The role of dietary breadth in national bumblebee (*Bombus*) declines: simple correlation?

Stuart CONNOP [a], Tom HILL [a], Jonathan STEER [a] and Phil SHAW [a]

[a] – School of Health and Bioscience, University of East London, Water Lane, E15 4LZ, UK.

Email addresses:
Tom HILL - bufus@hotmail.co.uk
Jonathan STEER - J.M.Steer@uel.ac.uk
Phil SHAW - p.j.shaw@btinternet.com

Author for correspondence:
Dr Stuart Connop, Environmental Research Group, School of Health and Bioscience, University of East London, Water Lane, E15 4LZ, UK.
Phone: +44(0)208 223 4985
Fax: +44(0)208 223 4959
Email: s.p.connop@uel.ac.uk

Abstract

Bumblebee species declines have been reported in Europe, North America and Asia. Loss of suitable habitat to agricultural intensification is considered to be the main cause of declines in Europe. Differential impacts on species have been recorded but insufficient knowledge of species ecology means that effective conservation management prescriptions cannot be put into place with certainty.

Dietary specialisation, specifically on flowers of Fabaceae, has been hypothesised as driving differential declines but the reliability of previous studies has been questioned. Here we present a three-year study of the foraging behaviour of two UK Biodiversity Action Plan bumblebee species. For the first time, analysis of nectar and pollen foraging was performed on sites where nationally rare UK bumblebees were as abundant as more nationally ubiquitous species.

Results indicated that the nationally rare *Bombus sylvarum* collected the majority of its pollen from flowers of *Odontites verna* and had a significantly narrower mean nectar dietary breadth than ecologically similar species *Bombus humilis* and *Bombus pascuorum* (p = 0.004 and 0.008 respectively). In contrast, the dietary breadth of the nationally rare *B. humilis* was similar to the more nationally ubiquitous species *B. pascuorum* and *Bombus lapidarius*. Moreover, *B. lapidarius* was recorded as having the narrowest pollen dietary breadth, collected pollen from the least number of floral taxa and was the most specialised of the *Bombus* species on pollen of Fabaceae.

Patterns of dietary specialization were inconsistent with national declines and results highlighted a need for further detailed investigation into the factors contributing to differential declines.

Keywords: *Bombus sylvarum*, *Bombus humilis*, habitat management, dietary breadth, specialization.

1. Introduction

World-wide studies of native bees, both solitary and social, have revealed disturbing trends of decline over the last 40-50 years (Williams 1982; Rasmont, 1995; Biesmeijer et al., 2006; Kosior et al., 2007; Williams and Osborne 2009). Evidence of declines can be demonstrated, although the effects of such declines are poorly understood (Ghazoul, 2005). A greater understanding of pollination ecology and the specific interactions between individual pollinator species and the plants they pollinate is a necessity if effective conservation strategies are to be developed (Williams, 1995; Williams and Osborne, 2009).

Bumblebees (*Bombus* species) have been recorded as a group demonstrating these trends (Williams, 1982; 1986; Fitzpatrick et al., 2007; Kosior et al., 2007; Goulson et al., 2008), with species declines reported in Europe, North America and Asia (Williams and Osborne 2009). In Europe these declines have been attributed to agricultural intensification and associated habitat loss and fragmentation leading to losses of wildflowers and nesting sites (Williams, 1986; Osborne and Corbet, 1994; Robinson and Sutherland, 2002; Goulson, 2003; Carvell et al., 2006). Rather than impacts being consistent across all bumblebee species however, differential declines between species have been occurring (Alford, 1980; Williams, 1982; Edwards, 1998; 2002). The means by which these impacts are driving differential declines remain poorly understood (Williams et al., 2007; 2009). This is due in part to a lack of understanding of the habitat requirements of individual species (Goulson, 2003). For effective restoration of lost foraging, nesting and hibernation habitat, this gap in knowledge must be addressed.

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 (Ranta and Lundberg 1980; Williams, 1989; Goulson and Darvill, 2004). Goulson and Darvill (2004) and Goulson et al. (2009) have suggested that longer-tongued bumblebees tend to forage preferentially on flowers of Fabaceae as a pollen resource, plants that have been particularly lost through agricultural change (Carvell et al., 2006; Kleijn and Raemakers, 2008). It has been theorised 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; Hanley et al., 2008; Goulson et al., 2009). 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 (Goulson et al., 2005).This theory is supported by reports of high incidences of nectar robbery of flowers with long corolla lengths by short-tongued bees (Stout et al., 2000).

This concept of dietary specialisation among rarer bumblebee species has however been challenged (Williams, 2005; Fitzpatrick et al., 2007) and theories of vulnerability among bumblebees being related to climatic niche position and breadth (Williams et al., 2007) and queen emergence time and habitat specialisation (Edwards and Williams, 2004; Fitzpatrick et al., 2007) have been proposed. Indeed, Williams (2005) questioned whether previous quantitative assessments of bumblebee specialisation data had adequately taken into account the sources of their data for analysis, arguing that for forage data to be comparable it must be from a comparable survey with all bumblebees being subjected to the same foraging options. In the UK there are few sites where most bumblebee species still occur together, where they might choose among the same plants, correspondingly therefore, there are few data sets that permit genuine quantitative comparisons of forager choice (Williams, 2005).

Williams (2005) investigated dietary breadth and dietary preference of bumblebees from historic data at one of the few UK sites where most bumblebee species were still occurring together. Results from the study were not consistent with the relative rarity or decline of bumblebee species in Britain. However, the author acknowledged that the data may have been unrepresentative of British bumblebee foraging in general and lacked information on pollen foraging. A need for further studies on sites where nationally rare species remained abundant was recognised.

More recently Kleijn and Raemakers (2008) and Goulson et al. (2009) were able to demonstrate a general trend between pollen dietary breadth and rarity in bumblebee communities. However, by generalising bumblebee declines, both studies may have overlooked the intricacies involved in individual species ecology occurring within their respective datasets. Whilst contention remains with regards to the dietary behaviour of rare versus ubiquitous bumblebees, and thus the direct drivers of decline for individual species, conservation management plans may be ineffective for all *Bombus* species when designed to target general bumblebee species.

This study was designed to: (1) identify the foraging behaviour in terms of nectar and pollen foraging of two UK Biodiversity Action Plan (UKBAP) species; the brown-banded carder bee, *Bombus humilis* Illiger (UKBAP, 1999), and the shrill carder bee, *Bombus sylvarum* (Linnaeus) (UKBAP, 1995); (2) compare patterns of dietary breadth and specialisation in foraging behaviour of these two species and more nationally ubiquitous bumblebees, *Bombus pascuorum* (Scopoli) and *Bombus lapidarius* (Linnaeus); (3) identify floral species and families of value for conservation habitat management.

The study was carried out according to the guidelines defined by Williams (2005) and Kleijn and Raemakers (2008) to ensure that data on foraging behaviour was all generated under the same foraging options.

2. Materials and methods

2.1 Study sites

Sites known to support a broad mix of bumblebee species were selected for the surveys (Table 1). The sites were distributed across approximately 60km^2 of fragmented habitat in South Essex, UK, comprising a mix of urban, semi-natural grassland, agricultural and post-industrial brownfield land. During 2003, 2004 and 2005 surveys were carried out across these sites to record the foraging behaviour of the UKBAP species *B. humilis* and *B. sylvarum*, and the more nationally ubiquitous *B. pascuorum* and *B. lapidarius*.

Surveys concentrated on nectar foraging and pollen foraging of worker bumblebees. It must be noted that studies of foraging queens, particularly during nest initiation are also necessary for an understanding of the foraging requirement of these bumblebees throughout their colony development (Connop, 2008).

2.2 Nectar foraging surveys

Nectar foraging surveys comprised of a modified version of the bee walk transects used by Banaszak (1980) and Saville et al. (1997). Modification of the method was necessary as forage distribution on the sites was too patchy and discontinuous for single straight-line transect walks to be effective. Thus, non-linear walks covering the whole site and encompassing the main flowering patches within each site were used. Length and approximate duration of walks were repeated within each site throughout the study period. Observations were made approximately 2m either side of the observer and walking speed was about 10m per minute.

Surveys recorded the number and species of *Bombus* workers observed and the floral species visited by each. Flower identification followed Stace (1997). Only foraging visits made by *Bombus* workers for nectar were used for this initial part of the study. No attempt was made to identify whether bumblebees were also collecting pollen from the flowers due to the intrinsic problems involved in identifying whether a flower is being visited for pollen (Connop, 2008). A separate pollen analysis survey was carried out to assess pollen use variation between the bumblebee species. All surveys were carried out by the same recorder to avoid observer bias (Westphal et al., 2008).

Bee walks were carried out from July to September each year. The survey dates corresponded with the peak flight periods for all four species in Southern England as reported by Edwards and Jenner (2005) and on observed peak timings from previous surveys (Connop, 2008). Forty-eight and seventy-one surveys were carried out across the sites in 2003 and 2004 respectively. In order to standardise search conditions, all searches were conducted between 9:30 and 17:00 BST and during warm dry weather favourable to bumblebee activity.

Identification of the bumblebees followed Prŷs-Jones and Corbet's key (1987). Bumblebees which could not be identified whilst foraging were captured using queen bee marking plunger cages (Kwak, 1987) and were identified by species morphology using a field lens. The resulting dataset was analysed to establish the relative nectar dietary breadth of each *Bombus* species (Williams, 2005).

2.3 Pollen foraging surveys

Observation of bumblebee behaviour on flowers has been used as an indication of whether a bumblebee is foraging for nectar or pollen (Carvell, 2002; Goulson et al., 2005). This method can be unreliable as it relies entirely upon assumptions of observed bumblebee behaviour (Connop, 2008). In addition, this method of pollen analysis provides no information on whether the bumblebee is collecting pollen from a single or several plant species or families. A more accurate method to assess which plant species bumblebees are using for pollen is to collect pollen from the hind tibia (pollen basket) of individual bumblebees during or after foraging flights. Pollen grain morphology varies between plant species so, in many cases, the plant species of origin of pollen grains can be identified using microscopy (Ranta and Lundberg, 1981).

To investigate the pollen foraging behaviour of *B. humilis* and *B. sylvarum* and to compare it to the more nationally ubiquitous *B. pascuorum* and *B. lapidarius*, further bumblebee surveys were carried out in 2005. Surveys were carried out between July and mid-August on the same sites and following the same bee-walk methodology as those in 2003 and 2004. During the surveys, 32 worker bumblebees of each species with full pollen loads on their hind tibia were randomly captured in plunger cages (Kwak, 1987). A pollen sample was then removed from one of their hind tarsi using a small plastic spatula. The pollen collected was placed into a small labelled sampling tube and stored at room temperature prior to analysis. The spatula was cleaned thoroughly with 70% ethanol between samples.

2.4 Survey design

Survey protocol was designed in such a way as to avoid the problems of studying forage choices in bees as defined by Williams (2005) and Kleijn and Raemakers (2008). The aim of this was to ensure that bumblebees surveyed were faced with the same foraging options. This was achieved by selecting a series of geographically close sites that supported diverse and abundant bumblebee populations of nationally rare and ubiquitous species. The surveys were carried out over a larger area than the Williams (1989) study, although the proximity of all sites to each other was considered to be within the distances over which bumblebees have been estimated to travel to forage (Walther-Hellwig and Frankl, 2000; Chapman et al., 2003; Knight et al., 2005; Osborne et al., 2008a). All surveys were carried out on sites containing areas of flower-rich grassland but within a landscape mosaic of urban gardens, ornamental parkland, agricultural land, scrub and woodland. This ensured that a broad range of flora was available to the foraging bumblebees. All *Bombus* species recorded were present across all of the sites sampled and foraging comparisons were only made between species when both species were abundant to ensure that the seasonality of forage availability did not influence dietary comparisons.

2.5 Nectar foraging data analysis

Relative dietary breadth is a comparison of the breadth of floral species or floral families that a particular *Bombus* species would be expected to visit during a standardised number of floral visits (Williams, 2005). This standardisation was achieved using a rarefaction procedure (Heck et al., 1975; Gotelli and Colwell, 2001), by creating sub-samples of 10 visits (Goulson et al., 2009) made from the observed frequency distribution of visits for each *Bombus* species, but chosen at random without replacement 1000 times (Williams, 2005). This technique allowed comparison of bumblebee foraging choices while reducing the influence of differing sample sizes (Williams, 2005).

Rarefied food-plant specialisation at the floral species and family level was calculated for each bumblebee species for each survey month (July, August and September) and for all observations within each study year. As sample sizes for rarefaction calculation of a population's dietary breadth were n=10, rarefaction values based on sample sizes of $n_1<10$ (where n_1=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.

To assess whether there were significant differences, monthly rarefied species and family dietary breadths of *B. humilis* and *B. sylvarum* were compared. Rarefied dietary breadths of *B. humilis* and *B. sylvarum* were also compared to those of the more nationally ubiquitous species, *B. lapidarius* and *B. pascuorum*. To reduce the effects of seasonality on worker foraging dynamics, comparisons of *Bombus* species pairs were only made for months in which $n_1\geq10$ observations were made for both species. Due to the small sample sizes (n_2=5 or 6, where n_2=the number of months for which a comparison was made), no assumption of distribution was made and the data was analysed using non-parametric Mann-Whitney U exact tests. Sequential Bonferroni Correction (Rice, 1989) was applied to allow for multiple testing.

2.6 Pollen foraging data analysis

Pollen was identified and quantified using microscopic analysis. Methodology followed that used by Westrich and Schmidt (1986) and pollen was identified to genus level and where possible to species level. If more than one type of pollen was found, the proportion of each type was calculated. This was done by homogenizing the sample by mixing it in glycerine gel, then counting the proportion of each grain type from a 200 grain subsample.

Although a rare occurrence, in some cases identification was not possible, under these circumstances the pollen was classified as ‘unknown’ for subsequent data analysis. For each *Bombus* species no more than a single pollen type was unidentified. Therefore, for the purpose of data analysis, unknown pollen was counted as a single additional floral species for species level analysis and, to ensure that calculations of dietary breadth were conservative for all species, the unknown pollen was not counted as an additional floral family. Such an occurrence was rare and occurred relatively equally between *Bombus* species (5% of *B. humilis*, 2% of *B. sylvarum*, 0% of *B. lapidarius* and 4% of *B. pascuorum* pollen samples), so was not considered to influence the results.

Total number of pollen taxa and proportion of pollen from each taxa at the floral family and species level was calculated cumulatively from the pollen collected from each *Bombus* species. The number of floral species and families visited for each pollen load was also compared to assess whether there were significant differences between the dietary breadths of *B. humilis* and *B. sylvarum*. Pollen load dietary breadths of *B. humilis* and *B. sylvarum* were also compared to those of the more nationally ubiquitous species, *B. lapidarius* and *B. pascuorum*. As the data represented discrete count data Mann-Whitney U exact tests were used to assess whether there were significant differences between the dietary breadths of *B. humilis* and *B. sylvarum* from *B. lapidarius* and *B. pascuorum* in terms of pollen load dietary breadth. A Sequential Bonferroni Correction was applied to allow for multiple testing (Rice, 1989).

3. Results

3.1 Nectar foraging

During the 2003 surveys, 1747 worker bumblebees were recorded foraging for nectar on 38 floral species. During the 2004 surveys, 2304 worker bumblebees were recorded foraging for nectar on 57 floral species. The following results are based on these observations:

3.2 Relative nectar dietary breadth

For floral species, *B. sylvarum* consistently recorded the lowest monthly and yearly rarefied dietary breadths, with the exception of *B. lapidarius* in August 2004 (Table 2). The largest dietary breadths recorded in 2003 were generally for *B. lapidarius*, and in 2004 for *B. humilis*. For floral families, again *B. sylvarum* generally recorded the lowest monthly and yearly rarefied dietary breadths, with the exception of *B. lapidarius* in August 2004 and *B. humilis* in September 2003. The largest dietary breadths were distributed between *B. pascuorum* and *B. humilis*.

Monthly rarefied estimates of *B. sylvarum* floral species dietary breadth were significantly lower than for *B. humilis* and *B. pascuorum* (p=0.004 and 0.008 respectively). No significant difference was found between the dietary breadths of *B.*

sylvarum and *B. lapidarius* (*p*=0.095). Similarly, no significant difference was found between *B. humilis* and *B. pascuorum* or *B. lapidarius* (*p*=0.222 and 1.000 respectively).

No statistically significant difference was recorded between mean rarefied plant family dietary breadths of each *Bombus* species.

3.3 Pollen foraging

Across the study sites, pollen loads were taken from 32 workers of each *Bombus* species. Due to mould development and sample damage during transport, not all samples collected were analysed. This was a rare occurrence, however, and in total 28 *B. humilis*, 30 *B. lapidarius*, 31 *B. pascuorum* and 30 *B. sylvarum* worker pollen samples were analysed

3.4 Total pollen loads

Pollen collected by each *Bombus* species was ranked in terms of relative abundance (Figure 1). *B. lapidarius* was most ‘specialised’ in terms of collecting pollen from a single floral family, with 60.6% of their pollen loads being collected from flowers of Fabaceae. *B. sylvarum* was the most specialized on pollen from a single floral species collecting 55.7% of their pollen loads from *Odontites verna* (Orobanchaceae).

B. humilis had the broadest dietary breadth in terms of total floral species and families from which pollen was collected (Figure 1). Of the four species analysed, *B. humilis* appeared to be the least specialised on a single floral species or family. Indeed, when ranking the pollen by abundance, if an arbitrary threshold of 80% most abundant sources is selected to represent the pollen on which the bee can be considered to be most 'specialised', the eighty percent most abundant pollen from *B. humilis* comprised four different floral species from three floral families. This result was similar for *B. pascuorum*, but seemingly far less specialised than either *B. sylvarum* or *B. lapidarius* (Figure 1).

In total, the 80% most abundant pollen collected by all four *Bombus* species originated from only five floral species (*Odontites verna*, *Lotus glaber*, *Galega officinalis*, *Trifolium pratense* and *Rubus fruticosus*).

3.5 Dietary breadth of pollen loads

B. humilis dietary breadth, in terms of the number of floral species represented in pollen loads, was significantly larger than for *B. sylvarum* (p=0.003) and *B. lapidarius* (p<0.001). No significant difference was recorded between *B. sylvarum* and *B. lapidarius* or *B. pascuorum (*p=0.04 and 0.55 respectively).

Analysis at the floral family level gave similar results. Although no significant difference was recorded between *B. humilis* and *B. sylvarum* after Sequential Bonferroni Correction, *B. humilis* recorded a significantly broader dietary breadth of pollen samples than *B. lapidarius* (*p*<0.001) and *B. pascuorum* (*p*=0.004). No significant difference was recorded between *B. sylvarum* and *B. lapidarius* or *B. pascuorum (p*=0.03 and 0.56 respectively).

4. Discussion

Of the two UKBAP species surveyed, *B. humilis* was no more specialised in terms of nectar or pollen foraging than the more nationally ubiquitous and similar tongue-length species *B. pascuorum* or the shorter-tongued *B. lapidarius*. In fact, *B. humilis* was recorded as having the broadest dietary breadth in terms of pollen foraging and visited the greatest number of pollen taxa of all of the *Bombus* species surveyed.

In contrast, the present study demonstrated that *B. sylvarum* had a narrow dietary breadth in terms of nectar and pollen foraging when compared to the other *Bombus* species. *B. sylvarum* had a significantly narrower nectar dietary breadth than *B. humilis* and *B. pascuorum*, and was the most specialised of the bumblebees in collecting pollen from a single floral species. However, the more nationally ubiquitous *B. lapidarius* was recorded as having the narrowest pollen dietary breadth at both the floral species and family level, was the most specialised in terms of collecting pollen of Fabaceae and collected pollen from the least number of floral taxa of the *Bombus* species surveyed.

Similarly to the Williams (2005) study, patterns of dietary specialisation and species decline recorded in this study did not fit the generic relationship predicted from correlational analyses reported by Kleijn and Raemakers (2008) and Goulson et al. (2009). Patterns also did not fit a relationship between species decline and Fabaceae specialisation (Goulson and Darvill, 2004; Goulson et al., 2009), nor a correlation between longer-tongued bumblebees and greater dietary specialisation (Inouye, 1980; Ranta and Lundberg, 1980; Goulson and Darvill, 2004; Goulson et al., 2005) based on the species tongue lengths reported by Goulson and Darvill (2004) and Goulson et al. (2005).

It has been argued that Williams' (2005) results are a product of the limited set of plant species present in the study area restricting food choice (Kleijn and Raemakers, 2008). In the absence of a complete inventory of floral species in the surrounding landscape, it is difficult to either prove or disprove this. However, the present study was carried out over a larger area, with a greater diversity of habitats and thus presumably a greater diversity of forage sources than in the Williams study (2005). This included native wildflowers, shrubs, trees and ornamental garden flora, both on-site and in surrounding urban gardens and parklands all within the flight distances of foraging bumblebees (Walther-Hellwig and Frankl, 2000; Chapman et al., 2003; Knight et al., 2005; Osborne et al., 2008a). Nevertheless, no correlation was recorded between pollen specialisation and species declines when this broad forage choice was available to all of the bees.

Moreover, closer analysis of the foraging behaviour recorded by Kleijn and Raemakers (2008) and Goulson et al. (2009) reveals that their results correspond with those in our study despite being generated over greater spatial-scales. Kleijn and Raemakers (2008) chose to generalise patterns of forage specialisation by grouping 'common' and 'rare' species to reveal patterns of pollen specialisation in rarer species. However, it appears from the data available within their article that despite meticulous data analysis, such arbitrary groupings may be masking individual *Bombus* species behaviour. Whilst the seemingly extreme generalist behaviour of *B. terrestris* and *B. pratorum* cannot be disputed, examination of the data presented for the four *Bombus* species studied here (*B. humilis*, *B. lapidarius*, *B. pascuorum* and *B. sylvarum*) reveals very similar foraging behaviour to that recorded in our data. Based on proportional pie-charts, *B. humilis* was recorded collecting from the highest number of plant taxa of the four *Bombus* species with *B. lapidarius* recording the fewest (Fig. 3; Kleijn and Raemakers, 2008) and, of the four study species, *B. pascuorum* appeared to be the most specialised on a single floral species (*Trifolium pratense*) (Fig. 3; Kleijn and Raemakers, 2008).

Whilst it is difficult to draw definitive conclusions in the absence of raw data, comparison of the data presented for *B. humilis*, *B. pascuorum*, *B. lapidarius* and *B. sylvarum* (Kleijn and Raemakers, 2008) appears to reveal no obvious differences in terms of their dietary specialisation despite two species being nationally rare in the UK and the other two being more nationally ubiquitous. Indeed, the same can be said of the Goulson et al. (2009) dataset which, whilst identifying a general trend of rarer species visiting fewer flowers, failed to explain why one of the most ubiquitous species in the study (and in the UK), *B. hortorum*, was the most specialised in terms of pollen dietary breadth. In addition, no explanation was given for why the UKBAP species *B. sylvarum* was one of the rarer species in the study and yet had the broadest dietary breadth of all of the nectar-collecting females investigated and had a very similar rarefied pollen dietary breadth to *B. pascuorum*, the most abundant species in the study.

Also of particular interest from the present study and the more widespread Kleijn and Raemakers (2008) study was the floral species and families on which the bumblebees were recorded foraging. The floral species comprising the majority of pollen collected by *B. humilis*, *B. lapidarius*, *B. pascuorum* and *B. sylvarum* in both studies corresponded with the floral species and families identified by Hanley et al. (2008) as having the greatest reward in terms of pollen quality. This indicates that all four of these *Bombus* species, rare and more ubiquitous, may forage to maximise their reward in terms of pollen quality.

Whilst these results still support the need for widespread high quality forage provision to counteract the effects of agricultural intensification and habitat fragmentation (Williams and Osborne, 2009), they question the role of dietary 'specialisation' in species declines. In particular, results provide a reason to question whether reported specialisation by declining UK *Bombus* species on floral families with high quality pollen reward is merely an illusion created by their populations only persisting in areas characterised by such high quality forage. And, if so, why are *B. lapidarius* and *B. pascuorum* able to persist in much of the landscape in which they previously co-existed with *B. humilis* and *B. sylvarum* whilst the latter two have declined?

If the more ubiquitous *Bombus* species, *B. lapidarius* and *B. pascuorum*, demonstrate similar foraging behaviour to *B. humilis* and *B. sylvarum*, it must be assumed that other environmental or ecological factors are contributing to differential declines. A range of potential contributing factors have been reported: the size and proximity to edge of climatic range adding additional stress on developing colonies (Williams et al., 2007; 2009); the nesting habitat preference and/or marginalisation of nesting habitat leading to increased competition for late emerging species (Fussel and Corbet, 1992; Edwards and Williams, 2004; Osborne et al., 2008b; Fitzpatrick et al., 2007); inter-species variation in colony size, worker size and/or foraging distance when faced with increasingly fragmented resources (Walther-Hellwig and Frankl, 2000; Chapman et al., 2003; Darvill et al., 2004; Knight et al., 2005; Peat et al., 2005; Connop, 2008); variation in susceptibility to pesticides and pathogens (Thompson, 2001; Williams and Osborne, 2009).

It may well be a combination of some or all of these factors driving world-wide differential declines. Certainly variation has been reported between the four *Bombus* species investigated here in terms of their climatic niches (Williams et al., 2007; 2009), emergence times and length of colony development in the UK (Goulson et al., 2005), but further investigation of individual species ecology is necessary if we are to fully understand the subtle factors contributing to individual species declines (Williams and Osborne, 2009).

4.1 Management implications

In terms of practical habitat management advice, the present study highlights a number of floral species and groups that can be targeted through landscape-scale forage provision programmes designed to counteract the loss of suitable forage to agricultural intensification and habitat fragmentation (Carvell et al., 2006). Specifically, the most abundant pollen sources *Odontites verna*, *Lotus glaber*, *Galega officinalis*, *Trifolium pratense* and *Rubus fruticosus* should be considered amongst the target species for *B. humilis* and *B. sylvarum* conservation habitat management.

Much of the data generated corresponded with existing survey data on *B. humilis* and *B. sylvarum* foraging behaviour. *B. humilis* has been reported previously as being most closely associated with flowers of Fabaceae (Harvey, 1999; Carvell, 2002; Goulson and Darvill, 2003; Chapman, 2004; Goulson et al., 2006) and, along with *B. lapidarius*, was recorded collecting the majority of their pollen from flowers of Fabaceae in this study (Figure 1). In addition, the rarest of the bumblebees in the UK, *B. sylvarum*, collected the majority of its pollen from flowers of *Odontites verna* (Figure 1), a species on which it has been consistently recorded foraging in South Essex (Harvey, 1999; 2000; Connop, 2008). The seemingly regional value of *Odontites verna* as a major forage source for *B. sylvarum* when compared to results from the Kleijn and Raemakers (2008) study indicates that local foraging surveys may also be of benefit to determine regional foraging variation.

Despite the majority of pollen in the present study being collected from a small number of floral species, all four *Bombus* species collected pollen from six different floral families (Fabaceae, Orobanchaceae, Rosaceae, Asteraceae, Lamiaceae, and Dipsacaceae). Similar pollen foraging preferences were also demonstrated in the Kleijn and Raemakers (2008) historical European dataset generated prior to agricultural intensification and the associated landscape-scale decline in bumblebee forage plants (Carvell et al., 2006). Thus, whilst the specific drivers of this behaviour are currently unknown, it seems prudent that conservation habitat management be designed to include a variety of floral families if diverse bumblebee communities are to be effectively supported.

4.2 Summary

Whilst our study provided some evidence of a narrower dietary breadth in the nationally declining bumblebee *B. sylvarum*, overall the patterns that emerged contradicted the reported correlations between patterns of dietary specialisation and species decline. Results indicated that a more species-based approach needs to be taken into investigating the role of dietary specialisation in bumblebee declines and that generic relationships may not encapsulate the complex interactions of autoecological traits and environmental factors that may be driving world-wide bumblebee declines.

5. Acknowledgements

This project was funded by the Cleanaway Pitsea and Canvey Marshes Trust. Analysis of the pollen samples was carried out by Paul Westrich (Institut für Biologie und Naturschutz, Kusterdingen, Germany). Our thanks to Paul Williams, John Rostron and Juliet Osborne for their advice on data analysis and on the write up. Thanks also to Peter Harvey, Andrew Woodhouse and Marcus Hotten for their help on the sites.

6. References

Alford, D.V. (ed.), 1980. Atlas of the Bumblebees of the British Isles. Institute of Terrestrial Ecology Publications, No. 30.

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

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, 351 – 354.

Carvell, C., 2002. Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. Biological Conservation 103, 33-49.

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, 481-489.

Chapman, R.E., 2004. PhD Thesis. Submitted to the University of London.

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, 2801-2808.

Connop, S.P., 2008. PhD Thesis. Submitted to the University of East London.

Darvill, B., Knight, M.E. and Goulson, D., 2004. Use of genetic markers to quantify bumblebee foraging range and nest density. OIKOS 107, 471-478.

Edwards, M., 1998. Annual UK Biodiversity Action Plan Bumblebee Working Group Reports. Unpublished annual report for the UK BAP Bumblebee Working Group, Midhurst, UK.

Edwards, M., 2002. Annual UK Biodiversity Action Plan Bumblebee Working Group Reports. Unpublished annual reports for the UK BAP Bumblebee Working Group, Midhurst, UK.

Edwards, M. and Jenner, M. 2005. *Field guide to the bumblebees of Great Britain & Ireland*. Ocelli, Eastbourne, UK.

Edwards, M and Williams, P.H., 2004. Where have all the bumblebees gone, and could they ever return? British Wildlife 15, 305-312.

Ellis, J., 2006. PhD Thesis. Submitted to University of Southampton.

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, 185-194.

Fussell, M. and Corbet, S.A., 1992. The nesting places of some British bumble bees. Journal of Apicultural Research 31, 32-41.

Ghazoul, J., 2005. Buzziness as usual? Questioning the global pollination crisis. TRENDS in Ecology and Evolution 20, 367-373.

Gotelli, N.J. and Colwell, R.K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters 4, 379-391.

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, 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, 55-63.

Goulson, D., Hanley, M.E., Darvill, B. and Ellis, J.S., 2006. Biotope associations and the decline of bumblebees (*Bombus* spp.). Journal of Insect Conservation 10, 95-103.

Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S. and Knight, M.E., 2005. Causes of rarity in bumblebees. Biological Conservation 122, 1-8.

Goulson, D., Lye, G.C. and Darvill, B., 2008. Decline and Conservation of Bumblebees. Annual Review of Entomology 53, 191-208.

Goulson, D., Lye, G.C. and Darvill, B., 2009. Diet breadth, coexistence and rarity in bumblebees. Biodiversity and Conservation 17, 3269-3288.

Hanley, M.E., Franco, M., Pichon, S., Darvill, B. and Goulson, D., 2008. Breeding system, pollinator choice and variation in pollen quality in British herbaceous plants. Functional Ecology 22, 592-598.

Harvey, P.R., 1999. A report on the status of the Shrill carder bee *Bombus sylvarum* in Essex. Essex Naturalist (New Series) 16, 79-82.

Harvey, P.R., 2000. Shrill Carder Bee: Essex 2000. Report produced for English Nature, Colchester Essex.

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, 1459-1461.

Inouye, D.W., 1980. The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebee. Oecologia 45, 197-201.

Kleijn, D. and Raemakers, I., 2008. A retrospective analysis of pollen host plant use by stable and declining bumble bee species. Ecology 89(7), 1811-1823.

Knight, M.E., Martin, A.P., Bishop S., Osborne, J.L., Hale, R.J., Sanderson, R.A. and Gouslon, D., 2005. An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. Molecular Ecology 14, 1811-1820.

Kosior, A., Celary, W., Olejniczak, P., Fijal, J., Krol, W., Solarz, W. and Plonka, P., 2007 The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. Oryx 41, 79-88.

Kwak, M.M., 1987. Marking bees without anaesthesia. Bee World 68, 180-181.

Osborne, J.L. and Corbet, S.A., 1994. Managing habitats for pollinators. Aspects of Applied Biology 40, 207-215.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J. and Sanderson, R.A., 2008a. Bumblebee flight distances in relation to the forage landscape. Journal of Animal Ecology 77, 406-415.

Osborne, J.L., Martin, A.P., Shortall, C.R., Todd, A.D., Goulson, D., Hale, R.J. and Sanderson, R.A., 2008b. Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. Journal of Applied Ecology 45, 784-792.

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, 145-151.

Prŷs-Jones, O.E. and Corbet, S.A., 1987. Bumblebees: Naturalists' Handbooks 6. The Richmond Publishing Co. Ltd, Slough.

Ranta, E. and Lundberg, H., 1980. Resource partitioning in bumblebees: the significance of differences in proboscis length. Oikos 35, 298-302.

Ranta, E. and Lundberg, H., 1981. Food niche analyses of bumblebees: a comparison of three data collecting methods. Oikos 36, 12-16.

Rasmont, P., 1995. How to restore the apoid diversity in Belgium and France? Wrong ways and right ways, or the end of a projection paradigm!. In: Banaszak, J. (ed) Changes in fauna of wild bees in Europe. Pedagogical University, Bydoszcz, pp 53-64.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223-225.

Robinson, R.A. and Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. Journal of Applied Ecology 39, 157-176.

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, 145-154.

Stace, C., 1997. Field Flora of the British Isles. Cambridge University Press, Cambridge, UK.

Stout, J.C., Allen, J.A. and Goulson, D. 2000. Nectar robbing, forager efficiency and seed set: Bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). Act Oecologica 21, 277-283.

Thompson, H.M., 2001. Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.), Apidologie 32, 305-321.

UKBAP, 1995. Biodiversity: The UK Steering Group Report - Volume II: Action Plans (December 1995, Tranche 1, Vol 2, p127). www.ukbap.org.uk. Accessed September 2007.

UKBAP, 1999. UK Biodiversity Group: Tranche 2 Action Plans - Volume IV: Invertebrates (March 1999, Tranche 2, Vol IV, p213). www.ukbap.org.uk. Accessed September 2009.

Walther-Hellwig, K. and Frankl, R., 2000. Foraging habitats and foraging distances of bumblebees, *Bombus* spp. (Hym., Apidae), in an agricultural landscape. *Journal of* Applied Entomology 124, 299-306.

Westrich, P. and Schmidt, K. 1986. Methoden und Anwendungs- gebiete der Pollenanalyse bei Wildbeinen (Hymenoptera Apoidea). Linz. Biol. Beiträge 18, 341-360.

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G., Roberts, S.P.M., Szentgyörgyi, H., Tscheulin, T., Vaissière, B.E., Woyciechowski, M., Biesmeijer, J.C., Kunin, W.E., Settele, J. and Steffan-Dewenter, I., 2008. Measuring Bee Diversity in Different European Habitats and Biogeographical Regions. Ecological Monographs 78, 653-671.

Williams, C.S., 1995. Conserving Europe's bees: why all the buzz? TREE 10, 309-310.

Williams, P.H., 1982. The distribution and decline of British bumble bees (*Bombus* Latr.). Journal of Apicultural Research 21, 236-245.

Williams, P.H., 1986. Environmental Change and the distribution of British bumble bees (*Bombus* Latr.). Bee World 67, 50-61.

Williams, P.H., 1989. Why are there so many species of bumblebees at Dungeness? Botanical Journal of the Linnean Society 101, 31-44.

Williams, P.H., 2005. Does specialization explain rarity and decline among British bumblebees? A response to Goulson et al. Biological Conservation 122, 33-43.

Williams, P.H., Araújo, M.B. and Rasmont, P., 2007. Can vulnerability among British bumblebee (Bombus) species be explained by niche position and breadth? Biological Conservation 138, 493-505.

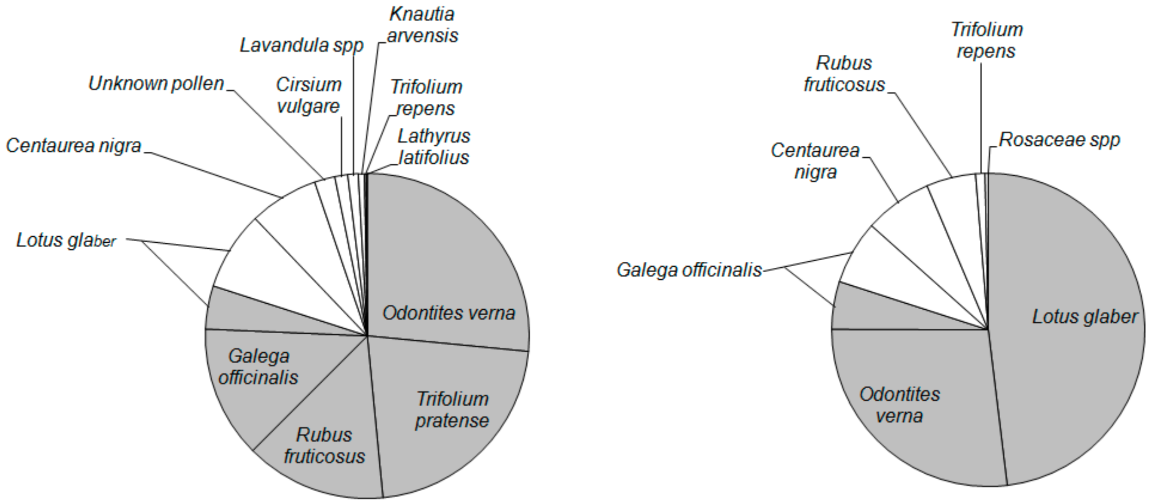
Williams, P.H., Colla, S. and Xie, Z., 2009. Bumblebee vulnerability: common correlates of winners and losers across three continents. Conservation Biology (in press, available online).

Williams, P.H. and Osborne, J.L., 2009. Bumblebee vulnerability and conservation world-wide. Apidologie 40, 367-387.

Caption

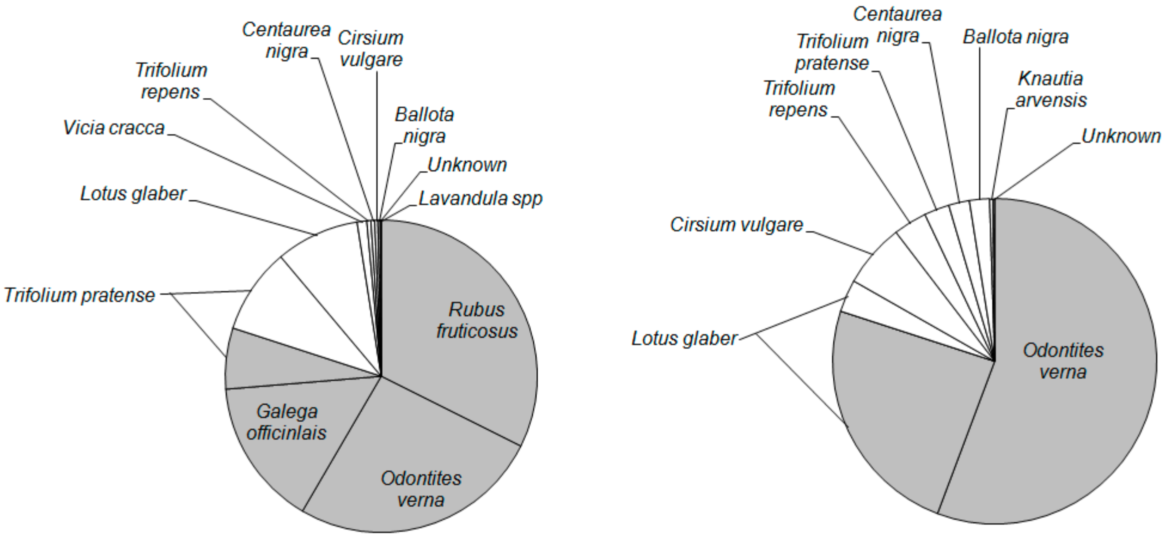
Fig 1. Cumulative pollen samples from foraging *Bombus* workers.

Charts represent pollen foraging in terms of floral species comprising a cumulative total of pollen identified from each *Bombus* species. Pollen was ranked in terms of abundance (most abundant through to least abundant sources). The most abundant 80% of pollen from the pollen totals is shaded in grey as a representation of the pollen on which each species was most ‘specialised’. n = the number of pollen samples on which each cumulative total is based.



Bombus humilis
n = 28
Total floral families = 6*
Total floral species = 12

Bombus lapidarius
n = 30
Total floral families = 4*
Total floral species = 7



Bombus pascuorum
n = 31
Total floral families = 5*
Total floral species = 12

Bombus sylvarum
n = 30
Total floral families = 5*
Total floral species = 9

Fig. 1.

Table 1. Location and description of survey sites.

Site name	*Location*	*Site description*
Belton Hills	51:32:34N, 0:38:13E	Semi-natural grassland with scrub and woodland areas
Canvey Wick SSSI	51:31:23N, 0:32:20E	Brownfield site: semi-natural grassland, scrub, woodland areas, bare sand and tarmac areas
Cleanaway Pitsea Landfill	51:32:25N, 0:30:49E	Active landfill with areas of bare topsoil, grassland, scrub
Creekside	51:32:47N, 0:33:15E	Semi-natural grassland with scrub areas
Hadleigh Castle Country Park	51:32:43N, 0:35:37E	Semi-natural grassland with scrub and woodland areas
Old County Council Landfill	51:33:08N, 0:29:51E	Brownfield site: semi-natural grassland and scrub
Two Tree Island	51:32:08N, 0:37:46E	Semi-natural grassland with scrub areas
Untidy Industry site	51:33:33N, 0:30:21E	Brownfield site: semi-natural grassland, scrub, bare sand and tarmac areas
Vange Hill	51:33:40N, 0:28:51E	Semi-natural grassland with scrub and woodland areas
Vange Marsh North	51:33:29N, 0:29:53E	Semi-natural grassland with scrub and marsh areas
Wat Tyler Country Park	51:32:59N, 0:30:08E	Semi-natural grassland with scrub and woodland areas

Table 2. Rarefied dietary breadth of floral species and families visited for nectar foraging. Rarefied estimates of the mean number of floral species and floral families a worker bumblebee would be expected to visit during 10 flower visits.

Floral species	Jul-03	Aug-03	Sep-03	**All 2003**	Jul-04	Aug-04	Sep-04	**All 2004**
B. humilis	5.13	5.42	3.54	**4.78**	5.77 *	6.56 *	7.03 *	**5.78 ***
B. lapidarius	5.55 *	5.52 *	3.64	**6.03 ***	5.58	2.92 **	N.A.	**5.21**
B. pascuorum	N.A.	4.09	4.16 *	**4.42**	4.7	4.72	5.02	**5.12**
B. sylvarum	1.48 **	3.3 **	3.35 **	**3.51 ****	3.09 **	3.53	3.61 **	**3.54 ****
Floral family								
B. humilis	3.59 *	3.8*	2.88 **	**3.58**	3.45 *	3.21	4.01 *	**3.30**
B. lapidarius	3.27	3.14	3.28	**3.30 ****	3.26	2.39 **	N.A.	**3.10**
B. pascuorum	N.A.	3.57	3.79 *	**3.85 ***	3.03	3.48 *	3.97	**3.47 ***
B. sylvarum	1.48 **	3.08**	3.17	**3.30 ****	2.67 **	2.98	2.54 **	**3.05 ****
Sample size								
B. humilis	n = 93	n = 332	n = 417	**n = 842**	n = 233	n = 360	n = 46	**n = 639**
B. lapidarius	n = 93	n = 161	n = 24	**n = 278**	n = 618	n = 158	N.A.	**n = 778**
B. pascuorum	N.A.	n = 59	n = 175	**n = 236**	n = 283	n = 216	n = 78	**n = 577**
B. sylvarum	n = 21	n = 117	n = 253	**n = 391**	n = 112	n = 192	n = 17	**n = 310**

* - Largest dietary breadth recorded within the defined period (month or year).

** - Smallest dietary breadth recorded within the defined period (month or year).

N.A. - Insufficient observations were made for this species in this month for rarefaction analysis of dietary breadth to be representative.