Supplement 1 Nov.

MAFF (1998) *The countryside stewardship scheme*. London: MAFF publications.

MAFF (1999) *Arable stewardship*. London: MAFF publications.

Magnuson, V.L., Ally, D.S., Nylund, S.J., Karanjawala, Z.E., Rayman, J.B., Knapp, J.I., Lowe, A.L., Ghosh, S. and Collins, F.S. (1996) Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polymerase: implications for PCR-based genotyping and cloning. Biotechniques 21(4), pp. 700-709.

Marletto, F., Patetta, A. and Manino, A. (2003) Laboratory assessment of pesticide toxicity to bumblebees. Bulletin of Insectology 56(1) 155-158.

Mikkola, K. (1978) Spring migrations of wasps and bumblebees on the southern coast of Finland (Hymenoptera: Vespidae and Apidae). Annales Entomologici Fennici 44(1), pp. 10-26.

Murray, V., Monchawin, C. and England, P. (1993) The determination of the sequences present in the shadow bands of a dinucleotide repeat PCR. Nucleic Acids Research 21(10), pp. 2395-2398.

Nickys Nursery (2007) Seed supplies. www.nickys-nursery.co.uk, accessed September 2007.

Opdam, P. (1990) Understanding the ecology of populations in fragmented landscapes. Transcript from the 19th IUGB Congress, Trondheim 1989, pp. 373-380.

Osborne, J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, A.D., Reynolds, D.R. and Edwards, A.S. (1999) A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. Journal of Applied Ecology 36, pp. 519-533.

Osborne, J.L. and Corbet, S.A. (1994) Managing habitats for pollinators. Aspects of Applied Biology 40, pp. 207-215.

Osborne, J.L., Martin, A.P., Shortall, C.R., Todd, A.D., Goulson, D., Knight, M. E., Hale, R.J. and Sanderson, R.A. (2007) Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. Journal of Applied Ecology, IN PRESS.

Osborne, J.L. and Williams, I.H. (2001) Site constancy of bumble bees in an experimentally patchy habitat. Agriculture, Ecosystems and Environment 83, pp.129- 141.

Osborne, J.L., Williams, I.H., Carreck, N.L., Poppy, G.M., Riley, J.R., Smith, A.D., Reynolds, D.R. and Edwards, A.S. (1997) Harmonic radar: a new technique for investigating bumblebee and honey bee foraging flight. Proceedings of the International Symposium on Pollination in Acta Horticulturae. 437, pp. 159-163.

Osborne, J.L., Williams, I.H. and Corbet, S.A. (1991) Bees, pollination and habitat change in the European Community. Bee World 72(3), pp. 99-116.

Owen, D.F. (1956) A migration of insects at Spurn Point, Yorkshire. The Entomologist's Monthly Magazine 92, pp. 42-44.

Pain, S. (2006) Wasps - sniffer dogs with wings? New Scientist issue 2548, pp. 42-45.

Paxton, R.J., Murray, E., Bradley, U. and Brown, M.J.F (2004) A new pollinator initiative on the island of Ireland. Bee World 85(4), pp. 83-84.

Peat, J., Darvill, B., Ellis, J. and Goulson, D. (2005) Effects of climate on intra- and interspecific size variation in bumble-bees. Functional Ecology 19, pp. 145-151.

Pelletier, L. and McNeil, J.N. (2003) The effect of food supplementation on reproductive success in bumblebee field colonies. OIKOS 103, pp. 688-694.

Pelletier, L. and McNeil, J.N. (2004) Do bumblebees always forage as much as they could? Insectes Sociaux 51, 271-274.

Pekkarinen, A. (1979) Morphometric colour and enzyme variation in bumblebees (Hymenoptera, Apidae, *Bombus*) in Fennoscandia and Denmark. Acta Zoologica Fennica 158, pp. 1-60.

Philp, E.G. (1957) Migrating bumblebees. Entomologist's Monthly Magazine 93, pp. 104.

Philp, E.G. (1998). Species account and map of British distribution of *Bombus sylvarum* (Hymenoptera: Apinae). BWARS Newsletter, Spring 1998:28.

Plowright, C.M.S., Plowright, R.C. and Williams, P.H. (1997) Replacement of *Bombus muscorum* by *Bombus pascuorum* in northern Britain? The Canadian Entomologist 129, pp. 985-990.

Plowright, R.C. and Laverty, T.M. (1984) The ecology and sociobiology of bumble bees. Annual Review of Entomology 29, pp. 175-199.

Potts, S.G., Petanidou, T., Roberts, S., O'Toole, C., Hulbert, A. and Willmer, P. (2006) Plant-pollinator biodiversity and pollination services in a complex Mediterranean landscape. Biological Conservation 129, pp. 519-529.

Pouvreau, A. (1974) The uptake of sugar solutions by Bumble-Bees. Apidologie 5(3), pp. 247-270.

Pouvreau, A. (1989) Contribution á l'étude du polyéthisme chez les bourdons, *Bombus* Latr. (Hymenoptera, Apidae). Apidologie 20, pp. 229-244.

Preston, C.D., Pearman, D.A. and Dines, T.D. (2002) *New Atlas of the British and Irish flora*. Oxford University Press, Oxford, UK

Prŷs-Jones, O.E. (1982) Ecological studies of foraging and life history in bumblebees. PhD thesis, University of Cambridge.

Prŷs-Jones, O.E. and Corbet, S.A. (1987) *Bumblebees: Naturalists' Handbooks 6.* The Richmond Publishing Co. Ltd, Slough.

Pyke, G.H. (1982) Local geographic distributions of bumblebees near Crested Butte, Colorado: competition and community structure. Ecology 63(2), pp.555-573.

Pywell, R.F., Warman, E.A., Carvell, C., Sparks, T.H., Dicks, L.V., Bennett, D., Wright, A., Critchley, C.N.R. and Sherwood, A. (2005) Providing foraging resources for bumblebees in intensively farmed landscapes. Biological Conservation 121, pp. 479-494.

Pywell, R.F., Warman, E.A., Sparks, T.H., Greatorex-Davies, J.N., Walker, K.J., Meek, W.R., Carvell, C., Petit, S. and Firbank, L.G. (2004) Assessing habitat quality for butterflies on intensively managed arable farmland. Biological Conservation 118(3), pp. 313-325.

Queller, D.C. and Goodnight, K.F. (1989) Estimating relatedness using genetic markers. Evolution 43(2), pp. 258-275.

Queller, D.C., Strassmann, J.E. and Hughes, C.R. (1993) Microsatellites and Kinship. Trends in Ecology and Evolution 8(8), pp. 285-288.

Raine N.E. and Chittka, L. (2005) Comparison of flower constancy and foraging performance in three bumblebee species (Hymenoptera: Apidae: *Bombus*). Entomologia Generalis 28, pp. 81-89.

Raine N.E. and Chittka, L. (2007) Pollen foraging: learning a complex motor skill by bumblebees (*Bombus terrestris*). Naturwissenschaften 94, pp. 459-464.

Ranta, E. and Lundberg, H. (1980) Resource partitioning in bumblebees: the significance of differences in proboscis length. Oikos 35, pp. 298-302.

Ranta, E. and Lundberg, H. (1981) Food niche analyses of bumblebees: a comparison of three data collecting methods. Oikos 36, pp. 12-16.

Rarefaction (2007) Online rarefaction calculator. www2.biology.ualberta.ca/jbrzusto/rarefact.php, accessed September 2007.

Raymond, M. and Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86, pp. 248-249 (available at http:// genepop.curtin.edu.au/).

Reed, D.H. (2004) Extinction risk in fragmented habitats, Animal Conservation 7, pp. 181–191.

Reid, W., Mooney, H.A., Cropper, A., Capistrano, D., Carpenter, S.R., Chopra, K., Dasgupta, P., Dietz, T., Duraiappah Kumar, A., Hassan, R., Kasperson, R., Leemans, R., May, R.M., McMichael, T.A.J., Pingali, P., Samper, C., Scholes, R., Watson, R.T., Zakri, A.H., Shidong, Z., Ash, N.J., Bennett, E., Kumar, P., Lee, M. J., Raudsepp-Hearne, C., Simons, H., Thonell, J. and Zurek, M.B. (2005) Millennium Ecosystem Assessment Synthesis. United Nations, pp. 1–219.

Ribeiro, M.F. (1994) Growth in bumble bee larvae: relation between development time, mass, and amount of pollen ingested. Canadian Journal of Zoology 72, 1978–1985.

Rice, W.R. (1989) Analyzing tables of statistical tests. Evolution 43, pp. 223-225.

Rich, T.C.G. and Woodruff, E.R. (1996) Changes in the vascular plant floras of England and Scotland between 1930-1960 and 1987-1988: the BSBI monitoring scheme. Biological Conservation 75, pp. 217-229.

Roach, J.L., Colayco, R., To, C., Batey, D. and May, B. (2003) *Microsatellite Genotyping Using the BaseStation DNA Fragment Analyser*. MJ Research Application Notes 2(7). MJ Research, San Francisco, USA.

Robinson, R.A. and Sutherland, W.J. (2002) Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology 39, pp. 157-176.

Rudebeck, G. (1965) On migratory movements of waspa, mainly *Vespula rufa* at Falsterbo, Sweden. Proceedings of the Royal Entomological Society of London A 40, pp. 1-8.

Sakagami, S.F. and Katayama, E. (1977) Nests of some Japanese Bumblebees (Hymenoptera; Apidae). Journal of the Faculty of Science Hokkaido University, series 6, Zoology 21(1), pp. 92-153.

Samways, M.J. (1993) Insects in biodiversity conservation: some perspectives and directives, Biodiversity and Conservation 2, pp. 258–282.

Saville, N.M., Dramstad, W.E., Fry, G.L.A. and Corbet, S.A. (1997) Bumblebee movement in a fragmented landscape. Agriculture, Ecosystems and Environment 61, pp. 145-154.

Sawchik, J., Dufrene, M., Lebrun, P., Schtickzelle, N. and Baguette, M. (2002) metapopulation dynamics of the bog fritillary butterfly: modelling the effect of habitat fragmentation. Acta Oecologica 23, pp. 287-296.

Schmitt, T. and Hewitt, G.M. (2004) The genetic pattern of population threat and loss: a case study of butterflies. Molecular Ecology 13, pp. 21-31.

Schmid-Hempel, P. (1998) *Parasites in social insects*. Princeton University Press, Princeton, New Jersey.

Schmid-Hempel, P. and Durrer, S. (1991) Parasites, floral resources and reproduction in natural populations of bumblebees. Oikos 62, pp. 342-350.

Schmid-Hempel, R. and Schmid-Hempel, P. (2000) Female mating frequencies in *Bombus* spp. from Central Europe. Insectes Sociaux 47, pp. 36-41.

Sladen, F.W.L. (1912) *The humble-bee, its life history and how to domesticate it, with descriptions of all the British species of Bombus and Psithyrus.* Reprinted 1989, Logaston Press.

Stace, C. (1997) Field Flora of the British Isles. Cambridge University Press, Cambridge, UK.

Standifer, L.N. (1967) A comparison of the protein quality of pollens for growth-stimulation of the hypopharyngeal glands and longevity of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Insectes Sociaux 14(4), pp. 415-425.

Steffan-Dewenter, I. (2003) Importance of habitat area and landscape context for species richness of bees and wasps in fragmented orchard meadows. Conservation Biology 17(4), pp. 1036-1044.

Steffan-Dewenter, I., Potts, S.G. and Packer, L. (2005) Pollinator diversity and crop pollination services are at risk. TRENDS in Ecology and Evolution 20(12), pp. 651-652.

Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C. and Tscharntke, T. (2002) Scale-dependent effects of landscape context on three pollinator guilds. Ecology 83(5), pp. 1421-1432.

Steffan-Dewenter, I. and Tscharntke, T. (2002) Insect communities and biotic interactions on fragmented calcareous grasslands – a mini review. Biological Conservation 104, pp. 275-284.

Stone, G.N. and Willmer, P.G. (1989) Warm-up rates and body temperatures in bees: the importance of body size, thermal regime and phylogeny. Journal of Experimental Biology 147, pp. 303-328.

Svensson, B. (2002) Foraging and nesting ecology of Bumblebees (*Bombus* spp.) in agricultural landscapes in Sweden. Unpublished doctoral thesis, Swedish University of Agricultural Sciences, Uppsala.

Svensson, B., Lagerlof, J. and Svensson, B.G. (2000) Habitat preferences of nest-seeking bumblebees (Hymenoptera: Apidae) in an agricultural landscape. Agriculture, Ecosystems and Environment 77, pp. 247-255.

Teras, I. (1985) Food plants and flower visits of bumblebees (*Bombus*: Hymenoptera, Apidae) in southern Finland. Acta Zoologica Fennica 179, pp. 1-120.

Thompson, H.M. (2001) Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). Apidologie 32, pp. 305-321.

Thorne (2007) Thorne equipment. www.thorne.co.uk, accessed September 2007.

Trimble (2007) Trimble equipment. www.trimble.com/index.aspx, accessed September 2007.

Trueman, I. and Millet, P. (2003) Creating wildflower meadows by strewing green hay. British Wildlife 15(1), pp. 37-44.

Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A. and Warman, M.L. (2000) Preparation of PCR – quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). Biotechniques 29, pp. 52-54.

UKBAP (1995) Biodiversity: The UK Steering Group Report - Volume II: Action Plans (December 1995, Tranche 1, Vol 2, p127). www.ukbap.org.uk

UKBAP (1999) UK Biodiversity Group: Tranche 2 Action Plans - Volume IV: Invertebrates (March 1999, Tranche 2, Vol IV, p213). www.ukbap.org.uk

UKBAP (2007) About UK Biodiversity Action Plans. www.ukbap.org.uk, accessed September 2007.

Usher, M.B. (1997) Small populations: fragmentation, population dynamics and population genetics. In Tew, T.G., Crawford, T.J., Spencer, J.W., Stevens, D.P., Usher, M.B. and Warren, J. (eds) *The role of genetics in conserving small populations*. JNCC, Peterborough. pp. 11-21.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P. (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, pp. 535-538.

Walther-Hellwig, K., Fokul, G., Frankl, R., Büchler, R., Ekschmitt, K. and Wolters, V. (2006) Increased density of honeybee colonies affects foraging bumblebees. Apidologie 37, pp. 1-16.

Walther-Hellwig, K. and Frankl, R. (2000a) Foraging distances of *Bombus muscorum*, *Bombus lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). Journal of Insect Behavior 13(2), pp. 239-246.

Walther-Hellwig, K. and Frankl, R. (2000b) Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. Journal of Applied Entomology 124, pp. 299-306.

Wang, J. (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166, pp. 1963-1979.

Weibull, A.C., Östman, O. and Granqvist, Å. (2003) Species richness in agroecosystems: the effect of landscape, habitat and farm management. Biodiversity and Conservation 12(7), pp. 1335-1355.

Weinberg, D. and Plowright, C.M.S. (2006) Pollen collection by bumblebees (*Bombus impatiens*): The effects of resource manipulation, foraging experience and colony size. Journal of Apicultural Research and Bee World 45(2), pp. 22-27.

Whittington, R. and Winston, M.L. (2003) Are bumblebee colonies in tomato greenhouses obtaining adequate nutrition? The Canadian Entomologist 135(6), pp. 883-892.

Wiegmann, D.D., Wiegmann, D.A. and Waldron, F.A. (2003) Effects of a reward downshift on the consummatory behaviour and flower choices of bumblebee foragers. Physiology and Behaviour 79, pp. 561-566.

Williams, C.S. (1995) Conserving Europe's bees: why all the buzz? TREE 10(8), pp. 309-310.

Williams, P.H. (1982) The distribution and decline of British bumble bees (*Bombus* Latr.). Journal of Apicultural Research 21(4), pp. 236-245.

Williams, P.H. (1985) On the distribution of bumblebees (Hymenoptera, Apidea) with particular regard to patterns within the British Isles. PhD thesis, pages 180. Department of Applied Biology, University of Cambridge, Cambridge.

Williams, P.H. (1986) Environmental Change and the distribution of British bumble bees (*Bombus* Latr.). Bee World 67, pp. 50-61.

Williams, P.H. (1988) Habitat use by bumble bees (*Bombus* spp.). Ecological Entomology 13, pp. 223-237.

Williams, P.H. (1989) Why are there so many species of bumblebees at Dungeness? Botanical Journal of the Linnean Society 101, pp. 31-44.

Williams, P.H. (2005) Does specialization explain rarity and decline among British bumblebees? A response to Goulson *et al.* Biological Conservation 122, pp. 33-43.

With, K.A. (2004) Metapopulation dynamics: perspectives from landscape ecology. In: Hanski, I. and Gaggiotti, O. E. (eds) *Ecology, Genetics and Evolution of Metapopulations*, Elsevier Academic Press, London, UK, pp. 23–44.

Wojtowski, F. (1963) Observations on the construction and arrangement of bumblebee nests (Bombinae). Zoologica Poloniae 13, pp. 137-152.

Chapter 12

Appendices

12.1 *Bombus humilis* and *B. sylvarum* forage use on individual sites during the 2003 and 2004 South Essex bumblebee surveys.

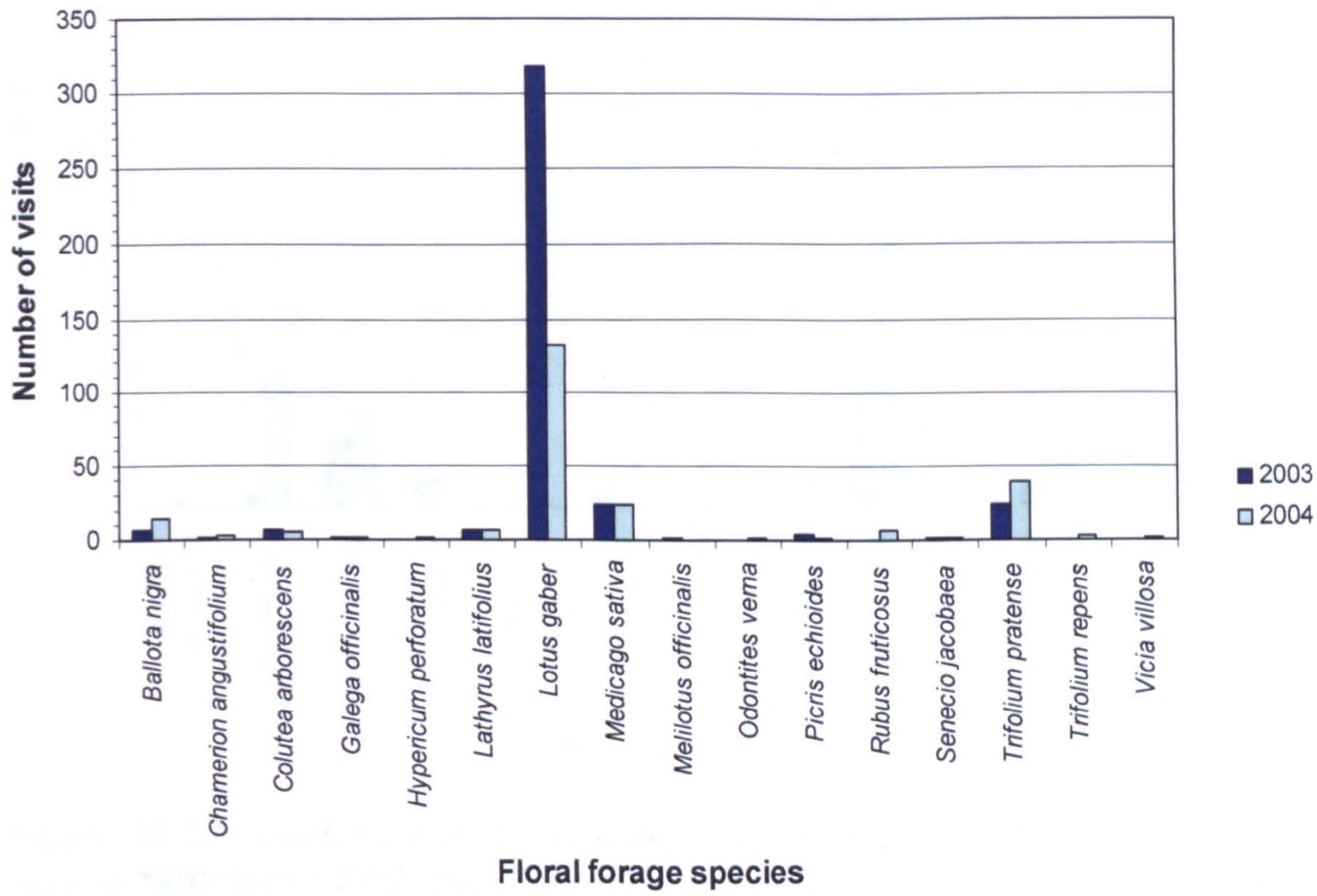


Figure 12.1.1. *Bombus humilis* sightings at the Canvey Northwick site, July to September 2003 and 2004. Based on sightings of all *B. humilis* flower visits during bee walk surveys at the site.

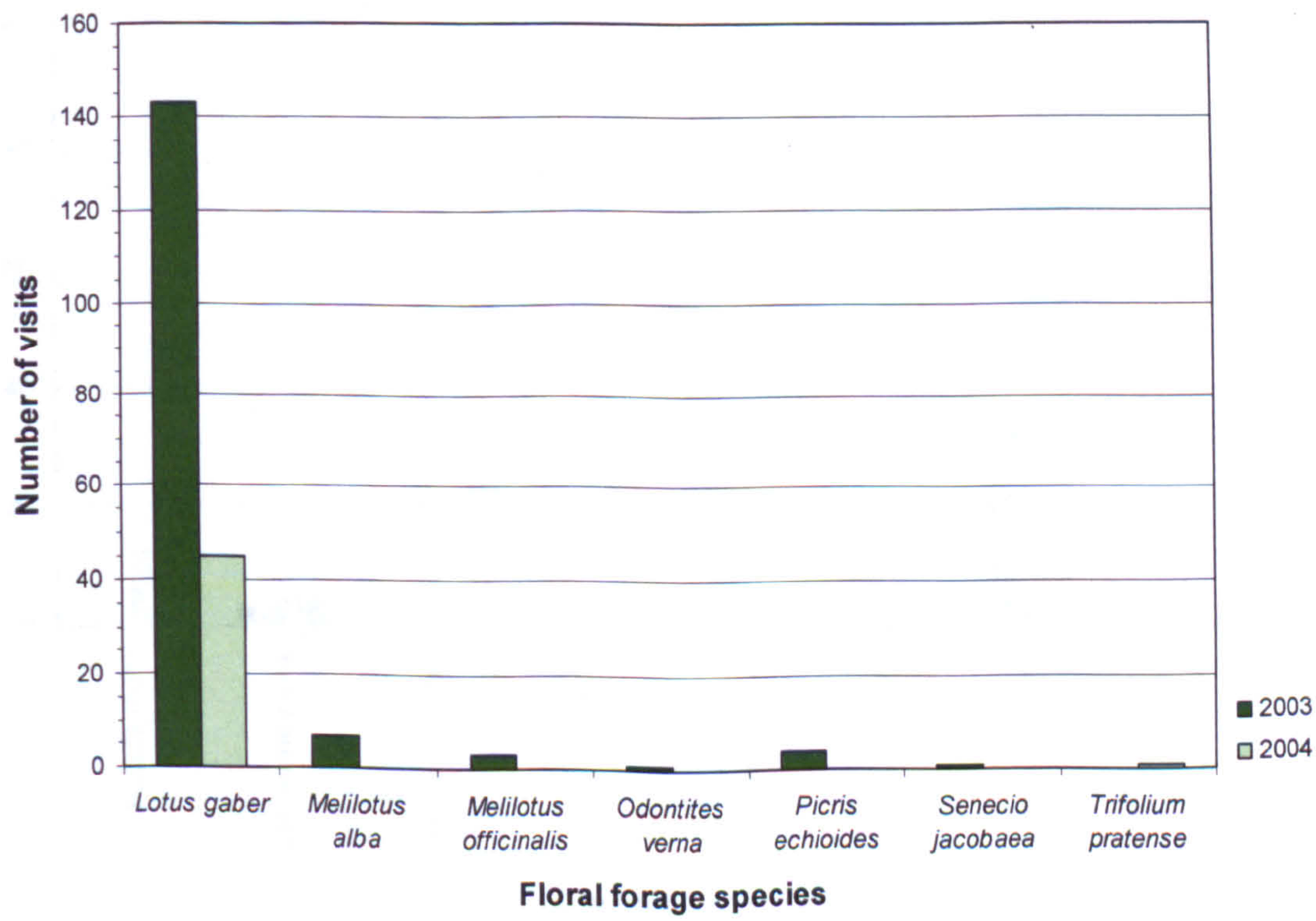


Figure 12.1.2. *Bombus sylvarum* sightings at the Canvey Northwick site, July to September 2003 and 2004. Based on sightings of all *B. sylvarum* flower visits during bee walk surveys at the site.

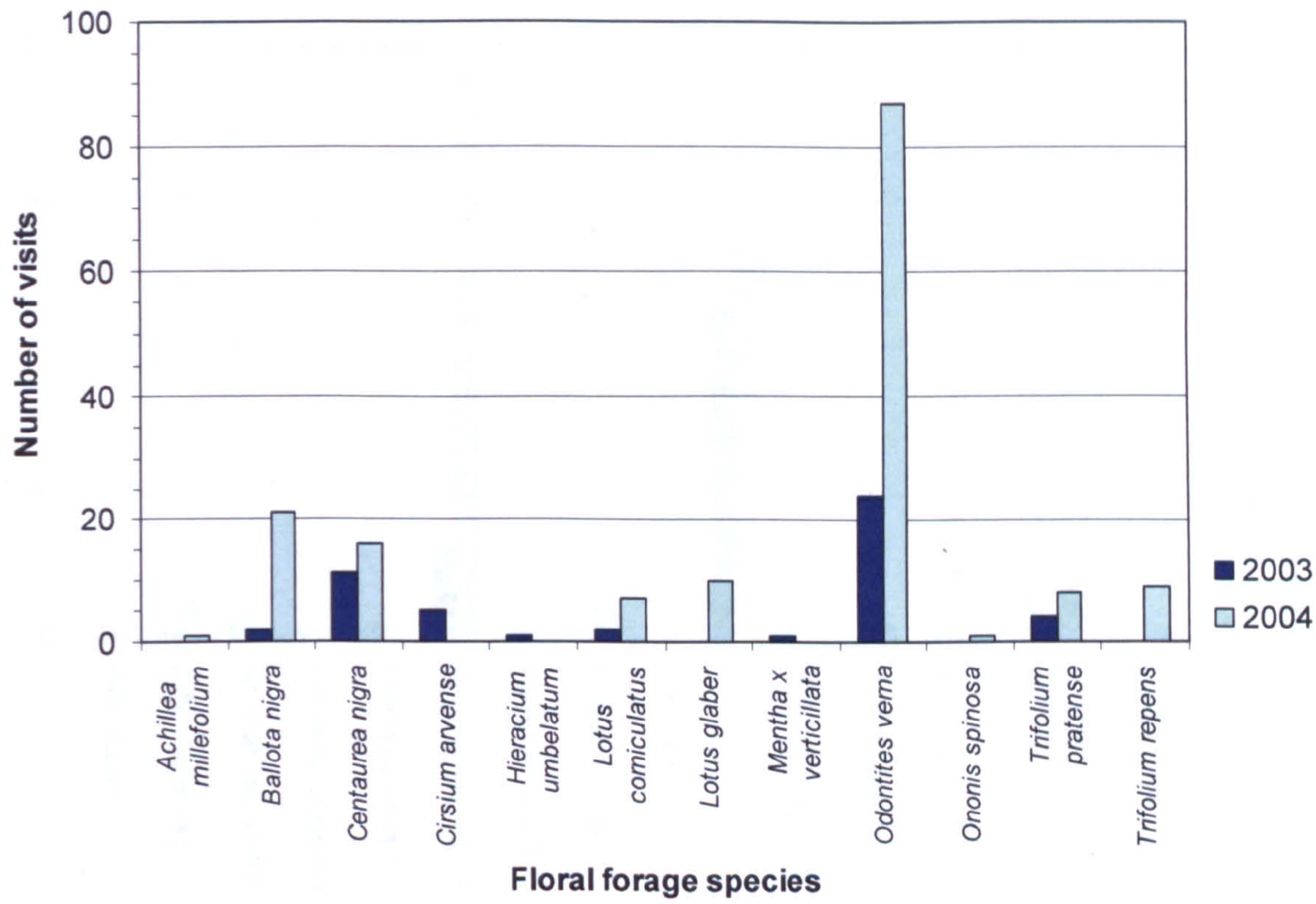


Figure 12.1.3. *Bombus humilis* sightings at Hadleigh Castle Country Park, July to September 2003 and 2004. Based on sightings of all *B. humilis* flower visits during bee walk surveys at the site.

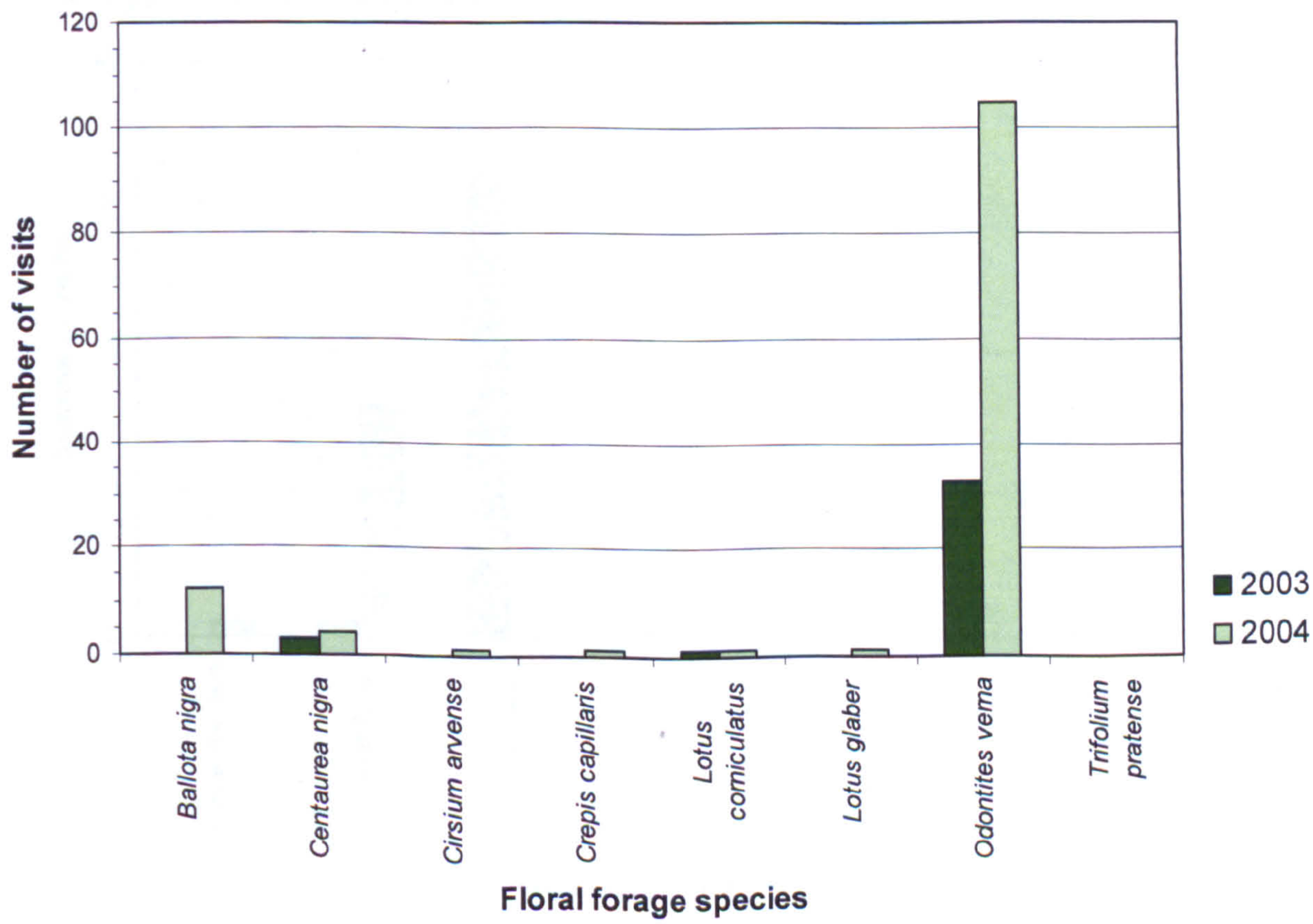


Figure 12.1.4. *Bombus sylvarum* sightings at Hadleigh Castle Country Park, July to September 2003 and 2004. Based on sightings of all *B. sylvarum* flower visits during bee walk surveys at the site.

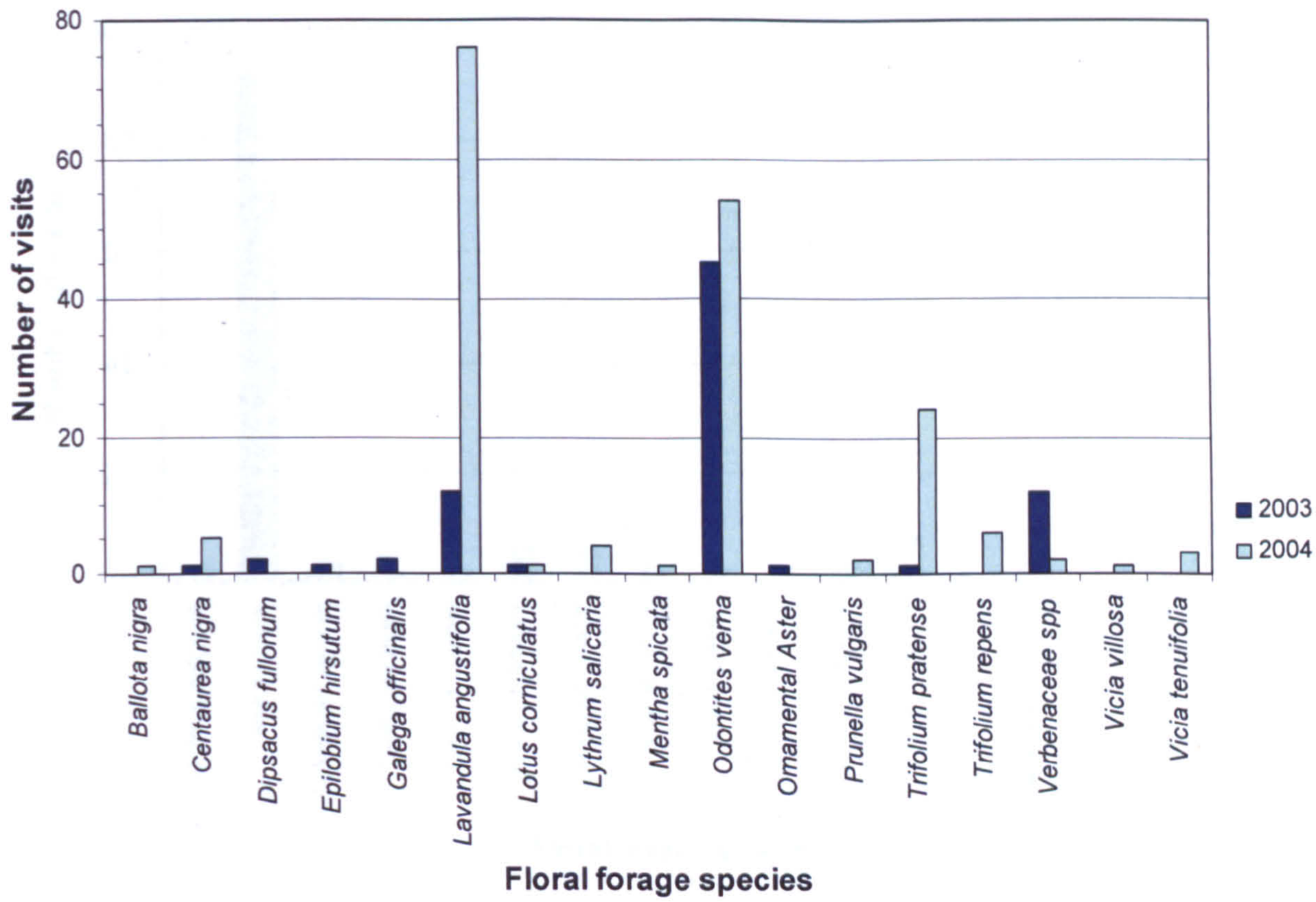


Figure 12.1.5. *Bombus humilis* sightings at Wat Tyler Country Park, July to September 2003 and 2004. Based on sightings of all *B. humilis* flower visits during bee walk surveys at the site.

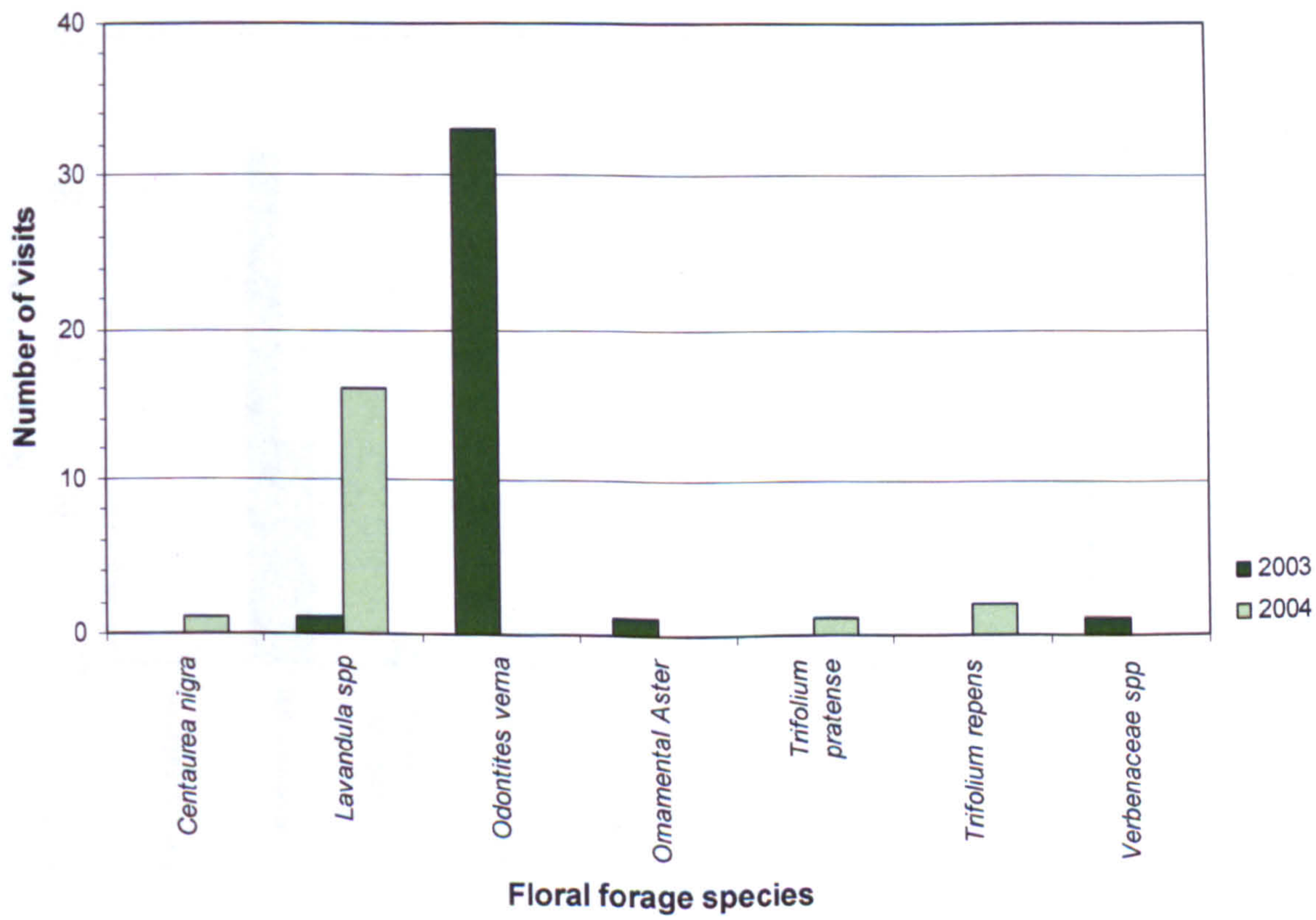


Figure 12.1.6. *Bombus sylvarum* sightings at Wat Tyler Country Park, July to September 2003 and 2004. Based on sightings of all *B. sylvarum* flower visits during bee walk surveys at the site.

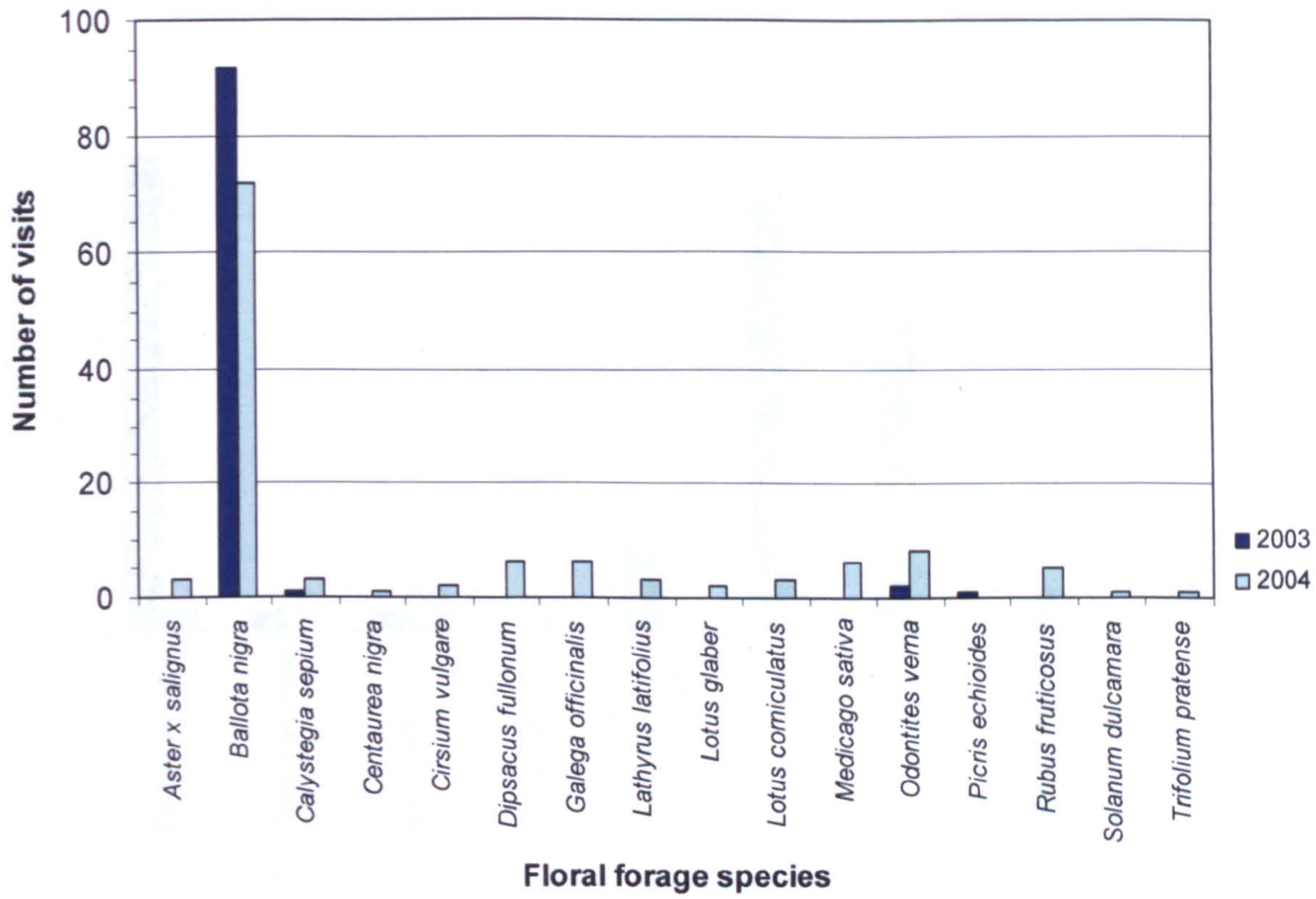


Figure 12.1.7. *Bombus humilis* sightings at Two Tree Island, July to September 2003 and 2004. Based on sightings of all *B. humilis* flower visits during bee walk surveys at the site.

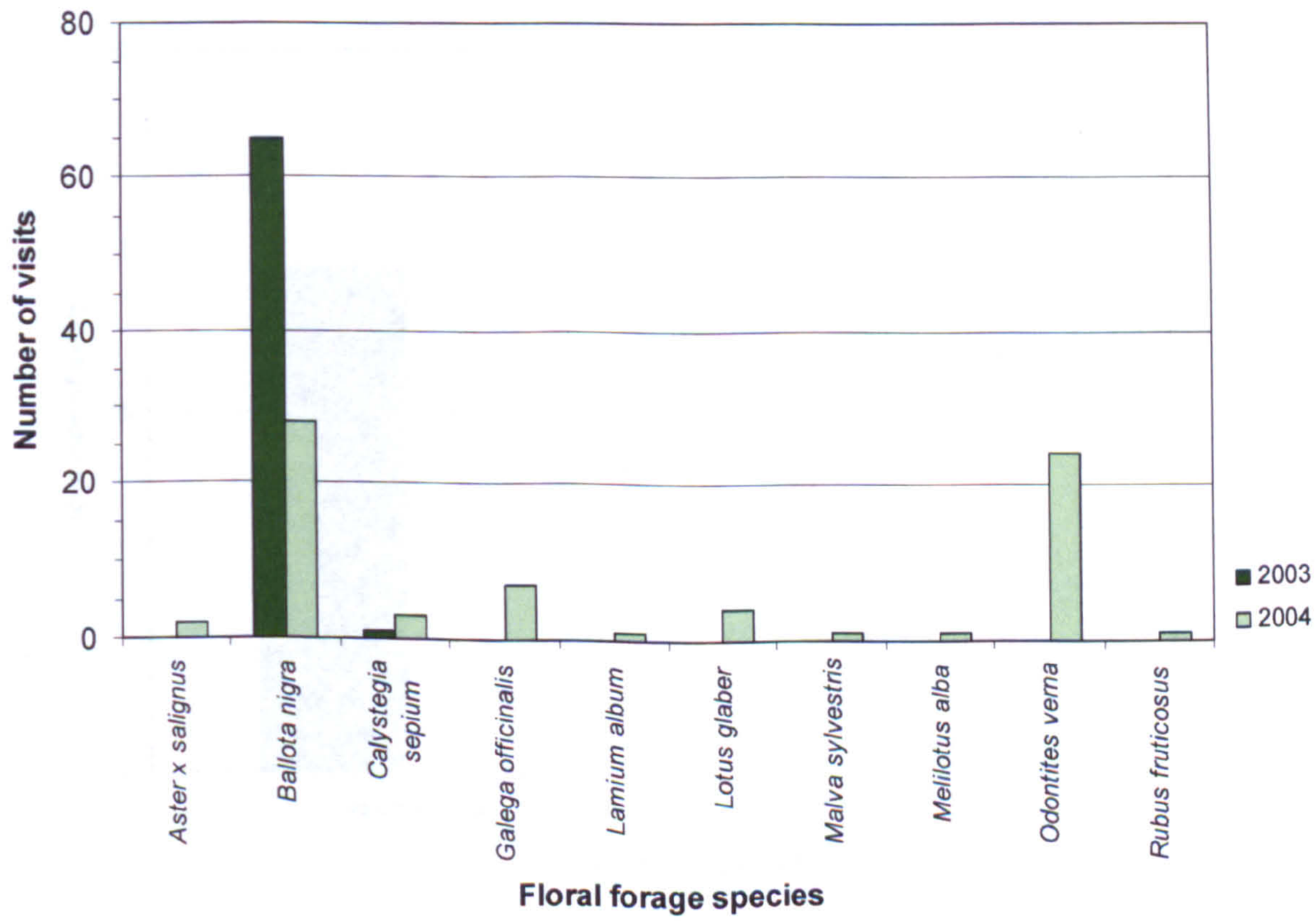


Figure 12.1.8. *Bombus sylvarum* sightings at Two Tree Island, July to September 2003 and 2004. Based on sightings of all *B. sylvarum* flower visits during bee walk surveys at the site.

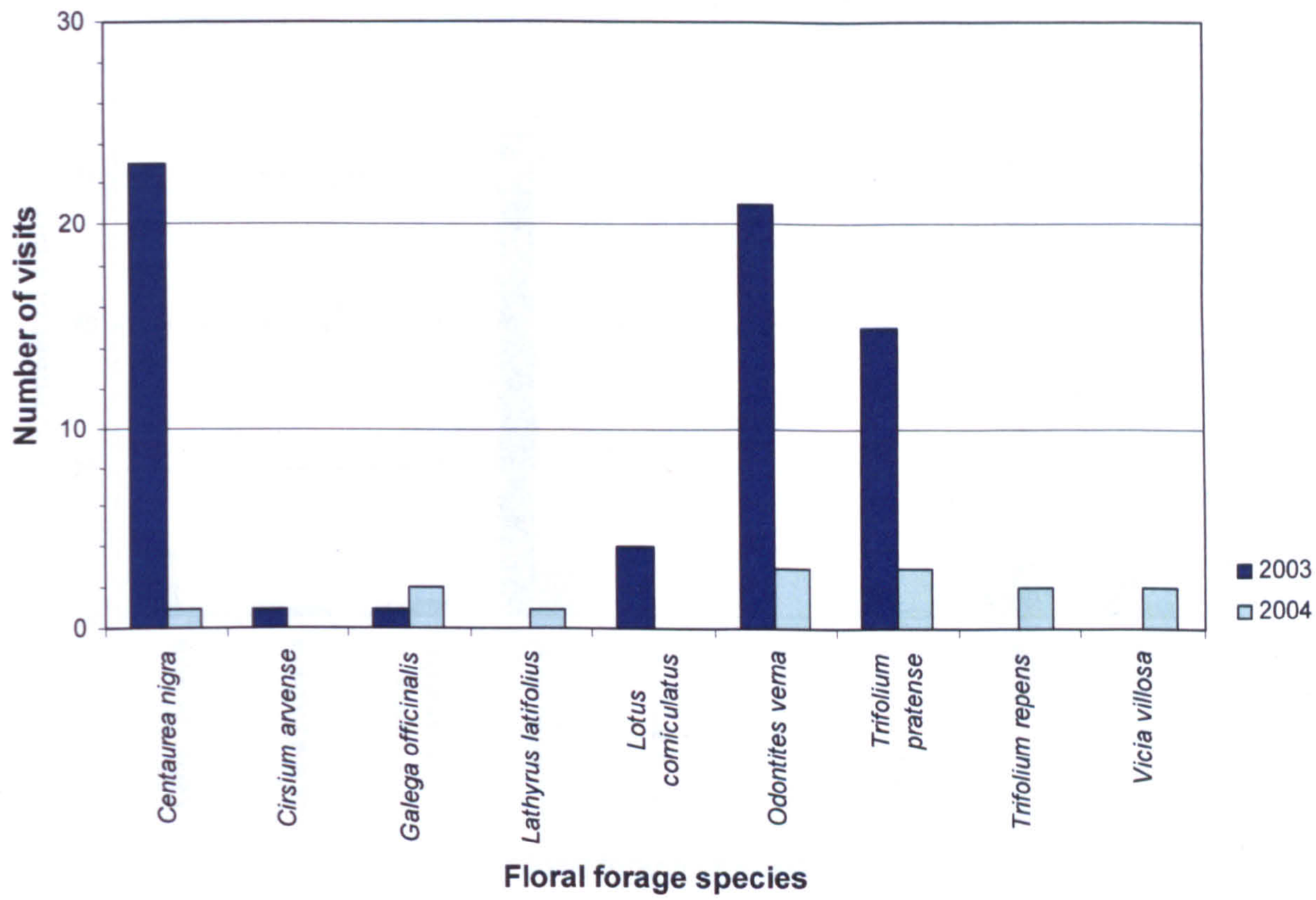


Figure 12.1.9. *Bombus humilis* sightings at Vange Hill, July to September 2003 and 2004. Based on sightings of all *B. humilis* flower visits during bee walk surveys at the site.

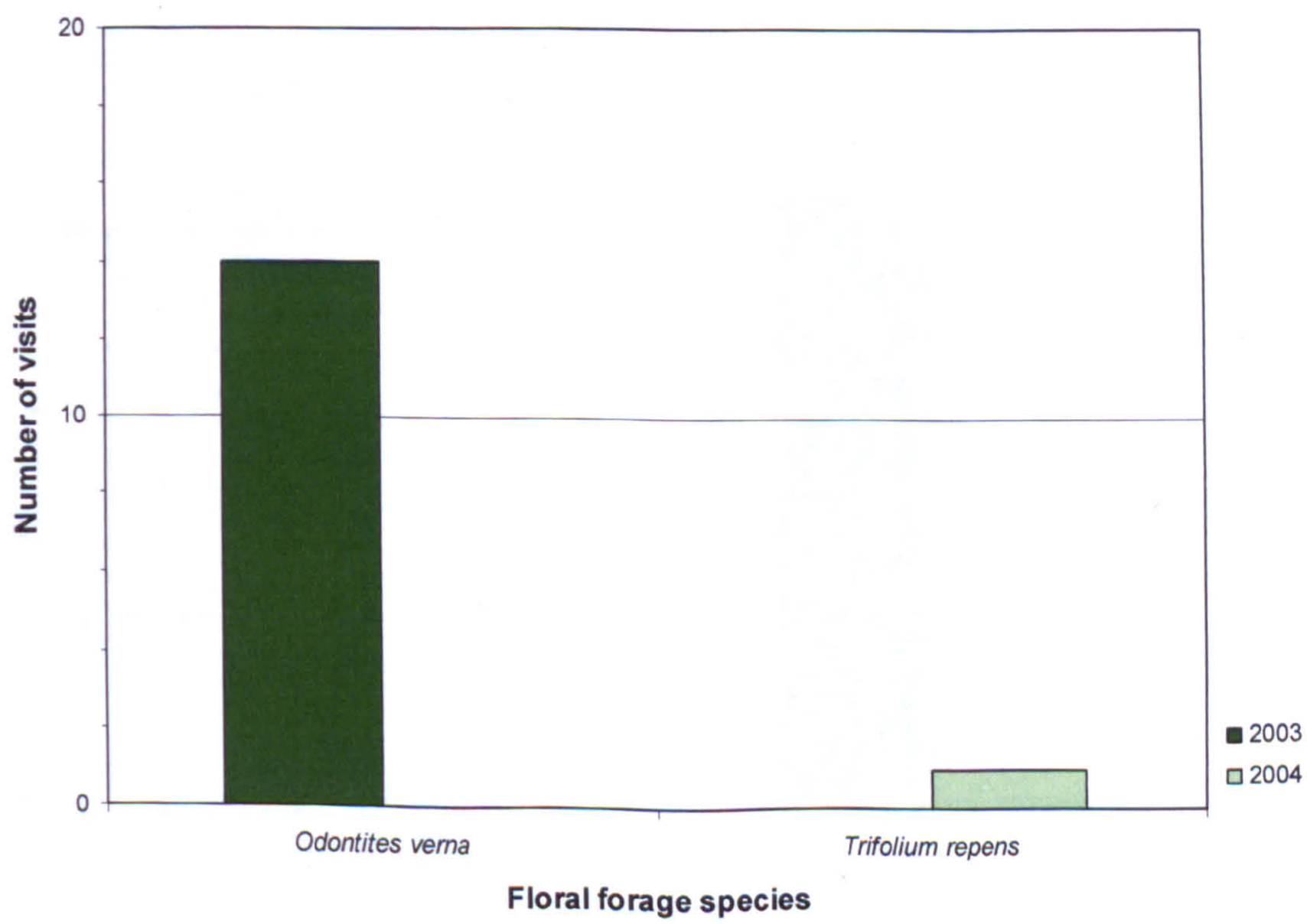


Figure 12.1.10. *Bombus sylvarum* sightings at Vange Hill, July to September 2003 and 2004. Based on sightings of all *B. sylvarum* flower visits during bee walk surveys at the site.

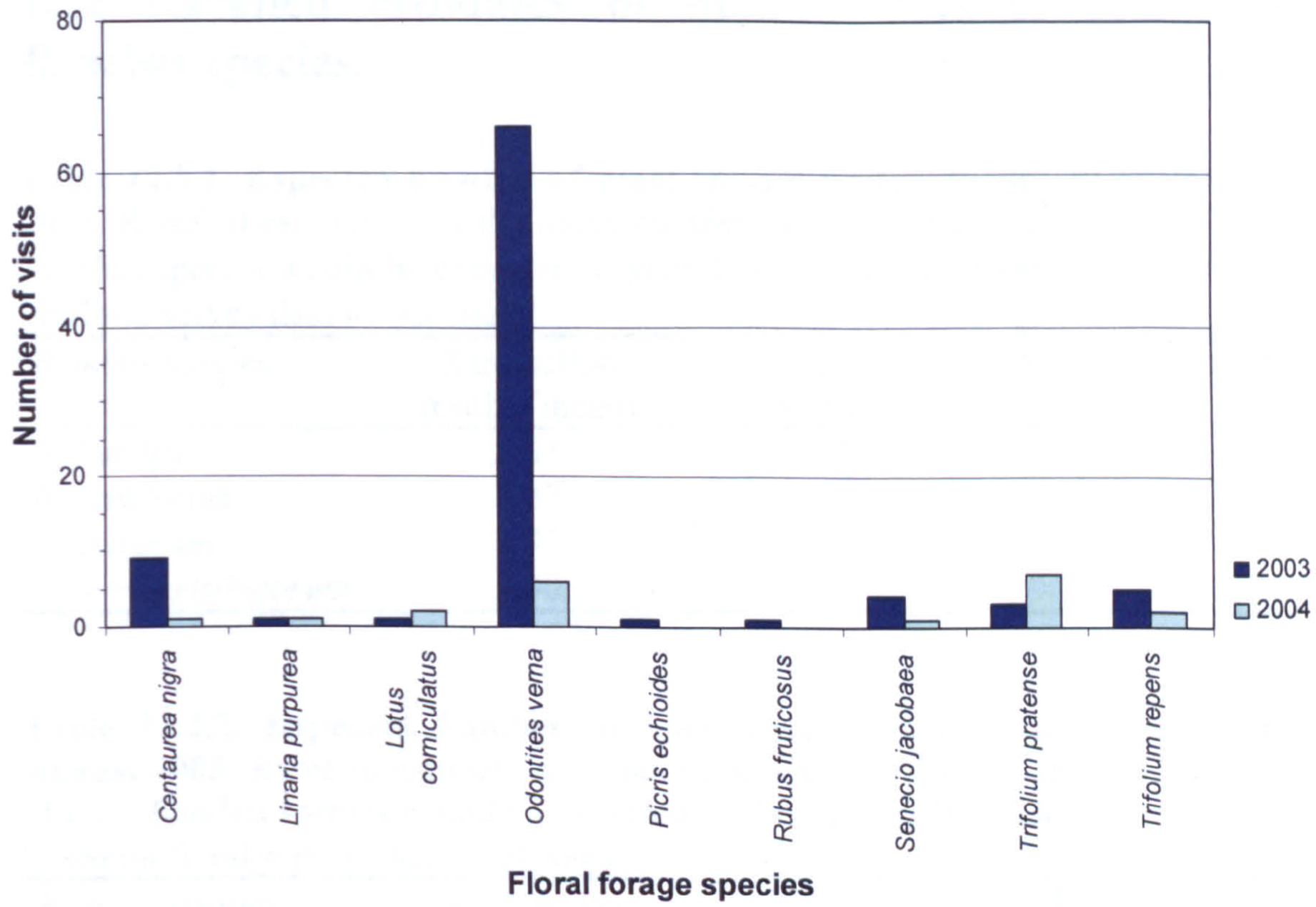


Figure 12.1.11. *Bombus humilis* sightings at Vange Marsh North, July to September 2003 and 2004. Based on sightings of all *B. humilis* flower visits during bee walk surveys at the site.

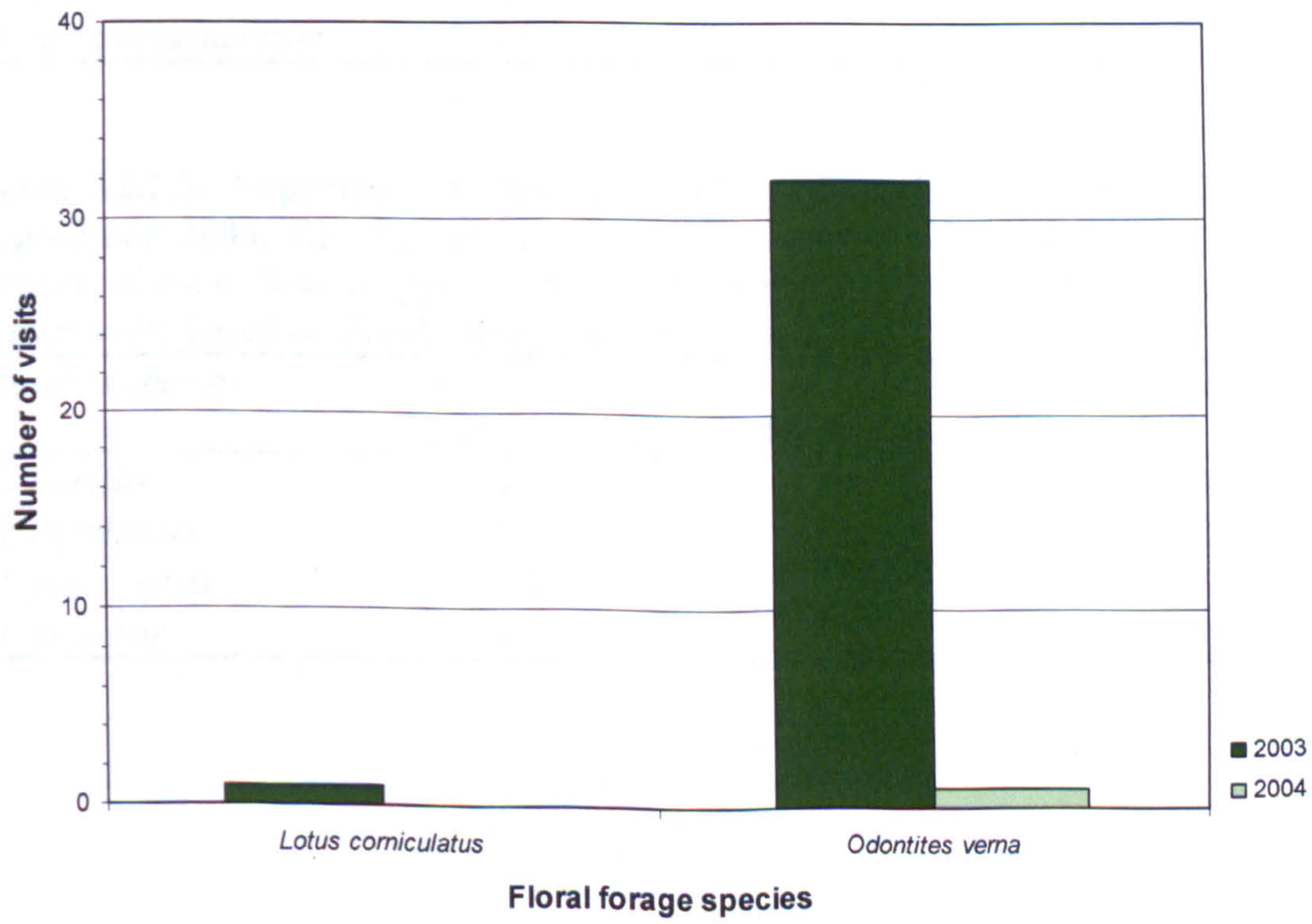


Figure 12.1.12. *Bombus sylvarum* sightings at Vange Marsh North, July to September 2003 and 2004. Based on sightings of all *B. sylvarum* flower visits during bee walk surveys at the site.

12.2 Rarefied estimates of dietary breadth for each *Bombus* species.

Table 12.2.1. Expected number of plant species visited during 20 visits, July 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	8.03	93	0.15
B. lapidarius	8.07	93	0.15
B. sylvarum	1.95	21	0.05
B. terrestris/lucorum	5.00	9	0.00

Table 12.2.2. Expected number of plant species visited during 20 visits, August 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	7.29	332	0.07
B. lapidarius	7.74	161	0.11
B. pascuorum	6.11	59	0.17
B. pratorum	3.81	24	0.08
B. sylvarum	3.91	117	0.07
B. terrestris/lucorum	11.00	17	0.00

Table 12.2.3. Expected number of plant species visited during 20 visits, September 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	4.87	417	0.06
B. lapidarius	4.67	24	0.11
B. pascuorum	5.45	175	0.09
B. sylvarum	4.16	253	0.06

Table 12.2.4. Expected number of plant species visited during 20 visits, July/August/September 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	6.61	826	0.05
B. lapidarius	8.85	248	0.10
B. pascuorum	6.04	236	0.08
B. pratorum	4.68	26	0.10
B. sylvarum	4.16	391	0.05
B. terrestris/lucorum	13.73	33	0.21

Table 12.2.5. Expected number of plant species visited during 20 visits, July 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. hortorum	9.54	32	0.16
B. humilis	8.25	233	0.09
B. lapidarius	8.20	618	0.06
B. pascuorum	6.78	283	0.08
B. ruderarius	8.00	13	0.00
B. sylvarum	4.45	112	0.11
B. terrestris/lucorum	7.39	219	0.11

Table 12.2.6. Expected number of plant species visited during 20 visits, August 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	6.56	360	0.07
B. lapidarius	4.41	158	0.10
B. pascuorum	6.65	216	0.09
B. sylvarum	4.56	192	0.07
B. terrestris/lucorum	10.53	40	0.23

Table 12.2.7. Expected number of plant species visited during 20 visits, September 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	7.03	46	0.16
B. pascuorum	7.14	78	0.14
B. sylvarum	5.00	17	0.00

Table 12.2.8. Expected number of plant species visited during 20 visits, July/August/September 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. hortorum	9.51	33	0.16
B. humilis	8.35	639	0.06
B. lapidarius	8.02	800	0.05
B. pascuorum	7.42	577	0.06
B. ruderarius	8.00	13	0.00
B. sylvarum	4.71	310	0.07
B. terrestris/lucorum	8.39	259	0.11

Table 12.2.9. Expected number of plant families visited during 20 visits, July 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	4.46	93	0.09
B. lapidarius	3.73	93	0.07
B. sylvarum	1.95	21	0.05
B. terrestris/lucorum	3.00	9	0.00

Table 12.2.10. Expected number of plant families visited during 20 visits, August 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	4.19	332	0.03
B. lapidarius	3.96	161	0.07
B. pascuorum	4.69	59	0.11
B. pratorum	2	24	0.00
B. sylvarum	3.42	117	0.05
B. terrestris/lucorum	6.00	17	0.00

Table 12.2.11. Expected number of plant families visited during 20 visits, September 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	3.63	417	0.02
B. lapidarius	3.83	24	0.08
B. pascuorum	4.43	175	0.05
B. sylvarum	3.79	253	0.05

Table 12.2.12. Expected number of plant families visited during 20 visits, July/August/September 2003. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	4.22	826	0.03
B. lapidarius	4.05	248	0.05
B. pascuorum	4.58	236	0.04
B. pratorum	2.95	26	0.04
B. sylvarum	3.7	391	0.03
B. terrestris/lucorum	5.91	33	0.14

Table 12.2.13. Expected number of plant families visited during 20 visits, July 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. hortorum	4.72	32	0.08
B. humilis	4.09	233	0.05
B. lapidarius	4.65	640	0.04
B. pascuorum	3.43	283	0.04
B. ruderarius	5.00	13	0.00
B. sylvarum	3.35	112	0.07
B. terrestris/lucorum	4.89	219	0.07

Table 12.2.14. Expected number of plant families visited during 20 visits, August 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	3.75	360	0.04
B. lapidarius	3.22	158	0.07
B. pascuorum	4.03	216	0.04
B. sylvarum	3.43	192	0.05
B. terrestris/lucorum	6.76	40	0.16

Table 12.2.15. Expected number of plant families visited during 20 visits, September 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. humilis	4.00	46	0.01
B. pascuorum	4.60	78	0.09
B. sylvarum	3.00	17	0.00

Table 12.2.16. Expected number of plant families visited during 20 visits, July/August/September 2004. Rarefied estimation of the mean number of floral species a worker of each *Bombus* species would be expected to visit during 20 random flower visits based on floral survey data for all sites.

Bombus species	Rarefaction results (mean)	Total observations	S.E. of the mean
B. hortorum	4.78	33	0.07
B. humilis	4.02	639	0.03
B. lapidarius	4.40	799	0.04
B. pascuorum	3.98	577	0.03
B. ruderarius	5.00	13	0.00
B. sylvarum	3.61	310	0.04
B. terrestris/lucorum	5.28	259	0.07

12.3 Summary statistics for rarefied estimates of dietary breadth for each *Bombus* species.

Table 12.3.1. Rarefied dietary breadth for each *Bombus* species at the floral species level. Summary data based on all months that >20 observation were made for the *Bombus* species. Observation made across all sites. [Valid n– number of months with >20 observations, S.E.– Standard error of the mean]

Bombus species	Valid n	Mean	Minimum	Maximum	S.E.
B. hortorum	2	9.52	9.51	9.54	0.01
B. humilis	6	7.01	4.87	8.25	0.50
B. lapidarius	5	6.62	4.41	8.2	0.85
B. pascuorum	5	6.43	5.45	7.14	0.30
B. pratorum	2	4.24	3.81	4.68	0.18
B. sylvarum	5	3.81	1.95	4.56	0.48
B. terrestris/lucorum	2	8.96	7.39	10.53	1.57

Table 12.3.2. Rarefied dietary breadth for each *Bombus* species at the floral family level. Summary data based on all months that >20 observation were made for the *Bombus* species. Observation made across all sites. [Valid n = number of months with >20 observations]

Bombus species	Valid n	Mean	Minimum	Maximum	Std. Dev.
B. hortorum	2	4.75	4.72	4.78	0.03
B. humilis	6	4.02	3.63	4.46	0.12
B. lapidarius	5	3.88	3.22	4.65	0.23
B. pascuorum	5	4.47	4.03	4.69	0.12
B. pratorum	2	2.48	2.00	2.95	0.47
B. sylvarum	5	3.19	1.95	3.79	0.32
B. terrestris/lucorum	2	5.83	4.89	6.76	0.93

12.4 Bumblebee survey results for dietary preference analysis.

Table 12.4.1. Bumblebee worker sightings June - Sept 2004. Summary of all flower visits observed across all sites during bee surveys for each *Bombus* species.

Floral species	*B. sylvarum*	*B. pascuorum*	*B. terrestris/lucorum*	*B. humilis*	*B. lapidarius*	**Totals**
Achillea millefolium	0	0	0	1	0	**1**
Aquilegia spp	0	0	1	0	0	**1**
Arctium lappa	0	0	1	0	0	**1**
Ballota nigra	37	71	2	44	4	**158**
Brassicaceae spp	0	0	0	0	2	**2**
Brassica nigra	0	0	5	0	10	**15**
Buddleja davidii	0	0	3	0	0	**3**
Calepina irregularis	0	0	0	0	1	**1**
Calystegia sepium	3	5	7	0	4	**19**
Carduus tenuiflorus	0	1	0	0	0	**1**
Centaurea nigra	3	14	2	14	32	**65**
Cirsium arvense	1	0	9	0	34	**44**
Cirsium spp	0	1	0	0	0	**1**
Cirsium vulgare	0	1	2	4	1	**8**
Colutea arborescens	0	1	8	7	0	**16**
Crataegus monogyna	0	0	1	0	0	**1**
Crepis capillaris	0	2	0	0	2	**4**
Digitalis purpurea	0	0	2	0	0	**2**
Dipsacus fullonum	0	0	0	4	0	**4**
Echium vulgare	0	1	2	0	0	**3**
Epilobium hirsutum	0	0	1	0	1	**2**
Galega officinalis	7	14	29	9	23	**82**

Floral species (cont.)	*B. sylvarum*	*B. pascuorum*	*B. terrestris/lucorum*	*B. humilis*	*B. lapidarius*	**Totals**
Geranium spp	0	0	0	0	1	**1**
Hypericum androsaemum	0	0	1	0	0	**1**
Hypericum perforatum	0	0	0	1	0	**1**
Inula crithmoides	0	0	1	0	0	**1**
Iris psuedacorus	0	0	1	0	0	**1**
Lamium album	1	0	0	0	0	**1**
Lathyrus hisutus	0	0	0	0	1	**1**
Lathyrus latifolius	0	4	55	40	0	**99**
Lathyrus nissolia	0	3	0	0	0	**3**
Lathyrus pratensis	0	2	1	1	0	**4**
Lavandula spp	16	68	6	75	29	**194**
Lilac spp	0	0	1	0	0	**1**
Linaria purpurea	0	4	1	1	0	**6**
Linaria vulgaris	0	0	0	0	1	**1**
Linum bienne	0	0	0	0	1	**1**
Lotus corniculatus	0	15	1	16	128	**160**
Lotus glaber	59	23	18	168	237	**505**
Lythrum salicaria	1	4	0	4	2	**11**
Malva sylvestris	2	0	4	1	12	**19**
Medicago sativa	0	2	6	6	0	**14**
Melilotus alba	1	0	0	0	1	**2**
Melilotus officinalis	1	1	6	1	6	**15**
Mentha spicata	0	1	0	1	0	**2**
Misopates orontium	0	0	3	0	0	**3**
Odontites verna	184	254	74	161	34	**707**
Ononis spinosa	0	0	0	1	1	**2**
Origanum vulgare	0	0	3	0	3	**6**

Floral species (cont.)	*B. sylvarum*	*B. pascuorum*	*B. terrestris/lucorum*	*B. humilis*	*B. lapidarius*	**Totals**
Ornamental rose	1	0	0	0	0	**1**
Ornamental Thistle	0	0	1	0	0	**1**
Picris echioides	0	1	2	0	10	**13**
Picris hieracioides	1	0	0	1	0	**2**
Potentilla reptans	0	0	1	0	5	**6**
Prunella vulgaris	0	2	0	2	0	**4**
Pulicaria dysenterica	0	0	1	0	0	**1**
Rubus fruticosus	1	14	184	11	60	**270**
Sedum reflexum	0	0	4	0	23	**27**
Senecio jacobea	0	0	0	1	0	**1**
Senecio squalidus	0	0	0	0	1	**1**
Solanum dulcamara	0	0	0	1	0	**1**
Taraxacum spp	0	0	0	0	1	**1**
Trifolium arvense	0	0	0	0	2	**2**
Trifolium pratense	2	64	3	73	16	**158**
Trifolium repens	2	24	6	21	121	**174**
Verbenaceae spp	0	5	0	0	0	**5**
Vicia cracca/villosa	0	63	142	4	20	**229**
Vicia sativa	0	1	1	0	0	**2**
Vicia tenuifolia	2	5	1	10	1	**19**
Totals	**325**	**671**	**603**	**684**	**831**	**3114**

12.5 Results of 2004/5 pollen sample analyses.

Table 12.5.1. 2004 pollen sample analyses. Percentage composition of 200 random pollen grains from each sample. [q – queen, w – worker]

Bombus species and caste	Pollen plants present (generally to family)
humilis q	Fabaceae: Vicia type 50%, Rosaceae: Rubus type 50%
humilis q	Fabaceae: Vicia type 100%
humilis q	Fabaceae: Vicia type 100%
humilis q	Fabaceae: Vicia type 50%, Rosaceae: Rubus type
humilis q	Fabaceae: Vicia type 100%
humilis q	very little pollen: Rosaceae
humilis q	Fabaceae: Vicia type 100%
humilis q	Fabaceae: Vicia type 100%
humilis q	Fabaceae: Vicia type 50%; Trifolium pratense type 50%
humilis w	Fabaceae: Trifolium repens type, Trifolium pratense type, Vicia type, Lotus type
humilis w	Fabaceae
humilis w	Fabaceae: Trifolium pratense type; Rosaceae; tiny unknown pollen A
humilis w	Fabaceae: Lotus type, Galega type; Asteraceae: Cirsium type
humilis w	Rosaceae: Rubus type 100%
humilis w	Fabaceae: Galega type 100%
humilis w	Fabaceae: Galega type 80%; Rosaceae: Rubus type 20%
humilis w	Fabaceae: Vicia type, Galega type; Rosaceae
humilis w	Fabaceae: Lotus type, Trifolium pratense type, Trifolium repens type
humilis w	Fabaceae: Trifolium pratense type 60%, Trifolium type 40%
humilis w	Fabaceae: Vicia type 100%
humilis w	Fabaceae: Trifolium repens type 90%; Asteraceae: Centaurea nigra type 10%
humilis w	Scrophulariaceae: Odontites type 90%; Fabaceae: Vicia type 10%
humilis w	Fabaceae: Trifolium pratense type 50%, Lotus type 50%

humilis w	Fabaceae: Trifolium pratense type 10%, Vicia type 40%, unknown triporate pollen 50%
humilis w	Fabaceae: Trifolium pratense type 100%
humilis w	Fabaceae: Galega type 100%
humilis w	Fabaceae: Lotus type 90%, Asteraceae: Centaurea nigra type 10%
humilis w	Scrophulariaceae Odontites type; Fabaceae: Lotus type, Trifolium pratense type, Trifolium repens type; Galega type
humilis w	Scrophulariaceae: Odontites type 90%; Fabaceae: Trifolium pratense type 10%
humilis w	Fabaceae: Lotus type 100%
humilis w	Fabaceae: Trifolium pratense type, Trifolium repens type, Lotus type
humilis w	Fabaceae: Lotus type 100%
humilis w	Fabaceae: Trifolium pratense type 100%
humilis w	Fabaceae: Galega type 95%, Trifolium pratense type 5%
humilis w	Fabaceae: Lotus type 90%,Trifolium pratense type 10%
humilis w	Fabaceae: Galega type 40%; Scrophulariaceae: Odontites type 30%; Asteraceae: Centaurea nigra type 30% unknown pollen 10%
humilis w	Fabaceae: Trifolium pratense type 50&, Lotus type 50%
humilis w	Fabaceae: Lotus type 90%; Rosaceae: 10%
humilis w	Fabaceae: Lotus type 100%
humilis w	Fabaceae: Galega type 80%; Scrophulariaceae: Odontites type 10%; rest a mixture of several pollen types, no species dominating
humilis w	Fabaceae: Trifolium pratense 100%
humilis w	Fabaceae: Trifolium pratense 100%
humilis w	Asteraceae: Centaurea nigra type 60%; Fabaceae: Trifolium pratense type 40%
humilis w	Fabaceae: Lotus type 90%; Lamiaceae: Lamium type 5%; Asteraceae: Centaurea nigra type 5%
humilis w	Fabaceae: Lotus type 90%, Trifolium pratense type 10%
sylvarum q	Fabaceae: Colutea type 95%, Trifolium pratense type 5%
sylvarum q	Fabaceae: Vicia type 100%
sylvarum w	Rosaceae: Rubus type; Fabaceae: Galega type; very large pollen A
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Fabaceae: Lotus type 90%; unknown pollen 10%

sylvarum w	Fabaceae: Lotus type 100%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Fabaceae: Galega type 33%; Lamiaceae: Lamium type 33%; Scrophulariaceae: Odontites type 33%
sylvarum w	Fabaceae: Lotus type 100%
sylvarum w	
sylvarum w	Fabaceae: Lotus type 90%; unknown Pollen 10%
sylvarum w	Fabaceae: Lotus type 100%
sylvarum w	Fabaceae: Lotus type 50%; Rosaceae: Rubus type 50%
sylvarum w	Fabaceae: Trifolium pratense type, Lotus type; Scrophulariaceae: Odontites type; Asteraceae: Cirsium type
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Scrophulariaceae: Odontites type 80%; Fabaceae: Trifolium pratense type 10%, Vicia type 10%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Scrophulariaceae: Odontites type 33%; Fabaceae: Trifolium pratense type 33%, Lotus type 33%
sylvarum w	Fabaceae: Lotus type 95%, rest Trifolium type, Lamium type, a few Trifolium pratense
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Scrophulariaceae: Odontites type 50%; Fabaceae: Galega type 50%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Fabaceae: Galega type 90%; unknown pollen 10%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Lamiaceae: Lamium type 33%; Fabaceae: Galega type 33%; Rosaceae 33%
sylvarum w	Scrophulariaceae: Odontites type 100%
sylvarum w	Scrophulariaceae: Odontites type 90%; Fabaceae: Trifolium pratense type 10%
sylvarum w	Mixture of Asteraceae: Cirsium type, Centaurea nigra type; Fabaceae: Lotus type; Scrophulariaceae: Odontites type
sylvarum w	Lamiaceae: Lamium type 40%; Fabaceae: Lotus type 50%; Asteraceae: Helianthus type 10%

Table 12.5.2. 2005 pollen sample analyses. Percentage composition of 200 random pollen grains from each sample. [All worker samples]

Bombus species	Pollen plants present (generally to family)
humilis	Fabaceae: Galega type 70%; Rosaceae: Rubus type 30%
humilis	Fabaceae: Trifolium pratense type 51%; Rosaceae: Rubus type: 49%
humilis	Fabaceae: Trifolium pratense type 90%, Lotus type 10%
humilis	Fabaceae: Galega type 55%, Trifolium pratense type 25%; Rosaceae: Rubus type: 20%
humilis	Fabaceae: Trifolium pratense type 10%, Trifolium repens type 5%, Galega type 80%; Rosaceae: Rubus type 5%
humilis	
humilis	Scrophulariaceae: Odontites type 95%; Fabaceae: Trifolium pratense type 5%
humilis	Scrophulariaceae: Odontites type 10%; Rosaceae: Rubus type 88%; Fabaceae: Trifolium pratense type 2%
humilis	Fabaceae: Trifolium pratense type
humilis	Fabaceae: Lotus type
humilis	Fabaceae: Galega type 72%; Asteraceae: Centaurea nigra type 11%: Scrophulariaceae: Odontites type 17%
humilis	Fabaceae: Lotus type 60%, Trifolium pratense 20%; Scrophulariaceae: Odontites type 15%; Asteraceae: Centaurea nigra type 5%
humilis	Rosaceae: Rubus type 75%; Asteraceae: Cirsium type 15%; Fabaceae: Trifolium pratense type 10%
humilis	Rosaceae: Rubus type 95%; Fabaceae: Trifolium pratense type 5%
humilis	Scrophulariaceae: Odontites type 97%; Fabaceae: Trifolium pratense type 3%
humilis	Lamiaceae: Salvia type 26%; Rosaceae: Rubus type 31%; Dipsacaceae: Knautia type 17%; Fabaceae: Trifolium pratense type 6% Unknown pollen 20%
humilis	Fabaceae: Lotus type 36%, Trifolium pratense type 3%; Scrophulariaceae: Odontites type 31%; Lamiaceae: Salvia type 3%; unknown pollen type 27%
humilis	Scrophulariaceae: Odontites type 80%; Fabaceae: Trifolium pratense type 20%
humilis	Fabaceae: Trifolium pratense type 80%, Scrophulariaceae: Odontites type 20%
humilis	Asteraceae: Centaurea nigra type
humilis	Scrophulariaceae: Odontites type
humilis	Scrophulariaceae: Odontites type

humilis	Fabaceae: Trifolium pratense type 56%, Scrophulariaceae: Odontites type 44%
humilis	Fabaceae: Galega type 92%; Asteraceae; Centaurea nigra type 6%; Fabaceae: Trifolium pratense type 2%
humilis	Fabaceae: Trifolium pratense type
humilis	Scrophulariaceae: Odontites type 69%; Asteraceae: Cirsium type 21%; Fabaceae: Lotus type 7%, Trifolium pratense type 3%
humilis	Scrophulariaceae: Odontites type 65%; Asteraceae: Centaurea nigra type 35%
humilis	Fabaceae: Lotus type 65%; Asteraceae: Centaurea nigra type 22%; Fabaceae: Trifolium pratense type 13%,
humilis	Fabaceae: Lotus type 63%, Trifolium pratense type 10%, Vicia type 3%; Asteraceae: Centaurea nigra type 14%; unknown pollen 10%
sylvarum	Fabaceae: Trifolium repens type
sylvarum	Lamiaceae: Lamium type 50%; Fabaceae: Trifolium pratense type 50%
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type 55%; Asteraceae: Cirsium type 45%
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Fabaceae: Lotus type 90%; Lamiaceae: Lamium type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type 55%; Fabaceae: Lotus type 30%; Asteraceae: Cirsium type 10%, Unknown 5%
sylvarum	Scrophulariaceae: Odontites type 50%; Asteraceae: Cirsium type 50%
sylvarum	Scrophulariaceae: Odontites type 66%; Asteraceae: Cirsium type 34%
sylvarum	Scrophulariaceae: Odontites type 70%; Asteraceae: Cirsium type 20%; Dipsacaceae: Knautia type 10%
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Fabaceae: Lotus type 90%; Scrophulariaceae: Odontites type 10%
sylvarum	Fabaceae: Lotus type
sylvarum	Fabaceae: Lotus type 95%; Scrophulariaceae: Odontites type 5%

sylvarum	Fabaceae: Lotus type
sylvarum	Fabaceae: Lotus type
sylvarum	Fabaceae: Lotus type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type
sylvarum	Scrophulariaceae: Odontites type 90%; Asteraceae: Cirsium type 10%
sylvarum	Scrophulariaceae: Odontites type 62%; Asteraceae: Cirsium type 17%, Centaurea nigra type 21%
sylvarum	Scrophulariaceae: Odontites type 40%; Fabaceae: Lotus type 25%, Trifolium pratense type 25%; Asteraceae: Centaurea nigra type10%
sylvarum	Scrophulariaceae: Odontites type 69%; Asteraceae: Centaurea nigra type 31%
sylvarum	Fabaceae: Lotus type 95%; Asteraceae: Cirsium type 5%
sylvarum	Scrophulariaceae: Odontites type
lapidarius	Fabaceae: Lotus type 91%; Rosaceae: Rubus type 9%
lapidarius	Fabaceae: Galega type 56%; Rosaceae: Rubus type 44%
lapidarius	Asteraceae: Centaurea nigra type
lapidarius	Fabaceae: Galega type
lapidarius	Fabaceae: Lotus type 90%; Asteraceae: Centaurea nigra type 10%
lapidarius	Rosaceae: Rubus type
lapidarius	Fabaceae: Lotus type 95%; Rosaceae 5%
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	Asteraceae: Centaurea nigra type
lapidarius	Fabaceae: Galega type
lapidarius	Scrophulariaceae: Odontites type
lapidarius	Scrophulariaceae: Odontites type
lapidarius	Fabaceae: Lotus type 71%, Trifolium repens type 29%
lapidarius	Fabaceae: Galega type 90%; Scrophulariaceae: Odontites type 10%
lapidarius	Scrophulariaceae: Odontites type
lapidarius	Scrophulariaceae: Odontites type

lapidarius	Scrophulariaceae: Odontites type
lapidarius	Scrophulariaceae: Odontites type
lapidarius	Scrophulariaceae: Odontites type
lapidarius	Scrophulariaceae: Odontites type
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type 95%; Rosaceae 5%
lapidarius	Fabaceae: Lotus type
lapidarius	Fabaceae: Lotus type
lapidarius	sample resp. tube missing
pascuorum	Fabaceae: Galega type
pascuorum	Rosaceae: Rubus type 98%; Fabaceae: Lotus type 2%
pascuorum	Fabaceae: Galega type 90%; Rosaceae: Rubus type 10%
pascuorum	Rosaceae: Rubus type
pascuorum	Rosaceae: Rubus type 47%; Fabaceae: Trifolium pratense 40%, Lotus type 6%; Lamiaceae: Lamium type 7%
pascuorum	Rosaceae: Rubus type 90%; Fabaceae: Lotus type 8%; unknown type 2%
pascuorum	Fabaceae: Trifolium pratense type 35%; Rosaceae: Rubus type 65%
pascuorum	Fabaceae: Galega type 93%; Fabaceae: Trifolium pratense type 7%
pascuorum	Fabaceae: Galega type
pascuorum	Rosaceae: Rubus type
pascuorum	Scrophulariaceae: Odontites type
pascuorum	Fabaceae: Lotus type 93%; Asteraceae: Cirsium type 5%; Centaurea nigra type 2%
pascuorum	Fabaceae: Trifolium pratense type 71%; Rosaceae: Rubus type 29%
pascuorum	Scrophulariaceae: Odontites type

pascuorum	Scrophulariaceae: Odontites type 65%; Fabaceae: Vicia type 13%, Trifolium pratense 9%, Lotus type 8%; Asteraceae: Centaurea nigra type 3%; Lavandula 1%; Unknown 1%
pascuorum	Fabaceae: Trifolium pratense type
pascuorum	Scrophulariaceae: Odontites type
pascuorum	Rosaceae: Rubus type 55%; Scrophulariaceae: Odontites type 45%
pascuorum	Scrophulariaceae: Odontites type
pascuorum	Scrophulariaceae: Odontites type
pascuorum	Fabaceae: Galega type 90%; Rosaceae: Rubus type 10%
pascuorum	Scrophulariaceae: Odontites type 97%; Asteraceae: Centaurea nigra type 3%
pascuorum	Rosaceae: Rubus type
pascuorum	Rosaceae: Rubus type
pascuorum	Rosaceae type
pascuorum	Rosaceae: Rubus type
pascuorum	Fabaceae: Lotus type 51%, Trifolium pratense type 31%, Vicia type 18%
pascuorum	Fabaceae: Lotus type 79%, Trifolium pratense type 12%; Asteraceae: Cirsium type 5%, Centaurea nigra type 4%
pascuorum	Fabaceae: Trifolium pratense type 97%, Trifolium repens type 3%
pascuorum	Fabaceae: Trifolium pratense type 69%, Lotus type 21%, Trifolium repens type 10%
pascuorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Rosaceae: Rubus type
terrestris/lucorum	Rosaceae: Rubus type
terrestris/lucorum	Rosaceae: Rubus type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Rosaceae: Rubus type 44%; Fabaceae: Galega type 56%
terrestris/lucorum	Scrophulariaceae: Odontites type 90%; Rosaceae: Rubus type 10%
terrestris/lucorum	Fabaceae: Lotus type
terrestris/lucorum	Rosaceae: Rubus type 95%; Fabaceae: Galega type 5%

terrestris/lucorum	Fabaceae: Medicago type
terrestris/lucorum	Fabaceae: Medicago type
terrestris/lucorum	Scrophulariaceae: Odontites type 97%; Asteraceae: Cirsium type 3%
terrestris/lucorum	Fabaceae: Medicago type 30%; Asteraceae: Cirsium type 10%; Onagraceae: Epilobium type 10%; rest non-expanded pollen
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Rosaceae: Rubus type 50%; Fabaceae: Galega type 50%
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Fabaceae: Lotus type
terrestris/lucorum	Fabaceae: Lotus type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Fabaceae: Lotus type
terrestris/lucorum	Fabaceae: Galega type 76%, Trifolium pratense 2%; Scrophulariaceae: Odontites type 14%; Asteraceae: Aster type 8%
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type
terrestris/lucorum	Scrophulariaceae: Odontites type 60%; unknown pollen type 40%

12.6 Bumblebee colonies identified at each forage patch

Table 12.6.1. *Bombus humilis* colonies at benfleet forage patch Hadleigh Castle Country Park, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches sisters from colony recorded on						
1	HCCP						
	Ben						
2	HCCP						
	Ben						
3	HCCP						
	Ben						
4	HCCP						
	Ben						
5	HCCP						
	Ben						
6	HCCP						
	Ben						
7	HCCP						
	Ben						
8	HCCP						
	Ben						
9	HCCP						
	Ben						
10	HCCP						
	Ben						
11	HCCP						
	Ben						
12	HCCP	HCCP					
	Ben	Ben					
13	HCCP	HCCP					
	Ben	Ben					
14	HCCP	HCCP					
	Ben	Ben					
15	HCCP	HCCP					
	Ben	Marsh					
16	HCCP	Canvey					
	Ben	West					
17	HCCP	Canvey					
	Ben	West					
18	HCCP	Canvey					
	Ben	West					
19	HCCP	Canvey					
	Ben	East					
20	HCCP	Canvey					
	Ben	East					
21	HCCP	Wat Outer					
	Ben						
22	HCCP	HCCP	HCCP				
	Ben	Ben	Marsh				
23	HCCP	Wat Outer	Canvey				
	Ben		East				
24	HCCP	HCCP	HCCP	HCCP			
	Ben	Marsh	Marsh	Marsh			
25	HCCP	Canvey	Canvey	HCCP			
	Marsh	East	West	Ben			
26	HCCP	HCCP	HCCP	HCCP	HCCP	HCCP	Canvey
	Ben	Marsh	Marsh	Marsh	Marsh	Marsh	West

Table 12.6.2. *Bombus humilis* colonies at marsh forage patch Hadleigh Castle Country Park, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches *sisters from colony recorded* on						
1	HCCP						
	Marsh						
2	HCCP						
	Marsh						
3	HCCP						
	Marsh						
4	HCCP						
	Marsh						
5	HCCP						
	Marsh						
6	HCCP						
	Marsh						
7	HCCP						
	Marsh						
8	HCCP						
	Marsh						
9	HCCP						
	Marsh						
10	HCCP						
	Marsh						
11	HCCP	HCCP					
	Marsh	Marsh					
12	HCCP	HCCP					
	Ben	Marsh					
13	HCCP	Wat					
	Marsh	Centre					
14	Wat	HCCP					
	Centre	Marsh					
15	HCCP	Canvey					
	Marsh	East					
16	HCCP	Canvey					
	Marsh	East					
17	HCCP	HCCP	HCCP				
	Ben	Ben	Marsh				
18	HCCP	Canvey	Canvey				
	Marsh	East	East				
19	HCCP	Canvey	Canvey				
	Marsh	West	West				
20	HCCP	Wat Outer	Canvey				
	Marsh		West				
21	HCCP	Canvey	Canvey	HCCP			
	Marsh	East	West	Ben			
22	HCCP	HCCP	HCCP	HCCP			
	Ben	Marsh	Marsh	Marsh			
23	HCCP	HCCP	HCCP	HCCP	HCCP	HCCP	Canvey
	Ben	Marsh	Marsh	Marsh	Marsh	Marsh	West

Table 12.6.3. *Bombus humilis* colonies at west forage patch Canvey Wick, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colonies	List of forage patches sisters from colony recorded on						
1	Canvey West						
2	Canvey West						
3	Canvey West						
4	Canvey West						
5	Canvey West						
6	Canvey West						
7	Canvey West						
8	Canvey West	Canvey West					
9	Canvey West	Canvey East					
10	Canvey West	Canvey East					
11	Canvey West	Canvey East					
12	Canvey West	Wat Centre					
13	Canvey West	Wat Outer					
14	Canvey West	Wat Outer					
15	HCCP Ben	Canvey West					
16	HCCP Ben	Canvey West					
17	HCCP Ben	Canvey West					
18	Canvey West	Canvey East	Canvey West				
19	Canvey West	Canvey East	Canvey East				
20	Canvey West	Canvey East	Canvey West				
21	Canvey West	Wat Centre	Canvey West				
22	HCCP Marsh	Canvey West	Canvey West				
23	HCCP Marsh	Wat Outer	Canvey West				
24	HCCP Marsh	Canvey East	Canvey West	HCCP Ben			
25	HCCP Ben	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh	Canvey West

Table 12.6.4. *Bombus humilis* colonies at east forage patch Canvey Wick, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches sisters from colony recorded on			
1	Canvey East			
2	Canvey East			
3	Canvey East			
4	Canvey East			
5	Canvey East			
6	Canvey East			
7	Canvey East			
8	Canvey East			
9	Canvey East			
10	Canvey East			
11	Canvey East	Canvey East		
12	Canvey East	Canvey East		
13	Canvey West	Canvey East		
14	Canvey West	Canvey East		
15	Canvey West	Canvey East		
16	HCCP Ben	Canvey East		
17	HCCP Ben	Canvey East		
18	HCCP Marsh	Canvey East		
19	HCCP Marsh	Canvey East		
20	Canvey East	Wat Outer		
21	HCCP Ben	Wat Outer	Canvey East	
22	Canvey West	Canvey East	Canvey West	
23	Canvey West	Canvey East	Canvey East	
24	Canvey West	Canvey East	Canvey West	
25	HCCP Marsh	Canvey East	Canvey East	
26	HCCP Marsh	Canvey East	Canvey West	HCCP Ben

Table 12.6.5. *Bombus humilis* colonies at central forage patch Wat Tyler Country Park, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches sisters from colony recorded on		
1	Wat Centre		
2	Wat Centre		
3	Wat Centre		
4	Wat Centre		
5	Wat Outer		
6	Wat Outer		
7	Wat Outer		
8	Wat Outer		
9	Wat Centre	Wat Centre	
10	Wat Centre	Wat Centre	
11	Wat Centre	Wat Centre	
12	Wat Centre	Wat Outer	
13	Wat Centre	Wat Outer	
14	Wat Centre	Wat Outer	
15	HCCP Marsh	Wat Centre	
16	Wat Centre	HCCP Marsh	
17	HCCP Ben	Wat Outer	
18	Canvey West	Wat Outer	
19	Canvey West	Wat Centre	
20	Canvey West	Wat Outer	
21	Canvey East	Wat Outer	
22	Canvey West	Wat Centre	Canvey West
23	HCCP Marsh	Wat Outer	Canvey West
24	HCCP Ben	Wat Outer	Canvey East

Table 12.6.6. *Bombus sylvarum* colonies at benfleet forage patch Hadleigh Castle Country Park, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches sisters from colony recorded on				
1	HCCP Ben				
2	HCCP Ben				
3	HCCP Ben				
4	HCCP Ben				
5	HCCP Ben				
6	HCCP Ben				
7	HCCP Ben				
8	HCCP Ben				
9	HCCP Ben				
10	HCCP Ben				
11	HCCP Ben	HCCP Ben			
12	HCCP Ben	HCCP Ben			
13	HCCP Ben	HCCP Marsh	HCCP Marsh		
14	HCCP Ben	Canvey West	Canvey West		
15	HCCP Ben	HCCP Ben	HCCP Marsh		
16	HCCP Ben	HCCP Marsh	HCCP Marsh	HCCP Marsh	
17	HCCP Marsh	HCCP Ben	HCCP Marsh	HCCP Ben	
18	HCCP Ben	HCCP Ben	HCCP Ben	HCCP Ben	
19	HCCP Ben	HCCP Ben	HCCP Ben	HCCP Ben	HCCP Ben

Table 12.6.7. *Bombus sylvarum* colonies at marsh forage patch Hadleigh Castle Country Park, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	*List of forage patches sisters from colony recorded on*					
1	HCCP Marsh					
2	HCCP Marsh					
3	HCCP Marsh					
4	HCCP Marsh					
5	HCCP Marsh					
6	HCCP Marsh					
7	HCCP Marsh					
8	HCCP Marsh	Canvey East				
9	HCCP Marsh	HCCP Marsh				
10	HCCP Ben	HCCP Ben	HCCP Marsh			
11	HCCP Ben	HCCP Marsh	HCCP Marsh			
12	Canvey East	Canvey East	HCCP Marsh			
13	HCCP Marsh	HCCP Ben	HCCP Marsh	HCCP Ben		
14	HCCP Ben	HCCP Marsh	HCCP Marsh	HCCP Marsh		
15	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh	
16	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh	HCCP Marsh

Table 12.6.8. *Bombus sylvarum* colonies at west forage patch Canvey Wick, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches sisters from colony recorded on				
1	Canvey West				
2	Canvey West				
3	Canvey West				
4	Canvey West				
5	Canvey West				
6	Canvey West				
7	Canvey West				
8	Canvey West				
9	Canvey West				
10	Canvey West				
11	Canvey West	Canvey West			
12	Canvey West	Canvey East			
13	Canvey West	Canvey East			
14	Canvey West	Canvey West	Wat Tyler		
15	Canvey West	Wat Tyler	Canvey West		
16	HCCP Ben	Canvey West	Canvey West		
17	Canvey West	Canvey West	Canvey West	Canvey East	
18	Canvey West	Canvey West	Wat Tyler	Wat Tyler	Wat Tyler
19	Canvey West	Canvey West	Canvey West	Canvey West	Canvey West

Table 12.6.9. *Bombus sylvarum* colonies at east forage patch Canvey Wick, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	*List of forage patches sisters from colony recorded on*			
1	Canvey East			
2	Canvey East			
3	Canvey East			
4	Canvey East			
5	Canvey East			
6	Canvey East			
7	Canvey East			
8	Canvey East			
9	Canvey East			
10	Canvey East			
11	Canvey East			
12	Canvey East			
13	Canvey East			
14	Canvey East			
15	Canvey East	Canvey East		
16	Canvey East	Canvey East		
17	Canvey West	Canvey East		
18	Canvey West	Canvey East		
19	HCCP Marsh	Canvey East		
20	Canvey East	Canvey East	Canvey East	
21	Canvey East	Canvey East	HCCP Marsh	
22	Canvey East	Canvey East	Canvey East	
23	Canvey West	Canvey West	Canvey West	Canvey East

Table 12.6.10. *Bombus sylvarum* colonies at centre forage patch Wat Tyler Country Park, 2005. Colonies identified by kinship analysis of microsatellite DNA data ($p \leq 0.01$).

Colony	List of forage patches sisters from colony recorded on				
1	Wat Tyler				
2	Wat Tyler				
3	Wat Tyler				
4	Wat Tyler				
5	Wat Tyler				
6	Wat Tyler				
7	Wat Tyler				
8	Wat Tyler				
9	Wat Tyler				
10	Wat Tyler				
11	Wat Tyler	Wat Tyler			
12	Wat Tyler	Wat Tyler			
13	Wat Tyler	Wat Tyler			
14	Wat Tyler	Wat Tyler			
15	Wat Tyler	Wat Tyler			
16	Wat Tyler	Wat Tyler			
17	Wat Tyler	Wat Tyler	Wat Tyler		
18	Canvey West	Wat Tyler	Canvey West		
19	Canvey West	Canvey West	Wat Tyler		
20	Canvey West	Canvey West	Wat Tyler	Wat Tyler	Wat Tyler