Nesting Ecology, Management and Population Genetics of Bumblebees: An Integrated Approach to the Conservation of an Endangered Pollinator Taxon

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SUMMARY ABSTRACT

Bumblebees have shown both long and short-term declines throughout their range. These declines may be attributed to a range of factors including changes in land use, alterations in climatic conditions and species introductions. However, management strategies for bumblebee conservation often focus on provision of summer forage resources and other factors are frequently overlooked. Provision of spring forage and nesting sites for bumblebee queens are rarely considered, though colony foundation and early colony growth are two of the most sensitive stages in bumblebee life history. Here, the efficacy of certain agri-environment prescriptions for providing spring forage and nest sites for bumblebees is assessed, highlighting a need for specific schemes targeted towards the provision of these vital resources in the rural environment. The nesting ecology of bumblebees is poorly understood because wild colonies are difficult to locate. However, a greater knowledge of the colony-level effects of environmental change is crucial to understanding bumblebee declines. Attracting bumblebee queens to nest in artificial domiciles could provide a valuable tool for studying colony-level responses. However, domicile trials and the findings of a literature review presented here demonstrate that this approach may be largely impractical for use in the UK. Conversely, a nationwide public bumblebee nest survey produced numerous data regarding nest site preferences among bumblebee species and also demonstrated that citizen science may also provide a sensitive method for detecting declines in currently common bumblebee species. An understanding of the ecology of species interactions and coexistence can provide valuable insights into factors that may influence declines. Data presented here suggest that coexistence between some bumblebee species may be maintained by resource partitioning based on diel activity patterns that are linked to species-specific environmental tolerances. If this is the case, the potential role of climate change in bumblebee declines may be severely underestimated. There is also increasing evidence that genetic factors may play a role in bumblebee losses, accelerating declines of small, fragmented populations as a result of reduction in genetic diversity and inbreeding depression. Here, the feasibility of reintroducing British *B. subterraneus* (now extinct in the UK) from New Zealand into England is assessed using population genetic techniques. The findings suggest that the population history of *B. subterraneus* in New Zealand has resulted in a dramatic loss of genetic diversity and high genetic divergence from the original UK population, suggesting that it may not be a suitable for use in the reintroduction attempt.

This work draws together some understudied aspects of bumblebee ecology with a particular focus on nest site requirements, availability of spring forage, mechanisms of avoidance of inter-specific competition and population genetic processes. The potential role of these in bumblebee declines is considered and new data relevant to the conservation of these important species is presented. It is hoped that this work will inform future management strategies for bumblebee conservation, highlight areas in need of further study and provide a sound starting point for future research in these areas.

DECLARATION

I declare that the thesis has been composed by myself and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

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Gillian Clare Lye

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General Introduction

1.1 A global decline in biodiversity

Biodiversity can be defined as any form of variation within living systems, ranging from ecosystem diversity, through species diversity to molecular and genetic diversity. The maintenance of biodiversity is of vital importance to humankind as it provides resources and ecosystem services upon which we are totally reliant. These include food, timber, pollination, water purification, nutrient cycling, aesthetic value and potential unknown future benefits such as novel compounds that could be used in medicine or pest control (Daily et al., 2000).

Understanding the mechanisms by which biodiversity is maintained and the factors that can interfere with them is one of the key challenges facing biologists today. The diversity of life on earth is believed to be greater now than ever before in earth's history (Benton, 1995), but losses are occurring at a rapid rate and these may have profound effects on global functioning (Balvanera et al., 2006). Records show that 1.2% of all mammal and bird species extant in the 1600s are now extinct (Primack, 2002) and of those that still persist, 24% and 12% respectively are considered to be threatened (at high risk of extinction within a short time frame) by the IUCN. This pattern is reflected across many taxa (table 1.1).

These declines have largely been attributed to man's influence on the planet in the form of habitat destruction, over-exploitation, pollution and ecosystem alteration (e.g. introduction of exotic species) (Frankham et al., 2004). With the inevitable continued increase in human population size, the extinction rate is set to accelerate, possibly reaching the point at which it is at a thousand fold that of normal

Table 1.1: Number of species described as threatened by the IUCN in 2006 (after http://www.iucnredlist.org/info/tables/table1)

Taxon	No. species described	No. species evaluated by the IUCN	No. species described as threatened	No. species threatened as % evaluated species
Mammals	5416	4856	20%	23%
Birds	9934	9934	12%	12%
Reptiles	8240	664	4%	51%
Amphibians	5918	5918	31%	31%
Fishes	29300	2914	4%	40%
Invertebrates	1190200	33978	0.18%	53%
Plants	287655	1901	3%	70%
Total of all species	1562663	40168	1%	40%

background levels (as deduced from the fossil record) (Balvanera et al., 2006). This phenomenon has been labelled as the 'sixth mass extinction' since its enormity is comparable to the five mass extinctions in Earth's history as evidenced by the fossil record (Primack, 2002).

1.2 Mitigation for declines in biodiversity

If declines are to be halted, the initial causes of these declines must be identified and these changes must either be reversed or mitigated. Initial causes of species decline are often deterministic factors resulting from human activities. Where such activities are directly responsible for these declines (such as in cases of over-exploitation), it is easy to understand what can be done to prevent further declines and allow populations to recover (although practical implementation may be more difficult as a result of societal or economic pressures). However, compensating for indirect effects of activities such as habitat destruction or species introduction can be more difficult since this often requires a precise understanding of all aspects of the ecology and ecosystem interactions of the system involved. Such knowledge is rarely if ever available.

When a population becomes small, secondary stochastic factors also begin to influence the likelihood of its survival and these must also be understood and addressed if conservation efforts are to be successful. These include demographic factors (e.g. chance changes in sex ratios or birth and death rates), environmental factors (e.g. chance variations in rainfall and food supply) and genetic factors (e.g. inbreeding, loss of genetic diversity and mutation accumulation).

1.3 Pollination service and the role of insects as pollinators

Pollination, the transfer of pollen between stamens and stigma of flowers, can occur by a variety of methods (e.g. via wind, birds or bats) but insects are one of the most important vectors of pollen. More than a third of human food is thought to be entirely dependent on insect pollination (McGregor, 1976; Corbet et al., 1991; Williams, 1995) and many crop plants require insect pollination to give good yields (Stoddard and Bond, 1987; Williams et al., 1987; Free, 1993; Goulson, 2003a; Klein et al., 2007).

Gaining a greater understanding of the role of animals in providing pollinator services and of methods for maintaining pollinator populations is currently a high priority for conservation research worldwide as a result of apparent ongoing declines of many pollinator species (Allen-Wardell et al., 1998; Kevan and Phillips, 2001; Steffan-Dewenter et al., 2005). This is reflected by the emphasis placed on pollinators within the framework of the recent EU funded ALARM (Assessing LArge scale Risks to biodiversity with tested Methods) project (http://www.fao.org/ag/agp/agps/C-CAB/Castudies/pdf/1-009.pdf). The desire to understand the dynamics of natural pollinator populations has become more urgent as a result of concerns over the spread of Colony Collapse Disorder in managed honeybee populations (Stankus, 2008). Pollination services provided by honeybees have been estimated at between 12-17 billion dollars (values calculated for 2003) per year in the US (Losey and Vaughan, 2006) and there is concern that the collapse of domesticated pollinator populations will result in a pollinator deficit that will not be adequately filled by impoverished wild pollinator populations (Kremen et al., 2002).

1.4 Bumblebees as keystone species

There are approximately 250 species of bumblebee (*Bombus* spp.) worldwide and these are present throughout Europe, Asia and North America, and also in South and Central America (Goulson, 2003a). Bumblebees are primitively eusocial insects, living in colonies consisting of one reproductive queen and her daughters, the workers. In the majority of species, the life-cycle is annual, and is characterized by colony founding, colony growth, production of males and new queens and colony expiration (figure 1.1). New queens leave the nest at the end of the colony cycle, mate and then pass anywhere up to nine months in a period of torpor known as the diapause (Alford, 1975) before emerging in late winter or spring to found a new colony.

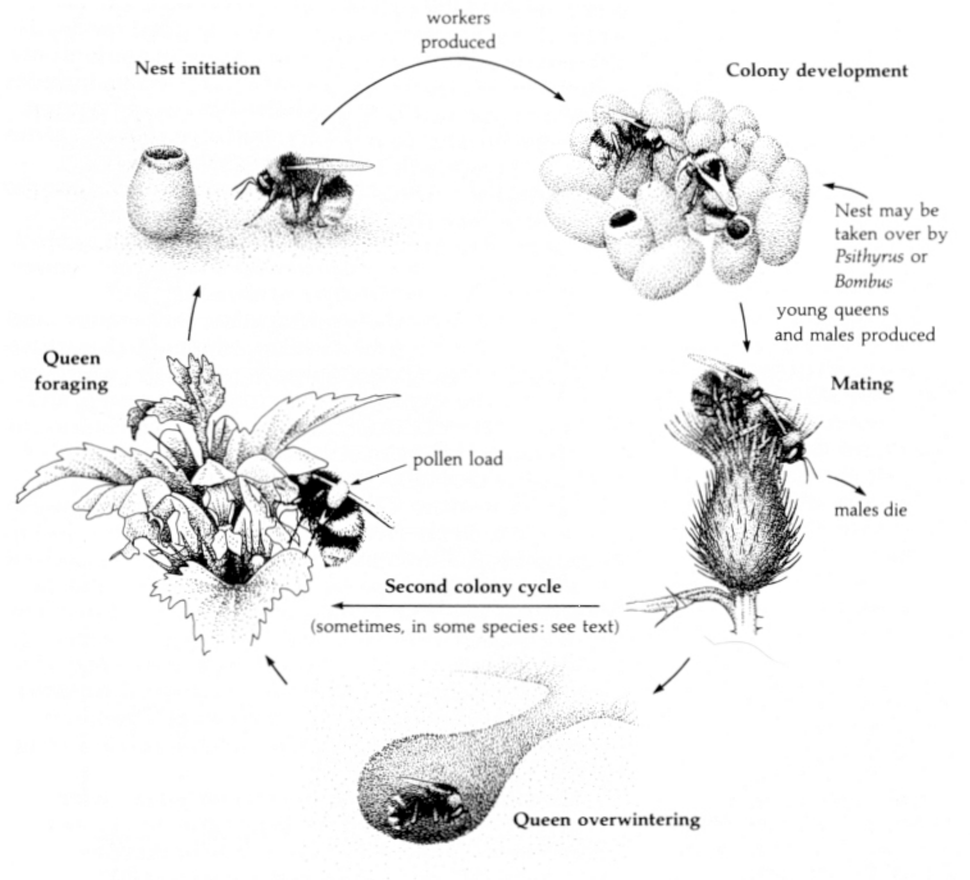

Figure 1.1: The life-history of a 'true' bumblebee (taken from Prŷs-Jones and Corbet, 1991).

Bumblebees are able to provide a particularly good pollinator service for several reasons. They are able to withstand a wide range of physical conditions, foraging earlier in the morning and later in the evening than other pollinator species and also foraging under adverse conditions such as wind and rain when other pollinators are inactive (Corbet et al., 1993). Bumblebee physiology is also very well suited to this role, since they are fatter and hairier than most other pollinator species, increasing the likelihood of contact with the reproductive organs of the plants they feed on and also allowing pollen to stick to their bodies more readily (Stanghellini et al., 1997 and 1998; Thompson and Goodell, 2001). Additionally, bumblebees are very variable in size both within and between species (Sladen, 1912; Peat et al., 2005) and different bumblebee species have different tongue lengths (Goulson et al., 2005 and 2008b) such that they are able to exploit an array of different flowers. Finally bumblebees are able to perform ‘buzz pollination’. This is the rapid vibration of the flight muscles at a frequency of about 400 Hz, which shakes the anthers of a plant to release pollen (King, 1993). Buzz pollination is vital for the pollination of certain crops, particularly those in the Solanaceae family such as tomatoes which have anthers that will only release large quantities of pollen in response to these vibrations (Van den Eijende et al., 1991). For these reasons, bumblebees are successful and efficient pollinators of a wide range of crop species and the financial implications of their loss could be severe (Goulson, 2003a).

Bumblebees are also proficient pollinators of wild flowers, a large number of which are pollinated exclusively or predominantly by bumblebees (Corbet et al., 1991; Osborne et al., 1991; Kwak et al, 1991a and 1991b; Rathcke and Jules, 1993) and plant families such as the Boraginaceae, Orchidaceae, Lamiaceae, Iridaceae, Ericaceae, Fabaceae, Scrophulariaceae, Solanaceae and Violaceae are thought to be highly dependent on bee pollination (Goulson 2003a). Some bumblebee species are also very generalist and may visit a range of species of which they are not the primary pollinator (Goulson, 2003b). Many wild flowers have shown declines in recent decades (Rich and Woodruff, 1996; Smart et al., 2005) and these often become restricted to small, fragmented populations (Osborne and Williams, 1996). The comparably large foraging range of bumblebees compared with many other pollinator species means that they are better able to reach these fragmented populations and provide a pollinator service. Loss of bumblebee fauna would be likely to result in reduced seed set and loss of genetic diversity via reduced out crossing thus accelerating declines of wild flower species, and almost certainly resulting in substantial numbers of these species being lost altogether. This would give rise to knock-on effects for associated herbivores and on up the trophic levels (Corbet et al., 1991).

Bumblebee nests often contain an array of commensals, many of which can be found only in this unique environment. There are thought to be around one hundred species of insect and mite which are found living in bumblebee nests and nowhere else (Goulson, 2003a). There are also many parasites, kleptoparasites and parasitoids which are specifically adapted to bumblebee hosts (Alford, 1975). If bumblebees are lost, then all of these organisms will be lost with them.

For these reasons, bumblebee losses would result in disproportionate alterations to the communities in which they live, and as a result, they have been described as keystone species (Kevan, 1991; Corbet, 1995). The conservation of keystone species is vital to prevent large-scale changes in community structures.

1.5 A global decline in bumblebee abundance and diversity

Many bumblebee species have shown declines over the last century and this pattern is reflected throughout their native range, with losses reported in Europe, North America and Asia (Kosier et al., 2007; Colla and Packer, 2008; Grixti et al., 2009; Williams et al., 2009). The majority of losses have occurred over several decades and are largely attributed to changes in land use, and particularly to changes in agricultural practices (Goulson et al., 2008a). For example, in the UK, the onset of bumblebee declines coincided with the agricultural revolution of the late 1940s. This period saw a drive toward higher productivity resulting in large areas of previously unfarmed land being brought into production, simplification of cropping patterns and removal of field margins such as hedgerows and field headlands to make way for larger fields bounded by simpler margin features (Goulson, 2003a; Pywell et al., 2005). The extensive use of artificial fertilizers replaced traditional rotations of leguminous crops such as red clover, usage of herbicides and organic pesticides increased dramatically and flower-rich hay meadows were replaced with monocultures of grass for use as silage (Goulson, 2003a). Since these changes began to be implemented, two out of the 19 true bumblebee species native to Britain have become extinct in the UK (*B. cullumanus* and *B. subterraneus*), at least six (*B. ruderatus*, *B. humilis*, *B. muscorum*, *B. distinguendas*, *B. sylvarum* and *B. monticola*) have suffered declines and only six species (*B. terrestris*, *B. lucorum*, *B. pascuorum*, *B. hortorum*, *B. lapidarius* and *B. pratorum*) remain common (Williams, 1982; Williams and Osborne, 2009). Similar land use alterations have occurred across the globe (Matson et al., 1997) and associated reductions in forage availability and potentially also nesting, hibernating and male patrolling habitat are implicated in bumblebee declines throughout Europe, North America and Asia (Williams, 1986; Kosier et al., 2007; Colla and Packer, 2008; Xie et al., 2008).

Climate change may also play a role in the alteration of bumblebee ranges and distribution either indirectly by effects on food plants or the small mammal populations that give rise to nest sites, or directly, as a result of the effects of changing weather conditions on bumblebee foraging activity, nest survival or over-wintering success (Williams and Osborne, 2009). Range contractions and extinctions of European frogs and butterflies have been linked to a general trend towards warming in western Europe (Thomas et al., 2006) and it is possible that similar responses may be reflected in bumblebee populations. The varying ranges of bumblebee species (Williams, 2005; Williams et al., 2009) suggest differences in environmental tolerances, and different bumblebee species are known to have different temperature optima for activity (Corbet et al., 1993). The apparent northerly expansion of *B. terrestris* and *B. lapidarius* in the UK has been suggested as evidence for the effects of climatic warming on bumblebees (MacDonald, 2001) but no conclusive evidence has yet been produced to demonstrate negative effects of climate change on bumblebee populations (Williams and Osborne, 2009).

1.6 Bumblebees as invasive species

Although some bumblebee species appear to be extremely susceptible to environmental changes, others show incredible resilience. For example, *B. terrestris* does not appear to be demonstrating significant declines in its native range and following introduction by man, has successfully become established in New Zealand, Tasmania, Israel, Japan, Chile and Argentina (Semmens et al., 1993; MacFarlane and Gurr, 1995; Ruz and Herrera, 2001; Goulson, 2003b; Matsumara et al., 2004; Torretta et al., 2006). These invasions are likely to have been facilitated by the great dietary plasticity of *B. terrestris* which has often been found to make use of a wide range of non-native plants where it is introduced (MacFarlane, 1976; Hingston et al., 2002). However, *B. impatiens*, *B. ruderatus, B. hortorum* and *B. subterraneus* have also been introduced outside their native ranges and many of these introductions have resulted in establishment of these species (Arretz and MacFarlane, 1986; MacFarlane and Gurr, 1995; Ruz, 2002). Bumblebees are generally introduced outside their native ranges for the pollination of crops such as tomato and red clover, and are often never intended to escape into the wild (Inari et al., 2005). The establishment of these non-native species poses a threat to biodiversity as a result of impacts on native bees. For example, recent, more precipitous declines of bumblebee species have been reported in Japan and the US and these have been attributed to negative impacts of bumblebee trafficking as a result of competition (Matsumara et al., 2004; Inoue et al., 2007; Nagamitsu et al., 2009) and/or transmission of novel bumblebee parasites and pathogens or pathogen spillover (Goka et al., 2001; Colla et al., 2006; Winter et al., 2006). Non-native subspecies of *B. terrestris* are imported into the UK for crop pollination (Ings et al., 2006) and there are concerns that this subspecies threatens the British subspecies as a result of hybridisation and/or out-competition (Ings et al., 2005a, 2005b and 2006).

As well as impacting on native bee species, the presence of non-native bees can facilitate the spread of alien weed species (Barthell et al., 2001; Stout et al., 2002; Goulson and Derwent, 2004) potentially having devastating impacts on native ecosystems (reviewed in Goulson, 2003b). It has also been shown that introduced bees may have direct negative impacts on native plant species since their visits can provide an inefficient pollination service compared to native pollinator species (Kenta et al., 2007).

Despite the negative effects of the introduction of bumblebee species outside their native ranges, there can be some unexpected benefits in terms of conservation. New Zealand populations of *B. subterraneus* and *B. ruderatus*, originally introduced from the UK at the turn of the last century, have survived far more successfully than those in the UK. British populations of *B. subterraneus* are now extinct and populations of *B. ruderatus* have shown clear declines (Edwards and Jenner, 2005). However, with more emphasis on conservation and habitat improvement in the UK, conditions may now be suitable to support *B. subterraneus* once again, and New Zealand populations could provide a source of the original British bumblebees for reintroduction. Indeed a project is underway to do just that (discussed in section 1.8.4).

1.7 Current trends in bumblebee conservation research

1.7.1 Farmland management schemes

Agri-environment schemes are government-subsidised projects designed to reverse the negative impacts of agricultural intensification on the environment and a principal aim of these is to promote habitat heterogeneity and biodiversity in the rural environment (Firbank et al., 1991; Dennis and Fry, 1992). Such schemes are now widespread in Europe and the US (Kleijn et al., 2001; Buskirk and Willi, 2004). Studies of the effects of agri-environment schemes have shown benefits for many insects, birds and small mammals (Boatman, 1992; Aebisher et al., 2000; Gardner et al., 2001; Peach et al., 2001; Reynolds, 2001; Marshall et al., 2006), and some prescriptions at least are likely to be valuable for a wide range of farmland flora and fauna.

Changes in land use associated with agricultural intensification have resulted in a decline in floral abundance and diversity in the rural environment and preferred bumblebee forage plants have been demonstrated to have suffered disproportionately (Carvell et al., 2006a). This reduction in forage availability has inevitable consequences for bumblebee success and this is generally regarded as the main factor influencing long-term bumblebee declines. In support of this, a recent UK study demonstrated that *B. terrestris* colonies placed in agricultural land perform less well than those placed out in urban areas suggesting that floral availability limits bumblebee populations in the rural environment (Goulson et al., 2002a).

Many agri-environment options promote floral abundance and diversity, for example, a popular management option of a 6m wide field margin kept free of crops and agrochemicals may contain six times as many flowering plants and ten times as many flowers than the equivalent cropped area. This increase in floral availability has been demonstrated to provide benefits for foraging bumblebees (Kells et al., 2001). Schemes found to be most successful in attracting a diversity and abundance of foraging workers are the sowing of either annual or perennial wildflowers or a pollen and nectar mix consisting of agricultural cultivars of legume species (Carreck and Williams, 2002; Meek et al., 2002; Carvell et al., 2004 and 2007; Pywell et al., 2005 and 2006). Some grassland management schemes have also been found to promote favoured bumblebee forage plants (Carvell, 2002) and long-term set-aside (lasting five years or more) can also be valuable, giving rise to the mid-successional communities preferred by bumblebees (Osborne and Corbet, 1994; Corbet, 1995).

1.7.2 Urban areas and public involvement

Urban areas cover approximately 4% of land area worldwide (approximately 7% in the UK) and this proportion is continuing to increase (Gaston et al., 2005a). In the US, 2.2 million acres of farmland and open space are converted into urban areas every year (McFrederick and LeBuhn, 2006). Urbanisation results in the loss and fragmentation of natural habitat and no doubt has negative consequences for many species (McKinney, 2006). It has been suggested that urban expansion may play a part in the declines of North American bumblebee species (Berenbaum et al., 2007) and may also be a contributing factor elsewhere.

However, in the impoverished agricultural environment, urban areas may also provide a haven for some bumblebee species and there are several that appear to thrive in urban parks and gardens (Tommasi et al., 2004; Goulson et al., 2006; McFrederick and LeBuhn, 2006; Fetridge et al., 2008). A recent study in the UK revealed that bumblebee density may be higher in urban areas than in rural areas (Osborne et al., 2007). Urban areas provide a mosaic of habitat types including parks and gardens that provide an abundance and diversity of plant species with an extensive flowering season, providing a season-long source of forage (Osborne et al., 2007). Many species of wildflower also flourish along road verges or in brownfield sites such that diverse bumblebee communities can be supported in these areas (Goulson et al., 2006).

Nest sites are also provided in abundance in the urban environment and bumblebees are often found nesting in sites specific to human activity including in compost heaps, in or under buildings and in bird boxes (Donovan and Weir, 1978; Fussell and Corbet, 1992). Indeed, *B. hypnorum* seems to show an association with urban areas (Løken, 1973) and it has been suggested that the spread of this species is facilitated by the provision of bird boxes by man (Rasmont et al., 2008).

The proportion of land dedicated to domestic gardens in the urban environment is often high, for example in Sheffield in the United Kingdom, the area given over to domestic gardens was estimated to be 23% (Gaston et al., 2005a). This presents an opportunity to involve members of the public in conservation with the ultimate goal of encouraging more wildlife friendly management in these areas. There has been an increasing trend for wildlife gardening and public interest in conservation in the UK and several bumblebee conservation schemes have been developed to exploit this. These aim to educate the public with regard to bumblebee declines, to promote bumblebee friendly management in urban gardens and to monitor bumblebee populations using records collected by the public (Williams and Osborne, 2009).

Public surveys are rising in popularity as a mechanism for gathering ecological data and can be a very effective method of doing so, since they allow the accumulation of large datasets of ecological information collected simultaneously across a wide geographic range (Silverton, 2009). They can also play an important role in stimulating public interest in important ecological issues and promote ecologically sensitive attitudes and behaviour (Cooper et al., 2007). Since bumblebees are charismatic and well-loved insects, the potential of public involvement to generate useful data on these species is high. In the UK, public surveys have generated useful data on bumblebee nesting ecology (Fussell and Corbet, 1992; Osborne et al., 2007), the volunteer-based organisation BWARS provides detailed records that facilitate the tracking of the temporal changes in bumblebee distributions, and a current public ‘transect walk’ scheme organised by the Bumblebee Conservation Trust is hoped to provide more detailed data on changes in abundance of bumblebee species over time (D. Goulson, pers. comm.). In this way, public involvement has provided Britain with good baseline data on its bumblebee species and could continue to aid conservation research in this area.

1.8 Future challenges for bumblebee conservation research

1.8.1 Bumblebee community structure

Although recent trends reflect a reduction in species diversity of bumblebee communities, little is understood about the mechanisms that maintain bumblebee community structure in the first place (Goulson et al., 2008b). A detailed understanding of the factors underlying the coexistence of multiple bumblebee species is crucial to allow a more accurate assessment of the processes that might be interfering with this balance.

Traditional competition theory dictates that there should be an upper limit on the similarity of the ecological niches occupied by coexisting species in order to prevent competitive exclusion (Pianka, 1974), yet coexisting bumblebee species often appear to occupy very similar niches (Goulson et al., 2008b). Tongue length is one way in which bumblebees partition resources amongst themselves, with different species exhibiting different tongue-lengths and visiting flowers with corolla-lengths that correspond to these (Ranta and Lundberg, 1980; Harder, 1985). Some evidence has been provided for competitive exclusion of species with the same tongue length in the US (Inouye, 1978; Pyke, 1982), but this does not always hold true. For example, in the UK, four of the six common and ubiquitous bumblebee species have very similar tongue-lengths (Goulson et al., 2005), and in Poland, coexisting bumblebees were also found to have high levels of overlap for tongue length (Goulson et al., 2008b).

The potential role of spatio-temporal heterogeneity in nest distribution and floral resources in promoting the coexistence of similar bumblebee species has also been highlighted with the premise being that the fate of each colony relative to others will vary across the season and also across the landscape as patches of various forage plants at different distances from each colony come in and out of flower (Ranta and Vepsäläinen, 1981).

Resources may also be partitioned as a result of behavioural differences between species. For example, Albrecht and Gotelli (2001) found that common species of ant in Oklahoma demonstrate diel niche partitioning in which different species were active at different times of day. Similar patterns have been observed in Swedish bumblebee communities (Hasselrot, 1960) and such partitioning has been attributed to differences in environmental optima for activity (Williams, 1986). Temperature thresholds for activity are known to vary between species (Corbet et al., 1993) and coexisting bumblebees that appear to have very similar ecological requirements often have different geographic ranges (Williams, 2005) suggesting differences in environmental tolerances. Thus, this mechanism of niche partitioning may be more widespread than suggested in the literature and if this is the case, changes in environmental conditions as a result of climate change may affect the balance of communities such that previously coexisting species become involved in competitive interactions which could result in competitive exclusion.

However, niche partitioning is only expected where competition is high. If resources are abundant then niche overlap for that resource will not affect fitness (Pianka, 1974). It is generally assumed that forage availability limits bumblebee populations and although this has been shown to be true in some cases (Pelletier and McNeil, 2003), this may not always be the case. Other factors such as nest site availability or abundance of natural enemies may also play important roles in determining bumblebee population sizes (McFrederick and LeBuhn, 2006; Carvell et al., 2008).

1.8.2 Nesting ecology of bumblebees

Whilst provision of forage resources is the main focus for most bumblebee conservation efforts, the nesting, hibernation and mating ecology of bumblebees receive relatively little attention. An understanding of all of these elements of ecology are required to ensure the provisioning of suitable habitat for bumblebees, but a particular focus on nesting ecology may be beneficial since a lack of suitable nesting sites is consistent with observed patterns of species declines.

The most convincing correlate of rarity in bumblebees is emergence time (Goulson et al., 2005; Williams, 2005; Fitzpatrick et al., 2007; Williams et al., 2009). Those species in which the queen emerges late from hibernation appear to be most prone to decline. Several hypotheses have been put forward to explain this pattern (Goulson et al., 2005; Williams et al., 2009) but all are linked to availability of forage resources. However, it is also possible that in some cases at least, these patterns reflect competition for other resources, the most likely of these being nest sites.

Choice of nest site is one of many aspects of bumblebee ecology that is species-specific. Certain considerations are probably common among all species, including the presence of suitable nest material for insulation of the brood, the degree of shelter, the drainage and heat absorption of a site and possibly the availability of proximate spring forage resources (Hobbs, 1967a). It is also a general feature that bumblebees are commonly found nesting in the abandoned homes of field mice, voles or other small mammals and birds (Sladen, 1912; Svensson and Lundberg, 1977; Donovan and Weir, 1978; Fussell and Corbet, 1992; Rasmont et al., 2008). Such sites are probably favourable as they provide an insulated cavity and ready prepared nesting material.

However, it is clear that different species of bumblebee will search for nest sites in different habitats, in different positions and in the vicinity of different landscape features (Fussell and Corbet, 1992; Svensson et al., 2000; Kells and Goulson, 2003). The most notable difference in nest site choice between bumblebee species is the distinction between those that nest below the ground and those that nest on or close to the soil surface. In the UK, *B. terrestris*, *B. lucorum*, *B. lapidarius* and *B. ruderatus* are known to prefer to nest underground, whilst *B. hortorum*, *B. pascuorum*, *B. sylvarum*, *B. humilis* and *B. muscorum* tend to nest on or just below the soil surface (Sladen, 1912; Fussell and Corbet, 1992). Some species, such as *B. hypnorum*, may also prefer aerial locations such as holes in trees (Hasselrot, 1960) and others, such as *B. pratorum*, appear to be very flexible in nest site positioning (Sladen, 1912).

Other species-specific differences in nest site choice are harder to observe since locating nests in the field is very difficult and only a small proportion of nests are ever found (Kells and Goulson, 2003). As a result of this, few data are available regarding the requirements of individual species, and this is especially true for those species that are rare and of conservation concern.

Agricultural intensification has resulted in the loss of large areas of natural and semi-natural habitat such as hedgerows, woodland and tussocky grassland, all of which attract the small mammals and birds that provide nest sites for bumblebees. This is likely to have resulted in a vast reduction in the availability of nest sites for bumblebees in the rural environment (Kells and Goulson, 2003).

Bumblebee abundance and diversity is often associated with presence and coverage of semi-natural or natural habitat, regardless of floral abundance in these areas (Kremen et al., 2002; Öckinger and Smith, 2007) suggesting that such habitat is providing resources other than forage. Greenleaf and Kremen (2006) report that although natural habitat was important in predicting abundance of *B. vosnesenkii*, this was not the case for the solitary bee species *Anthophora urbana* which is able to create its own nest sites, suggesting that nest site availability may be the limiting factor for bumblebees in this situation. Even within the urban environment, evidence has been produced to suggest that nest sites may limit bumblebee populations. In urban parks in San Francisco, bumblebee abundance was found to be positively correlated with number of rodent holes (McFrederick and LeBuhn, 2006), known to provide nest sites for bumblebees.

Although species-specific differences are apparent among bumblebees, there appears to be a strong overlap in nest site choice among species (Richards, 1978) such that if nest sites were limiting, interspecific competition for these resources might occur and could result in competitive exclusion of later emerging species. For example, in the UK, the early emerging and ubiquitous *B. pascuorum* and the late emerging and declining *B. humilis* are both known to have a preference for nesting amongst dense vegetation on the surface of the ground (Alford, 1975). Similarly the early emerging and common *B. terrestris* and the late emerging and rarer *B. soroeensis* are both known to nest below ground (Alford, 1975), often in the abandoned homes of small mammals. If this were the case, this provides an alternative or complementary explanation for the tendency of later emerging species to be in decline.

1.8.2.1 The role of agri-environment schemes in providing nest sites for bumblebees

In addition to providing sources of forage for bumblebees, agri-environment schemes may also be beneficial in providing hibernation sites, male patrolling routes and nest sites for bumblebees. For example long-term set-aside, uncropped field margins and beetlebanks and field corner management will provide the tussocky grass favoured for nesting sites by surface nesting species whilst suitable management of field boundary features such as hedgerows will provide suitable nesting sites for subterranean nesting species (Goulson, 2003a). All are likely to attract the small mammals that often make homes for bumblebees.

1.8.2.2 The potential for the use of artificial domiciles in bumblebee conservation

If bumblebee populations are limited by nest site availability then the provision of suitable artificial nesting sites would be an ideal way to enhance populations in areas where natural nest sites might be scarce. The same technique could also be used to enhance bumblebee nest density adjacent to flowering crops, reducing or eliminating the need for commercially reared bumblebee colonies. Studies on other bee species (e.g. the alkali bee (*Nomia melanderi*) and the leafcutter bee (*Megachile rotunda*)), have shown that the provision of suitable nest sites can significantly enhance local populations (Peck and Bolton, 1946; Parker et al., 1987). If bumblebees could be induced to nest in artificial domiciles, this would also allow close monitoring of the colonies as well as the option of artificial protection against natural enemies and artificial provisioning during times of forage scarcity.

Artificial domiciles have been used with some success in Canada, New Zealand and the US (Frison, 1926; Fye and Medler, 1954; Donovan and Weir, 1978; Richards, 1978 and 1987; Pomeroy, 1981), with uptake rates of 30-50% often reported in these studies. The most commonly used design consists of a wooden box with an entrance hole drilled into the side, provisioned with fine, insulating nest material (Hobbs et al., 1962). These boxes can be placed on the surface of the ground, suspended above the ground or adapted with the addition of an entrance tube to be buried beneath the ground depending on the nesting preferences of the target species. However, many other designs have also been trialled with comparable success.

Recent artificial domicile trials in the UK (Fussell and Corbet, 1992; Carvell, 2000; Gaston et al., 2005b) have been far less successful than those carried out elsewhere, with occupancy rates ranging from 0-4%. There are several possible explanations for the discrepancy between results obtained in the UK and elsewhere. Firstly, the nest site preferences of bumblebee species present in the UK may be such that artificial domiciles such as those used elsewhere are simply less attractive to British bumblebees. This may be true of some species, but it seems unlikely that this is the full story since Sladen (1912) commonly achieved uptake rates of ~30% in UK artificial domicile trials, and all bumblebees present in New Zealand are of UK origin (Hopkins, 1914). It is notable that the work of Sladen and that of most other authors reporting high uptake rates was carried out several decades ago, and given ongoing declines in bumblebee populations, it is possible that differences in success between studies simply represent differences in bumblebee abundance in the study regions and changes in abundance over time. Another explanation is that nest sites may limit bumblebee populations elsewhere, but that this is not the case in the UK. This explanation may be particularly relevant for explaining differences between New Zealand and the UK, since the small mammal fauna of New Zealand is very much reduced compared to that of the UK (Fussell and Corbet, 1992). Site selection for placing artificial domiciles is likely to be of vital importance in influencing uptake rates and experimenter experience may play an important role in effective domicile siting (Frison, 1926; Donovan and Weir, 1978), so it is possible that the lack of success in the UK can be explained by a lack of experimenter experience rather than real differences in bumblebee populations between studies.

However, almost all studies using artificial domiciles for bumblebees report relatively low uptake rates compared to those sometimes achieved with artificial domiciles designed for other types of wildlife; for example artificial domiciles designed for solitary bees commonly achieve uptake rates of between 50-100% and can attract a wide range of different species (Gaston et al., 2005b). It is unknown whether rates of uptake of domiciles for bumblebees are a result of active rejection by queens, or simply because they are not discovered. It is possible that cues used by bumblebee queens to locate suitable nest sites are simply not found in association with artificial domiciles.

1.8.2.3 The potential for enhancement of artificial domicile success using odour cues

Nest site searching bumblebee queens demonstrate a very characteristic behaviour flying close to the ground, adopting a zigzag trajectory and stopping to investigate potential nest sites on foot. This behaviour would be ideal for the detection of short-range olfactory cues and it has often been hypothesised that bumblebee queens may use odour cues as a method of locating suitable nest sites.

Since bumblebee colonies are often found in the abandoned homes of small mammals, it has been suggested that nest site searching queens specifically search for such sites using odour cues associated with old small mammal nests. Few studies have examined this theory, although baiting artificial domiciles for bumblebees with mouse nests or associated odours appeared to have no effect on the uptake rate of the boxes by nest-founding queens (Hobbs et al., 1960; Barron et al., 2000; Carvell, 2000). However, Djegham et al. (1994) reported that queens of *B. terrestris* were more likely to initiate colony foundation in the presence of odours associated with the vole *Microtus arvalis*. Odour detection by insects can be very specific and since odour bouquets released from the nest material of small mammals are likely to be specific to the mammal species and also to alter with age, these factors may be important.

Several studies indicate that bumblebees may have a preference for nesting in sites which have been occupied by bumblebee colonies in previous years (Donovan and Weir, 1978; Pomeroy, 1981; Barron et al., 2000) and this may be because a site that has been successful in previous years is likely to be successful again in subsequent years. Suggested mechanisms by which consecutive occupancy of nest sites is achieved include the return of daughter queens to the site of their maternal nest (Donovan and Weir, 1978; Pomeroy, 1981) or the detection of cues associated with old bumblebee nest material by queens during nest site searching (Barron et al, 2000).

If odour cues are used by bumblebee queens to locate suitable nest sites, it may be possible to increase artificial domicile success by baiting domiciles with these odours in order to promote location and exploration of the domiciles by nest site searching bumblebee queens.

1.8.3 The need for an integrated approach to habitat restoration for bumblebees

Whilst the provision of nest sites for bumblebees is an understudied and important aspect to be considered when devising conservation management strategies for these species, increasing nest site availability alone is unlikely to be sufficient to boost bumblebee populations. Habitat heterogeneity has been shown to be beneficial for bumblebee assemblages (Greenleaf and Kremen, 2006; Pywell et al., 2006; Rundlöf et al., 2008) and this is likely to be because bumblebees rely on a range of different habitat types throughout the season to provide forage, nest sites, hibernation sites and male patrolling sites.

Since bumblebees are unable to store large quantities of food resources within the nest as honeybees do, they require a continuous supply of forage throughout the spring and summer (Carvell et al., 2006b). A diverse range of habitat types is likely to be beneficial in providing this since different habitat types support different plant species with different flowering phenology. To date, few studies report on the season-long forage requirements of bumblebees and whilst forage usage in the mid- to late- stages of colony development are well studied (e.g. Carvell, 2002; Goulson and Darvill, 2004; Pywell et al., 2005 and 2006), early forage use by newly emerged and nest founding queens is as yet, poorly studied (Goulson et al., 2005).

There are also likely to be important interaction effects between the availability of different components of the ecological requirements of bumblebees on their survival, particularly early in the year. For example, during the four to five weeks following nest foundation, a bumblebee queen must feed, incubate and defend the first brood of workers unaided, so that proximity and continuity of forage resources to the nest is likely to be particularly important at this time (Alford, 1975). The success of current management strategies is generally assessed by counting foraging workers but since workers may travel long distances from their nests to locate high quality forage resources (Chapman et al., 2003) and multiple workers may originate from the same nest, this approach provides no indication of the success of the management strategy for promoting bumblebee nest density and success in the local area. Since nests are the reproductive unit of bumblebee populations, such approaches are extremely limited (Williams and Osborne, 2009). Management strategies targeting bumblebees should aim to meet all of the ecological requirements of bumblebees and ensure that resources are available at the appropriate spatial scale, and assessment of the success of strategies at the population level should focus on colony success rather than worker abundance.

1.8.4 Reintroduction of B. subterraneus into the United Kingdom from New Zealand

The UK Biodiversity Action Plan (BAP) was devised in order to meet objectives laid down in the Convention on Biological Diversity, signed by the United Kingdom at the Rio Earth Summit in 1992 (http://www.ukbap.org.uk). The goal of this initiative is to describe the UK's biological resources and to produce detailed plans for the protection of these. The UK BAP currently includes 391 Species

Action Plans developed for species considered to be priorities for conservation. Amongst these are plans for five bumblebee species (*B. distinguendas*, *B. humilis*, *B. subterraneus, B. ruderatus* and *B. sylvarum*) and a further two (*B. muscorum* and *B. ruderarius*) are now listed as priority species. Action specified by the bumblebee plans includes regular monitoring, ecological research to identify specific causes of decline, promoting awareness of the species, protecting remaining areas of suitable habitat, and in some cases restoring habitat and re-establishing populations in those areas from which they have disappeared (http://www.ukbap.org.uk). The aims of these BAPs were to maintain existing populations of these rare species at all known sites and to have significantly enhanced their representation in Britain by 2010.

One of the species listed under the UK BAP, *B. subterraneus*, has since been declared extinct in the UK (Edwards and Jenner, 2005). However, reintroduction of native British *B. subterraneus* is still possible as a result of the presence of *B. subterraneus* of British origin in New Zealand (MacFarlane and Gurr, 1995) and several conservation organisations in the UK have recently embarked on a collaborative project funded by the government body, Natural England, to do just that.

Bumblebees were originally introduced into New Zealand for the pollination of the fodder plant, red clover (*Trifolium pratense*) (Hopkins, 1914). Following these introductions, four species of bumblebee became established in New Zealand: *B. terrestris*, *B. hortorum*, *B. ruderatus* and *B. subterraneus*. These species spread rapidly throughout large areas of the South Island and by 1960 all but *B. subterraneus* had also colonized the North Island (Gurr, 1964). The most recent survey of the distribution of these species found that *B. terrestris* and *B. hortorum* are still ubiquitous and thriving, whilst *B. ruderatus* is locally abundant, and *B. subterraneus* persists within restricted ranges (Goulson and Hanley, 2004).

Although both *B. subterraneus* and *B. ruderatus* have done poorly in the United Kingdom over the last century, bumblebee populations in New Zealand have thrived and spread during the majority of this period. This may be partially explained by the freedom of these populations from natural enemies (Donovan and Weir, 1978) but the most convincing explanation is the presence of an abundance of introduced European plant species which can provide a rich source of forage for New Zealand bumblebee populations (Goulson and Hanley, 2004). However, a pattern of range restriction is now beginning to emerge for both *B. subterraneus* and *B. ruderatus* in New Zealand (Goulson and Hanley, 2004) and this is likely to be linked to the same processes believed to be responsible for declines elsewhere. Withdrawal of government subsidies for the sowing of *T. pratense* and *Lotus corniculatus* on agricultural land have resulted in reduction and fragmentation of suitable foraging habitat for bumblebees.

1.8.4.1 Maximising the success of the reintroduction of B. subterraneus to Britain from New Zealand

The presence of British bumblebees in New Zealand provides a unique opportunity to compare the success of different species between these two regions and to assess the factors likely to be responsible for these differences. This information could provide important insights for future conservation efforts for bumblebees. In particular, a detailed understanding of the ecology of *B. subterraneus* in New Zealand would be extremely beneficial in aiding the development of suitable land management strategies to maximise the likelihood of success of the reintroduction attempt.

However, as in all reintroduction attempts, secondary factors that could affect survival such as founder effects and the effect of small population sizes must also be considered (Olech and Perzanowski, 2002). This is of particular relevance to the reintroduction of *B. subterraneus* to the UK from New Zealand since the New Zealand population may already be suffering from negative effects associated with their initial introduction and the subsequent low density at which they have persisted. In each introduction of bumblebees into New Zealand, relatively few individuals were introduced giving rise to small initial populations (although the precise numbers of each species are unknown). When a population undergoes an extreme reduction in numbers it is known as a population bottleneck (Frankham et al., 2004). Severe bottlenecks such as this inevitably result in loss of genetic diversity, but also increases susceptibility to genetic drift (the chance changes in allele frequency between generations) which can result in the fixation of deleterious, or loss of beneficial alleles. Since the New Zealand bumblebee populations have experienced relaxed selection for defences against British natural enemies and have been exposed to different environmental conditions to those in the UK, it is vital that the adaptive potential of the population to be reintroduced is high. However, the effects of a bottleneck and small population sizes are likely to have rendered the population less able to adapt to the new environmental conditions that will be faced upon reintroduction to the UK.

Small population sizes can also lead to inbreeding depression. Inbreeding depression is any negative effect arising as a result of individuals with similar genetic make-up reproducing with one another. This includes the production of deleterious allele combinations and the reduction of heterozygosity within the population. (There is evidence to suggest that heterozygotes have selective advantages over homozygotes, a phenomenon known as heterozygote vigour). Inbreeding depression has variously been found to lower survival, growth rate and fecundity as well as to cause greater susceptibility to disease, predation and environmental stress in a wide range of animal species (reviewed in Keller and Waller, 2002).

There have been varying reports on whether or not bumblebees suffer from inbreeding depression For example, Gerloff and Schmid-Hempel (2005) found no effect of inbreeding on the reproductive output or overall fitness of laboratory reared colonies of *Bombus terrestris* whilst Beekman et al. (1999) found a negative effect on the fecundity of queens of the same species. Since such studies have always focused on common species it is also possible that rarer bumblebee species may suffer more pronounced consequences of inbreeding.

In many hymenopteran species including bumblebees, there is a further cost of inbreeding in that homozygosity at the sex determining locus in diploid individuals will give rise to diploid males rather than workers or queens. A study on the sex determination mechanisms in the bumblebee, *Bombus terrestris*, has shown that in bumblebees, sex is determined by a process known as parthenogenetic arrhenotoky (Crozier and Pamilo, 1996). This means that sex is determined by one or more sex determining loci. Those individuals that are homozygous at these loci are male, and those that are heterozygous at one or more of these loci are female. As there are always either a large number of loci, or a large number of alleles at a single locus (the latter is true of *B. terrestris*), most diploid individuals are female. Males arise from unfertilized (thus haploid) eggs. However diploid males are theoretically possible and have been observed in inbred lab populations (Duchateau et al., 1994) and also in the wild (Darvill et al., 2006).

Diploid males are unable to produce viable offspring, confer no benefit to the colony and halve the worker force of a colony (approximately 50% of fertilized eggs will become diploid males and are reared to adulthood within the colony using up valuable resources [Duchateau et al., 1994; Cook and Crozier, 1995]). Thus, diploid male production represents a considerable cost to the colony. Plowright and Pallett (1979) found that in the laboratory diploid male producing colonies showed reduced growth rates compared to normally reproducing colonies and this has recently been demonstrated to be true under field conditions (Whitehorn et al., 2009).

Genetic diversity in New Zealand bumblebee populations has not been assessed for three out of the four species present, and it would be of interest and value to use this information to draw conclusions regarding the genetic processes that have affected these populations and on the survival of these species in light of this knowledge. If the reintroduction of *B. subterraneus* is to succeed it is of vital importance to assess the genetic processes that may have shaped this population, and to prevent further degradation of genetic diversity during the reintroduction of this species into Britain.

1.9 Aims and objectives

Bumblebee conservation research has primarily focused on the link between population declines and changes in forage plant availability. Consequently, most conservation strategies for these species are based on the provision of floral resources. Whilst forage availability is important for explaining bumblebee losses, other factors may also contribute to their declines and an understanding of these may be vital for the development of successful management strategies for these species. The aim of this thesis is to draw together understudied aspects of bumblebee ecology, supplying new data relevant to conservation strategies for these species and providing a basis for further study into these areas. Specific aims are:

1. To assess methods of artificially increasing nest site availability for bumblebees (chapters 2, 3 and 4).

2. To investigate in detail the nest site requirements of British bumblebee species and assess the potential impacts of species-specific differences in nest site preference on susceptibility to decline (chapter 5)

3. To determine the effectiveness of current British agri-environment schemes for providing nest sites and spring forage for bumblebees as a basis to inform future management strategies (chapter 6)

4. To identify mechanisms by which ecologically similar bumblebee species are able to partition forage resources (chapter 7)

5. To investigate the influence of population history on population genetic structure and genetic diversity in British bumblebee species introduced into New Zealand (chapter 8)

6. To provide ecological, distributional and population genetic data relevant to the current attempt to reintroduce *B. subterraneus* into the UK from New Zealand (chapters 7 and 8)

Each chapter is presented as a stand-alone paper so that reference to general introduction should not be required for interpretation of the work.

Chapter 2

A review of the use of artificial domiciles for bumblebees for research, conservation and commercial benefit

2.1 Abstract

The use of artificial domiciles for bumblebees has the potential to provide wide-ranging benefits: for conservation, by providing a method of boosting nest site availability where natural nesting habitat is degraded and allowing monitoring of colonies of rare species; for agriculture, by allowing positioning of colonies in beneficial locations for the pollination of flowering crops, and for research, by facilitating much-needed study into bumblebee colony dynamics and colony level responses to environmental change. Over the past century, numerous attempts have been made across the world to attract bumblebee queens to nest in artificial domiciles, often with good results, however more recent studies generally report very low success rates. Many studies such as these are never published, perhaps due to a reluctance to publish negative results. This review summarises the history of the use of artificial domiciles for bumblebees incorporating information from published and unpublished studies in an attempt to draw conclusions regarding the potential of such domiciles for use in conservation, research and for commercial gain. Factors to be considered when using artificial domiciles for bumblebees are discussed and recommendations are made for future work. The findings of this review suggest that use of artificial domiciles for bumblebees may be more appropriate in some parts of the world compared to others, perhaps depending on ecological differences between bumblebee species found in these localities. However, there is also evidence for an overall decline in uptake rates of artificial domiciles in recent decades, potentially reflecting general declines in bumblebee abundance throughout their range.

2.2 Introduction

There are around 250 bumblebee species worldwide and these provide economically and ecologically important services throughout their native range as pollinators of crops and wildflowers (Osborne and Williams, 1996; Javorek et al., 2002; Biesmeijer et al., 2006). However, many bumblebee species have suffered severe declines in recent years (Williams and Osborne, 2009) and this may have important implications for conservation and agriculture.

A detailed understanding of bumblebee ecology is crucial in order to prevent further declines of these important species. Foraging behaviour is a much studied and well understood area of bumblebee ecology but relatively little is known of other aspects of their ecology including mating behaviour, hibernation ecology and, importantly, nesting ecology (Goulson, 2003a; Benton, 2006). A better understanding of nesting requirements would not only inform conservation management strategies but also present many other opportunities for the management and exploitation of these species (Corbet et al., 1994).

For over a century, there have been many attempts worldwide to induce bumblebees to nest in man-made domiciles. Some of this work has been published, while other studies, particularly those with poor success, are not mentioned in the scientific literature. This review provides a summary of previous work, both published and unpublished, on the development of artificial domiciles for bumblebees in order to assess the potential of this approach for future research, management and conservation.

2.3 Practical uses for artificial domiciles for bumblebees

2.3.1 Artificial domiciles to boost crop pollination

Bumblebees are very efficient pollinators of a wide range of important crop plants (Corbet et al., 1991; Free, 1993; Osborne and Williams, 1996; Stubbs and Drummond, 2001) and are therefore of great commercial importance to agriculture. Studies on other bee species (e.g. the alkali bee, *Nomia melanderi* and the leafcutter bee, *Megachile rotunda*), have shown that the provision of suitable nest sites can significantly augment local populations thus enhancing pollination service (Peck and Bolton, 1946; Parker et al., 1987). The potential of artificial domiciles for bumblebees either to promote favourable positioning of bumblebee colonies in relation to flowering crops, or to allow relocation of colonies to crop fields as the need arises was recognised several decades ago and has been the main driver behind many studies into the use of artificial domiciles (Fye and Medler, 1954; Hobbs et al., 1960, 1962; Hobbs, 1967a; Donovan and Weir, 1978; MacFarlane et al., 1983). Until recently, the only known methods for rearing bumblebees in captivity were highly time consuming and labour intensive and did not guarantee results, so the development of artificial domiciles for field placement was deemed to be an easier and more manageable method of boosting local bumblebee populations (Fye and Medler, 1954).

The development of methods for rearing bumblebees on a commercial scale and the rapid development of a thriving trade in bumblebee colonies, coupled with often disappointing results from the use of artificial domiciles saw a drop in interest in artificial domicile research. Import and export of commercially reared bumblebee colonies for pollination of crops such as greenhouse tomatoes and soft fruits is now a huge global industry (Asada and Ono, 2002; Hingston, 2005; Ings et al., 2006; Winter et al., 2006). However, the development of artificial domiciles that would reliably be accepted by wild bumblebee queens as nest sites would provide three important advantages over the current reliance on commercially reared colonies.

First, there could be significant financial benefits. Ensuring efficient pollination of crops by commercially reared bumblebee colonies comes at a huge financial cost to growers. For example, Koppert Biosystems recommend a density of 6-9 colonies per hectare for the pollination of raspberries and at £126 for a three colony unit (quoted in July 2009), adequate provision of bumblebee colonies may cost as much as £378 per hectare. Colonies are guaranteed for six weeks only so that repeat orders may be required within a year for crops flowering over a long period of time. A single outlay for artificial domiciles that could be used year on year and would ensure a certain number of colonies would considerably reduce these costs (although commercially reared bumblebees would still be required for crops grown out of season in polytunnels or glasshouses.)

Second, the design and usage of artificial domiciles can be engineered such that target species are preferentially attracted (Frison, 1926; Hobbs et al., 1962 and Hobbs, 1967a) and these can be chosen to suit the pollinating requirements of specific crops. This would confer huge advantages over the current system, because only a handful of bumblebee species are reared commercially and these are not always the most suitable for pollinating the crops being grown. For example, only the short-tongued bumblebee species, *B. terrestris*, is commercially reared for distribution in Europe, but it is unsuited to the pollination of crops with deep flowers, being more likely to rob these flowers, biting into the nectaries from behind and conferring no pollination benefit (Free, 1968). An example of such a crop is field bean (*Vicia faba*), yields of which have been shown to benefit from adequate pollination by long-tongued bumblebees such as *B. hortorum* (Free and Williams, 1976). Many long-tongued species are so-called ‘pocket-makers’ which feed their larvae directly on pollen collected in the field, and these species are notoriously more difficult to rear than the so-called ‘pollen storers’ which are able to feed their larvae from pollen stores within the nest (Griffin et al., 1991). As a result of these differences, commercial rearing of these species is unlikely to be feasible. However, several studies have demonstrated that pocket-makers such as *B. hortorum* and *B. ruderatus* will readily found nests in artificial domiciles (Sladen, 1912; Palmer, 1968; Barron et al., 2000).

Thirdly, several conservation concerns have recently been raised regarding the transportation of bumblebees outside their native ranges for pollination purposes. For example, two European sub-species of the buff-tailed bumblebee, *B. terrestris dalmitinus* and *B. terrestris terrestris*, are currently imported into the United Kingdom in vast quantities every year and there are concerns that these may be outcompeting or introgressing with the native British subspecies *B terrestris audax* (Ings et al., 2005a, 2005b, 2006). There have also been concerns in Japan, where *B. terrestris* is imported for glasshouse pollination. Glasshouse escapees are now living in the wild in Japan (Inari et al., 2005) and there is evidence to suggest that this species has the potential to outcompete native bumblebee species (Matsumara et al., 2004, Inari et al, 2005) and also that reproduction of native species may be inhibited by interspecific matings with *B. terrestris* (Kondo et al., 2009). As with any system in which a species exists at high density, the commercial rearing of bumblebees also raises issues with spread of parasites and/or pathogens (Pie et al., 2003) and it is believed that a major cause of precipitous declines observed in many US bumblebee species over the past twenty years is the accidental introduction of European bumblebee parasites and/or pathogens with bumblebees exported to Europe for rearing and then re-imported for crop pollination (Thorp et al., 2003; Colla et al., 2006; Winter et al., 2006).

If artificial domiciles for bumblebees could be designed such that there was a high probability of colonisation by bumblebees, strategic positioning of these boxes on farmland either before or after colonisation would allow farmers to boost local bumblebee populations ensuring a high quality pollination service by suitable bee species and vastly reducing the need for expensive and environmentally hazardous commercially reared bumblebee colonies.

2.3.2 Artificial domiciles for research

Since the colony is the reproductive unit of a bumblebee population (Wilson, 1975), knowledge of nesting ecology (e.g. species' requirements, density) is vital if we are to understand the dynamics of these species. However, currently, there is no reliable and accurate method for assessing bumblebee nest density in a given area (Osborne et al., 2007) and though it is evident that colony success is limited and that a large proportion of colonies never produce reproductive individuals (Cumber, 1953), few quantitative data are available on colony growth, survival rates or why some colonies fail. This lack of knowledge is mainly due to the difficulty in locating natural bumblebee nests and the propensity of those nests that are discovered to have been built in such a location as to make study or manipulation difficult or impossible. As a result, many studies requiring colonies use commercially reared bees (e.g. Goulson et al., 2002a; Morandin and Winston, 2002; Lopez-Vaamonde et al., 2004; Carvell et al., 2008), which may not be representative of the local wild population. However, well-designed artificial domiciles for bumblebees can provide reasonable numbers of colonies of wild bees for study (Pomeroy, 1981; Richards, 1987; R. Cartar, pers. comm.) and these can be relocated or manipulated as required. Perhaps the earliest account of bumblebee nest founding behaviour and the stages of colony growth is that of F.W.L. Sladen (1912). Many of his observations, which remain accurate and useful sources of information to this day, were made as a result of experimentation with the provision of artificial nest sites for bumblebees (Sladen, 1912). Since this time, studies using artificial domiciles for bumblebees have been successfully used to study niche breadth and overlap of nesting habitat (Richards, 1978) and pollination efficiency (Richards, 1987) as well as providing novel insights into colony behaviour, nest survival and the dynamics of some natural enemies of bumblebee nests (Hobbs et al., 1962; Richards, 1978). This demonstrates that artificial domiciles can provide a valuable tool for the study of bumblebee ecology at the level of the colony.

2.3.3 Artificial domiciles for conservation

Bumblebees pollinate a very wide range of wildflower species worldwide (Goulson et al., 2008a) and as a result, are likely to play an important role in the maintenance of wildflower populations. However, many bumblebee species have suffered severe declines over the past century, and this is true throughout their range in Europe (Williams, 1982; Kosier et al., 2007), North America (Grixti et al., 2009; Colla and Packer, 2008) and Japan (Xie et al., 2008). The reasons for these declines may differ in different localities and are still poorly understood, although in most places, the key factors are likely to relate to reduction in habitat quality as a result of changes in agricultural practices (Williams and Osborne, 2009). In the United Kingdom, bumblebee forage plants have declined disproportionately when compared to trends in the overall flora of the UK (Carvell et al., 2006a). The use of artificial domiciles for bumblebees near known populations of certain rare plant species may enhance pollination and out-crossing rates promoting the persistence of these plant species in a fragmented and impoverished environment.

Although the general reduction in forage availability for bumblebees is likely to have greatly contributed towards bumblebee declines, a reduction in nesting habitat may also have resulted in increased competition for nest sites, which would be particularly detrimental to later emerging species. Agricultural intensification has resulted in huge losses of natural and semi-natural habitat including the grasslands, woodland-edge habitat and field boundary features (Fuller, 1987; Robinson and Sutherland, 2002) favoured by nesting bumblebee queens (Kells and Goulson, 2003; Osborne et al., 2007). There is some evidence to support the hypothesis that availability of nesting sites may limit bumblebee populations. For example, although specific nest site preferences of individual species are still unclear, broad patterns indicate high levels of niche overlap for nesting habitat between species (Richards, 1978) and those species that have shown the most severe declines tend to be those that emerge later in the year (Goulson et al., 2005; Williams et al., 2009). McFrederick and Lebuhn (2006) found that bumblebee abundance was positively correlated with numbers of rodent holes which are known to provide nesting sites for bumblebees (Sladen, 1912; Svensson and Lundberg, 1977), suggesting that in this case nest sites were limiting. If nest site availability does limit bumblebee populations, the provision of successful artificial domiciles could be very valuable in boosting these populations. Many rare and/or declining species will found nests in artificial domiciles for example, *B. subterraneus, B. sylvarum* and *B. ruderatus* in Europe (Sladen, 1912; Palmer, 1968) and *B. fervidus, B. vagans, B. pennsylvanicus* and *B. borealis* in North America (Frison, 1926; Fye and Medler, 1954; Hobbs et al., 1962; Richards, 1978).

Artificial domiciles could also be used for monitoring and for supplementary feeding of species targeted in conservation action plans. This would be particularly useful for schemes involving species' reintroduction, as the effective population size will be particularly low in the early stages rendering the population more susceptible to extinction.

2.3.4 Artificial domiciles for public sale

Bumblebees are a charismatic and well-loved group of insects and as a result, there is a lucrative market for domiciles designed for members of the public to entice bumblebees to nest in their gardens and allow them to observe colony life. Indeed, several thousand bumblebee nest boxes are sold per year from garden centres and wildlife-friendly retailers in the UK alone. Sales in products related to wildlife gardening continue to increase at a rate of between 4 and 10% per year regardless of the financial climate (data collected by the Garden Centre Association in 2008) and these trends are due to increasing public awareness of declines in biodiversity and enhanced public interest in ‘gardening for wildlife’. Many people also appreciate the importance of pollinators for their garden plants and feel privileged to have a bumblebee nest in the garden.

There are currently a range of artificial domiciles designed for bumblebees available to the public, the most popular of which consists of a single-chambered wooden box supplied with a handful of straw to be used as nesting material. The boxes are generally intended to be placed on the surface of the ground. Several variations on this design are also available, including two-chambered boxes and boxes with entrance tunnels for use underground. However, there is little evidence to suggest that any of these commercially available domiciles provide attractive nesting sites for bumblebees, and anecdotal evidence suggests that uptake rates of such domiciles are extremely low (Bumblebee Conservation Trust, unpublished data). With appropriate marketing, the sale of more successful artificial nesting sites for bumblebees to the general public could be a huge commercial success.

2.4 A history of the use of artificial domiciles

2.4.1 Early artificial domicile trials

The first published trial of artificial domiciles for bumblebees is that of Sladen (1912). Sladen trialled four subterranean domicile designs (referred to here as Sladen basic, Sladen cover, Sladen tin and Sladen terracotta – appendix 2.1) in the United Kingdom. All of Sladen's domicile designs involved the creation of a subterranean cavity into which a handful of suitable nesting material was placed. This usually consisted of shredded grass, shredded moss and/or unravelled rope fibres. The domiciles were not self-contained in that the bottom of the domicile was always open and the nesting cavity was therefore in contact with the earth.

Sladen's domiciles achieved some success with an overall uptake rate of 29% (table 2.1). However, descriptions of his experiments are anecdotal and as a result, the proportion of occupancies that gave rise to successful colonies is unknown. It is likely that this was lower. Queens occupying Sladen's domiciles represented six different species. Two of these (*B. sylvarum* and *B. ruderatus*) are now rare and *B. subterraneus* is extinct in the UK.

In 1915, T.H. Frison, attempted to use artificial domiciles as a means of obtaining bumblebees for study in Urbana, Illinois (Frison, 1926). He designed three different styles of artificial domicile, the first two of which were influenced by Sladen's 'tin domicile' and are referred to here as 'Frison large tin' and 'Frison small tin' (see appendix 2.1 for details). These essentially consisted of tin cans with entrance tunnels consisting of metal spouts welded onto holes cut into the side of the cans. Sand and paint were poured down the spouts to provide a rough surface for grip. In Frison's original designs, the tins had sealed bottoms so that unlike Sladen's domiciles, the nest chamber was not in contact with the earth. However, an

Table 2.1: Occupancy rates for different artificial domicile designs for bumblebees, achieved across different studies.

(*B. app* = *Bombus appositus*, *B. aur* = *B. auricomus*, *B. bif* = *B. bifarius*, *B. bim* = *B. bimaculatus*, *B. bor* = *B. borealis*, *B. cal* = *B. californicus*, *B. cent* = *B. centralis*, *B. ferv* = *B. fervidus*, *B. flav* = *B. flavifrons*, *B. frig* = *B. frigidus*, *B. hort* = *B. hortorum*, *B. hunt* = *B. huntii*, *B. imp* = *B. impatiens*, *B. lap* = *B. lapidarius*, *B. luc* = *B. lucorum*, *B. mel* = *B. melanopygus*, *B. mix* = *B. mixtus*, *B. nev* = *B. nevadensis*, *B. occ* = *B. occidentalis*, *B. pasc* = *B. pascuorum*, *B. penn* = *B. pennsylvanicus*, *B. pratic* = *B. praticola*, *B. prator* = *B. pratorum*, *B. rud* = *B. ruderatus*, *B. ruf* = *B. rufocinctus*, *B. sep* = *B. separatus*, *B. subt* = *B. subterraneus*, *B. sylv* = *B. sylvarum*, *B. tern* = *B. ternarius*, *B. terr* = *B. terrestris*, *B. vag* = *B. vagans*.)

Box style	**Country**	**Year**	**Number of boxes***	**Occupancy**	**Workers**	**Reproductives**	**Species attracted****	**Reference**
Sladen basic	United Kingdom	1906	40	9 (23%)	1 (2.5%)		*B. lap* (56%), *B. terr* (11%), *B. hort* (11%), *B. rud* (11%), unknown (11%)	Sladen, 1912
Sladen cover	United Kingdom	1910-1912	79	24 (30%)			*B. lap* (63%), *B. subt* (17%), *B. terr* (4%), *B. rud* (4%), *B. hort* (4%), *B. sylv (4%)*, unknown (4%)	Sladen, 1912
Sladen tin	United Kingdom	1910-1912	40	13 (33%)			*B. lap* (77%), *B. subt* (15%), *B. sylv* (8%)	Sladen, 1912
Sladen terracotta	United Kingdom	1911	12	4 (33%)		3 (25%)	*B. terr* (25%), *B. lap* (75%)	Sladen, 1912
Frison large tin	Illinois, US	1915	9	1 (11%)	0 (0%)	0 (0%)	*B. penn* (100%)	Frison, 1926
Frison small tin	Illinois, US	1916	3	2 (67%)		1 (33%)	*B. aur* (50%), unknown (50%)	Frison, 1926
Frison small tin (improved)	Illinois, US	1917	4	2 (50%)		1 (25%)	*B. bim* (50%), unknown (50%)	Frison, 1926
Wooden box underground	Illinois, US	1917/1919	20	12 (60%)		4 (20%)	*B. bim* (8%), *B. penn* (17%), *B. sep* (8%), *B. imp* (8%), unknown (58%)	Frison, 1926
Fye and Medler flower pot	Wisconsin, US	1952	7	1 (14%)			*B. bor* (100%)	Fye and Medler, 1954
Fye and Medler metal can	Wisconsin, US	1952	4	3 (75%)			*B. bor* (67%), *B. vag* (33%)	Fye and Medler, 1954

Box style	Country	Year	Number of boxes*	Occupancy	Workers	Reproductives	Species attracted**	Reference
Fye and Medler tile	Wisconsin, US	1952	10	2 (20%)			*B. ruf* (100%)	Fye and Medler, 1954
Fye and Medler cone	Wisconsin, US	1952	18	0 (0%)			N/A	Fye and Medler, 1954
Wooden box surface	Wisconsin, US	1952	130	52 (40%)			*B. ruf* (27%), *B. sep* (6%), *B. bor* (35%), *B. ferv* (17%), *B. vag* (6%), unknown (10%)	Fye and Medler, 1954
Wooden box aerial	Wisconsin, US	1952	3	1 (33%)			*B. ferv* (100%)	Fye and Medler, 1954
Wooden box surface	Alberta, Canada	1955/1959	334	25 (7%)			*B. nev, B. bor, B. ferv, B. hunt, B. ruf, B. occ, B. app* (Relative occupancy rates unknown.)	Hobbs et al, 1960
Wooden box surface	Alberta, Canada	1960-1961	618	197 (32%)			*B. ferv* (1%), *B. nev* (2%), *B. hunt* (1%), *B. ruf* (36%), *B. app* (25%), *B. frig* (12%), *B. cal* (4%), *B. cent* (2%), *B. vag* (3%), *B. bif* (2%), *B. mel* (1%), unknown (13%)	Hobbs et al, 1962
Wooden box semi-underground	Alberta, Canada	1960-1961	180	57 (32%)			*B. nev* (2%), *B. hunt* (4%), *B. ruf* (19%), *B. app* (42%), *B. frig* (4%), *B. cal* (9%), *B. cent* (2%), *B. aur* (2%), unknown (18%)	Hobbs et al, 1962
Wooden box underground	Alberta, Canada	1960-1961	225	101 (45%)			*B. ferv* (4%), *B. bor* (2%), *B. nev* (12%), *B. hunt* (9%), *B. ruf* (11%), *B. app* (29%), *B. frig* (4%), *B. cal* (1%), *B. cent* (1%), *B. occ* (5%), *B. bif* (2%), *B. aur* (1%), *B. pratic* (1%), *B. tern* (1%), unknown (18%)	Hobbs et al, 1962
Wooden box surface	Alberta, Canada	1961-1966	1233	315 (26%)			unknown	Hobbs et al, 1967
Wooden box underground	Alberta, Canada	1961-1966	465	272 (58%)			unknown	Hobbs et al, 1967
Wooden box false underground	Alberta, Canada	1961-1966	500	255 (51%)			unknown	Hobbs et al, 1967
Wooden box aerial	Alberta, Canada	1961-1966	100	35 (35%)			unknown	Hobbs et al, 1967
Wooden box aerial	Holland	unknown	30	13 (43%)			unknown	Wilcke, 1953

Box style	**Country**	**Year**	**Number of boxes***	**Occupancy**	**Workers**	**Reproductives**	**Species attracted****	**Reference**
Wooden box surface or semi-underground	Holland	unknown	14	6 (43%)			unknown	Wilcke, 1953
Wooden box underground	Holland	unknown	28	10 (36%)			unknown	Wilcke, 1953
Wooden box surface	South Island, New Zealand	1967	100	17 (17%)	7 (7%)	2 (2%) only males	*B. rud* (94%), *B. terr* (6%)	Palmer, 1968
Pomeroy plastic underground	North Island, New Zealand	1974-1979	54	48 (88%)	31 (57%)	relocated for study	*B. rud* (54%), *B. terr* (21%), unknown (25%)	Pomeroy, 1981
Pomeroy brick surface	North Island, New Zealand	1974-1979	48	11 (23%)	9 (19%)	relocated for study	*B. rud* (91%), *B. terr* (9%)	Pomeroy, 1981
Pomeroy pumice-concrete surface	North Island, New Zealand	1974-1979	25	2 (8%)	2 (8%)	relocated for study	*B. rud* (50%), *B. terr* (50%)	Pomeroy, 1981
Pomeroy semi-underground	North Island, New Zealand	1974-1979	13	8 (62%)	8 (62%)	2 (15%)	*B. rud* (100%)	Pomeroy, 1981
Wooden box underground	Alberta, Canada	1970-1971	535	206 (39%)			*B. frig* (12%), *B. bif* (30%), *B. occ* (31%), *B. flav* (9%), *B. tern* (4%), *B. cal* (3%), *B. ruf* (5%), *B. app* (6%), *B. hunt* (<1%)	Richards, 1978
Wooden box false underground	Alberta, Canada	1970-1971	535	121 (23%)			*B. frig* (12%), *B. bif* (17%), *B. mix* (4%), *B. occ* (7%), *B. flav* (7%), *B. tern* (3%), *B. cal* (13%), *B. ruf* (21%), *B. app* (13%), *B. vag* (<1%)	Richards, 1978
Wooden box surface	Alberta, Canada	1970-1971	535	175 (33%)			*B. frig* (11%), *B. bif* (9%), *B. mix* (17%), *B. occ* (6%), *B. flav* (1%), *B. tern* (1%), *B. cal* (11%), *B. ruf* (19%), *B. app* (22%), *B. hunt* (1%), *B. cent* (1%), *B. vag* (1%)	Richards, 1978
Wooden box aerial	Alberta, Canada	1970-1971	535	207 (39%)			*B. frig* (29%), *B. bif* (5%), *B. mix* (16%), *B. occ* (2%), *B. flav* (2%), *B. tern* (<1%), *B. cal* (8%), *B. ruf* (19%), *B. app* (15%), *B. hunt* (<1%), *B. mel* (1%), *B. nev* (<1%)	Richards, 1978

Box style	**Country**	**Year**	**Number of boxes***	**Occupancy**	**Workers**	**Reproductives**	**Species attracted****	**Reference**
Wooden box surface	Alberta, Canada	1978-1979	500	99 (20%)	168 (17%) across both types	127 (13%) across both types	*B. nev* (42%), *B. hunt* (3%), *B. ruf* (32%), *B. cent* (6%), *B. app* (15%), *B. ferv* (1%)	Richards, 1987
Wooden box underground	Alberta, Canada	1978-1979	500	218 (44%)			*B. nev* (50%), *B. hunt* (23%), *B. ruf* (7%), *B. cent* (7%), *B. occ* (6%), *B. app* (5%), *B. tern* (<1%), *B. bif* (<1%)	Richards, 1987
Wooden surface or underground	South Island, New Zealand	1982	60	21 (35%)			*B. rud* (100%)	MacFarlane et al, 1983
Wooden surface or false underground	South Island, New Zealand	1971	43	8 (19%)	56 (16%) across all types	32 (9%) across all types produced queens	*B. hort* (52%), *B. rud* (20%), *B. terr* (10%), *B. subt* (7%)	Donovan and Weir, 1978
Polystyrene box white surface/false underground	South Island, New Zealand	1971	62	7 (11%)				Donovan and Weir, 1978
Wooden box surface	South Island, New Zealand	1972	65	9 (14%)				Donovan and Weir, 1978
Donovan and Weir polystyrene box black	South Island, New Zealand	1972-1973	107	45 (42%)				Donovan and Weir, 1978
Donovan and Weir polystyrene 'hives'	South Island, New Zealand	1973	63	15 (24%)				Donovan and Weir, 1978
Wooden surface 'hotels'	South Island, New Zealand	1995-1998	1280	67 (5%)	46 (4%)	11 (1%) queens	*B. hort* (61%), *B. terr* (25%), *B. rud* (2%), *unknown* (12%)	Barron et al, 2000
Gaston terracotta pot	United Kingdom	2000-2002	60	0 (0%)			N/A	Gaston et al, 2005
Gaston buried terracotta pots	United Kingdom	2000-2002	60	0 (0%)			N/A	Gaston et al, 2005
Two-chamber wooden surface boxes	United Kingdom	2000-2002	120	0 (0%)			N/A	Gaston et al, 2005
Wooden box surface and aerial	United Kingdom	1989-1991	532	5 (1%)			*B. pasc* (80%), *B. prator* (20%)	Fussell and Corbet, 1992
Fussell and Corbet brick domiciles	United Kingdom	1990-1991	122	5 (4%)			*B. pasc* (80%), *B. prator* (20%)	Fussell and Corbet, 1992
Carvell flower pot surface	United Kingdom	1999	16	0 (0%)			N/A	Carvell, 2000
Carvell flower pot subterranean	United Kingdom	1999	16	0 (0%)			N/A	Carvell, 2000

Box style	**Country**	**Year**	**Number of boxes***	**Occupancy**	**Workers**	**Reproductives**	**Species attracted****	**Reference**
Wooden box surface	United Kingdom	unknown	30-40	0 (0%)			N/A	Carvell, pers.comm.
Wooden box surface	Colorado, US	2006-2007	200	~20 (~10%)			*B. app* (~91%), *B. bif* (~9%)	Elliot, 2008
Complex underground	United Kingdom	2007-2008	170	13 (8%)	10 (6%)	4 (2%) queens***	*B. terr* (23%), *B. luc* (23%), *B. hort* (8%), *B. terr/B. luc* (23%), unknown (23%)	Chapter 3
Roosting pocket	United Kingdom	2007-2008	120	3 (3%)	2 (2%)	2 (2%) queens	*B. prator* (67%), unknown (33%)	Chapter 3
Subterranean slab domicile	United Kingdom	2007	100	2 (2%)	2 (2%)	1 (1%) queens, 1 (1%) males	*B. luc* (50%), *B. lap* (50%)	Chapter 3
Semi-subterranean flowerpot domicile	United Kingdom	2007	100	0 (0%)	N/A	N/A	N/A	Chapter 3
Wooden box semi-underground	United Kingdom	2007	100	1 (1%)	0 (0%)	0 (0%)	*B. luc* (100%)	Chapter 3
Wooden box surface	United Kingdom	2008-2009	26	0 (0%)	N/A	N/A	N/A	Chapter 3

*defined as number of opportunities to occupy i.e. the same domicile over two years is counted as 2

**number in brackets refers to percentage contribution to total occupancy where known

***two other thriving colonies were and these may also have progressed to reproductive production ‘improved’ version of the ‘small tin’ domicile had an open bottom with fine copper mesh acting as a barrier between the nest chamber and the earth. Frison’s domiciles achieved reasonable success obtaining 31% occupancy in total with occupants representing three different bumblebee species. However, only 13% of the colonies founded progressed to reproductive production (table 2.1).

Frison’s third domicile design was the first published ‘wooden box’ domicile and was also designed for subterranean use with an entrance tunnel and open bottom as the ‘improved’ small tin domicile (appendix 2.1). These obtained 50% occupancy and occupants represented four different bumblebee species (table 2.1).

2.4.2 Wooden boxes as artificial domiciles for bumblebees

Following the success of Frison’s wooden box domiciles, several artificial domicile studies were published based on similar designs. However, all subsequent wooden box designs have had closed bases so that the nest chamber is self-contained. Fye and Medler (1954) trialled the first wooden box domiciles to be positioned on the surface of the ground (appendix 2.1) in Wisconsin, US. Of 130 surface wooden boxes placed out, 52 (40%) were occupied and five different bumblebee species were represented (table 2.1). In addition, they placed three wooden boxes on buildings (termed ‘aerial domiciles’), one of which was occupied by a queen of *B. fervidus*.

In Canada, underground, surface, aerial, semi-underground and false underground wooden domiciles were trialled in the 1960s with the aim of providing a source of bumblebee colonies for crop pollination (Hobbs et al., 1960, 1962; Hobbs, 1967a) (appendix 2.1). Occupancy rates were variable, ranging from 7% to 58%, but subterranean domiciles generally attracted the greatest numbers of bumblebee queens. Sixteen species of bumblebee occupied these domiciles across the three studies (table 2.1).

Later, Richards (1978, 1987) used similar domiciles for studies on niche overlap of nesting habitat in bumblebees and on pollinator efficiency and effectiveness, also in Canada. He used underground, surface and aerial domiciles as well as false-underground domiciles based on those of Hobbs (1967a). Occupancy rates ranged from 20 to 44% and sixteen different bumblebee species were represented across these studies (table 2.1). Colony success was reported for just two domicile styles trialled in 1978-1979 for which 40% of colonies founded went on to produce reproductive individuals.

More recently, Elliot (2008) also attempted to collect colonies for study using wooden box artificial domiciles in North America. She reports occupancy rates of roughly 10% but this probably indicates successful colony foundation rather than queen occupancy rates, which may have been higher.

Wooden box artificial domicile designs are still being used in North America to collect colonies for study. Uptake rates are reported to be consistently around 50%, although these often fail at the queen stage (R. Cartar, pers. comm.).

Wooden box domiciles have also been trialled in Europe and in New Zealand. Wilcke (1953) set out 72 wooden domiciles in the Netherlands, 30 of which were aerial, 14 of which were surface or semi-underground and 28 of which were subterranean. Positioning did not appear to affect uptake rates with domiciles in all positions achieving around 40% occupancy rates (table 2.1). Seven different bumblebee species were represented.

In New Zealand, surface and underground wooden domiciles have been trialled with the intention of finding a method for providing bumblebee colonies for crop pollination (Palmer, 1968; MacFarlane et al., 1983). Across the two studies, 24% occupancy was achieved, although colony success was low in the former and unreported in the latter (table 2.1).

Also in New Zealand, Barron et al. (2000) trialled the 'bumblebee hotel', a long wooden box divided into four compartments each designed to house one bumblebee colony. Of these, only 5% were occupied, although all three species present at the study site were represented (table 2.1). Although it is tempting to attribute the comparatively low success rate to the proximity of domiciles to each other within the same 'hotel', no evidence was found for competition between adjacent colonies. There were three incidences of double occupancy and the authors note that in one of these cases, both colonies became large and progressed to queen production. Instead, the relatively low success rate achieved was attributed to small local bumblebee populations, plentiful natural nest sites, poor domicile design or poor placement.

2.4.3 Studies involving the development of novel domicile designs

Alongside trials with wooden box domiciles, Fye and Medler (1954) trialled a range of other domicile designs in the US, most of which were heavily influenced by the previous work of Sladen and Frison (see appendix 2.1 for details). These achieved variable success, with occupancy ranging from 0% for the ‘Fye and Medler cone’ design to 75% for the ‘Fye and Medler metal can’ design. Three bumblebee species were represented in their trials (table 2.1).

In New Zealand, Donovan and Weir (1978) compared traditional wooden surface and underground domiciles against novel designs based on a polystyrene box with the goal of developing a successful method for boosting bumblebee populations for crop pollination. These trials led to the development of the ‘polystyrene hive’ which consisted of a commercially available polystyrene box with holes for access, drainage and ventilation (appendix 2.1). The outside was white and the inside was black to optimise the internal temperature. In this study, polystyrene domiciles achieved higher uptake rates than wooden box designs (overall, 29% versus 16% respectively – see table 2.1). All four species present in New Zealand (*B. terrestris, B. hortorum, B. ruderatus* and *B. subterraneus*) colonised domiciles during this study.

Between 1974 and 1979, Pomeroy (1981) also conducted trials with artificial domiciles in New Zealand, using four original designs. The aim of the work was to provide a source of colonies for study, and considerable success was achieved. The domiciles were described as ‘plastic-underground’, ‘brick-surface’, ‘pumice-concrete' and 'semi-underground' (see appendix 2.1). The 'plastic-underground' domiciles achieved a maximum uptake rate in one year of 93% and when colonies were removed from domiciles, it was not uncommon for the domiciles to become occupied for a second time in the same year.

In the UK, little has been published on the use of artificial domiciles since Sladen (1912) although this may simply reflect a lack of success with inducing queens to nest within artificial domiciles in the UK combined with a reluctance to publish negative results. Fussell and Corbet (1992) report a trial of several different domicile designs (see appendix 2.1) in which occupancy rates were reported to be extremely low (table 2.1) and Gaston et al., 2005b achieved 0% occupancy for three designs trialled in urban gardens (appendix 2.1, table 2.1). Similarly, artificial domiciles designed with the aim of providing nesting sites for the Biodiversity Action Plan listed British bumblebee species *B. sylvarum* and *B. humilis* (see appendix 2.1 for details) and trialled in South Wales, achieved no occupancy by any species (Carvell, 2000). The same author also trialled 30-40 wooden surface boxes with mesh ventilation placed out in suitable habitat at Monkswood, UK but again, none were colonised (C. Carvell, pers. comm.).

There has been some success with artificial domiciles in the UK. Two styles have reportedly resulted in some 30 colonies per year within one garden, and although numbers of domiciles put out are not recorded, this is likely to reflect reasonable uptake rates (Intenthron and Gerrard, 1999). However, some of these colonies were probably established after the forced introduction of queens into the domiciles (see section 2.5.8), and Intenthron's description of his work suggests that without queen introduction, uptake rates are generally low.

Lye (chapter 3) also trialled several domicile styles based on successful designs by Intenthron and Gerrard (1999) and other authors (table 2.1). Occupancy rates varied dramatically, ranging from 0% to 45%, but were generally low. The domiciles trialled were colonised by five different British bumblebee species, suggesting that under some circumstances, domiciles can be successfully used to attract nesting bumblebee queens in the UK.

2.4.6 Reasons for differences in occupancy – Does country and date have an effect?

Occupancy rates seem to be generally higher in Canada and the US than in Europe and New Zealand (table 2.2). This may be a result of species-specific differences in nest site preference between North American bumblebees and European bumblebees. The greater number of common species present at the North American study sites as compared to Europe and New Zealand also presumably gives rise to a wider range of nesting preferences, perhaps resulting in greater overall uptake of domiciles. However, New Zealand contains only four bumblebee species, all of European origin, yet studies conducted in New Zealand tend to give higher uptake rates than those conducted in Europe. Patterns in uptake rates across countries may also be linked to differences in the availability of natural nest sites for bumblebees between the different locations (Fussell and Corbet, 1992). In many parts of North America, the landscape is more homogenous than that in Europe such that there are large expanses of intensive agricultural land which are probably poor in suitable nesting habitat for bumblebees. Under these conditions, domicile uptake might be expected to be high. In New Zealand, the limited diversity of small mammals may result in a paucity of suitable nesting sites for bumblebees. Rats and house mice are present in New Zealand, but *Sorex*, *Apodemus*, *Clethrionomys* or *Microtus,* all of which may provide nest sites for bumblebees in Europe, are absent (Fussell and Corbet, 1992).

Table 2.2: Combined occupancy rates of studies using artificial domiciles for bumblebees, split by country (taken from data presented in table 4.1)

Country	**Domiciles**	**Occupied**	**Percent occupancy**	**Number of studies**
Europe	1815	108	6%	7
New Zealand	1920	258	13%	5
US	408	86	21%	3
Canada	6795	2283	34%	5

Recent studies seem to report lower occupancy rates than older studies (table 2.3) and this may represent the general decline in bumblebee populations in recent years (Williams and Osborne, 2009). It is possible that whilst nest sites may once have limited bumblebee populations, other factors such as forage availability are now more important determinants of bumblebee abundance. This is concerning since it suggests a general decline in bumblebee abundance regardless of species, perhaps demonstrating that even those species currently regarded as not at risk may be suffering losses.

However, published studies of artificial domiciles are few, so care must be taken when making comparisons between countries and time periods. Uptake rates are likely to be strongly affected by yearly fluctuations in weather conditions, local bumblebee abundance and small mammal population sizes, and differences observed may be strongly influenced by these chance factors. Since many North American studies are conducted by the same authors, it is also possible that differences are influenced by experimenter experience and ability to design and site artificial domiciles such that they will be attractive to nest founding queens.

Table 2.3: Combined occupancy rates of studies using artificial domiciles for bumblebees, split by date (taken from data presented in table 2.1)

Year	**Domiciles**	**Occupied**	**Percent occupancy**	**Number of studies**
1900-1920	207	67	32%	2
1950s	678	130	19%	4
1960s	3321	1232	37%	2
1970s	3620	1179	33%	4
1980-1999	2026	98	5%	3
2000s	1086	19	2%	4

2.5 Maximising domicile uptake rates – attracting queens to nest

A successful artificial domicile has two functions. Firstly, it must be attractive to nest site searching bumblebee queens and secondly, it must provide conditions conducive to colony development. Bowers (1985) suggested that the factors used by bumblebee queens when locating nest sites are not necessarily those which will determine colony survival, and this is supported by the fact that several artificial domicile studies report queen occupancy in domiciles that are unsuitable for colony development (Sladen, 1912; Pomeroy, 1981). Section 2.4 deals with attracting bumblebee queens to initiate colony foundation within a domicile whilst section 2.5 addresses some factors that are important for maximising colony survival and success within artificial domiciles.

2.5.1 Habitat type

The habitat into which artificial domiciles are placed is important in terms of both how many domiciles will become occupied and which species will colonise them. Higher rates of occupation would be expected in areas where bumblebees are plentiful, but might also be predicted where natural nest site availability is limiting (Fussell and Corbet, 1992). Similarly, since there are species-specific differences in the preferred nesting habitat of bumblebees (Frison, 1926; Svensson et al., 2000; Kells and Goulson, 2003), placing domiciles out in different habitat types should target different species.

Some of the most successful artificial domicile studies are those in which domicile location has been dictated by the author’s prior knowledge of where there have previously been high densities of bumblebee nests (Sladen 1912; Frison, 1926; Pomeroy, 1981; chapter 3). Therefore, in order to maximise artificial domicile success it is of value to scope out potential sites in advance, using indirect measures such as abundance of nest site searching queens or direct evidence such as nest searches as indicators of good habitat in which to place domiciles.

If a particular species is to be targeted, knowledge of habitat preferences is required. For example, species such as *B. impatiens, B. bimaculatus* and *B. occidentalis* are known to be associated with woodland, so domiciles placed in woodland edge habitat are more likely to attract these species, but *B. nevadensis, B. borealis, B. fervidus* and *B. huntii* are more often found occupying domiciles placed out in open grassland, since this is the type of habitat with which these species tend to associate (Frison, 1926; Hobbs et al., 1962).

The least successful artificial domiciles are often those placed out in areas of intensively managed agricultural land (Hobbs, et al., 1960; chapter 3) and Barron et al., (2000) obtained a significantly lower rate of uptake on intensively managed farms than low-intensity agricultural sites. Modern intensive farming methods are believed to have dramatically reduced the quality of habitat for bumblebees in agricultural land and it is believed that this is one of the main drivers behind bumblebee declines, particularly across Europe (Goulson et al., 2008a). Today, bumblebee colonies in the UK appear to be more successful in the urban environment than the rural environment, and the increased prevalence of the bumblebee wax moth *Aphomia sociella* in the urban versus rural environment suggests that bumblebees are more abundant in urban areas (Goulson et al., 2002a). This would explain the low occupancy rates observed in the intensive agricultural environment.

The relatively poor performance of domiciles on farmland has important implications for those wishing to use artificial domiciles to boost pollinator abundance for crop pollination. The most effective method for the use of artificial domiciles to increase pollinator abundance on farmland may be to place the domiciles elsewhere and subsequently relocate colonies to the site of the crop during the flowering period (as Hobbs et al., 1962).

In some cases, the most suitable habitat in which to place domiciles to maximise uptake by a target species may not be the habitat type with which nests of that species are most strongly associated. For example, *B. terrestris* is known to thrive in the urban environment and is commonly found nesting in urban areas, making use of compost heaps, buildings and other man-made objects (Fussell and Corbet, 1992; Donovan and Weir, 1978). However, in New Zealand, *B. terrestris* showed higher rates of domicile occupancy in rural areas than in suburban habitat (Donovan and Weir, 1978), suggesting that in this case, high occupancy rates may represent low nest site availability in the rural environment rather than a preference for this particular habitat type.

2.5.2 Positioning relative to the ground

Depending on where domiciles are to be used and which species are to be targeted, different positions will provide different success rates. Some species e.g. *Bombus terrestris, B. ternarius, B. terricola, B. nevadensis, B. borealis, B. fervidus, B. occidentalis* and *B. huntii* (Sladen, 1912; Fye and Medler, 1954; Hobbs et al., 1960, 1962; Richards, 1978) prefer to nest underground, whilst others (such as *B. pascuorum* and *B. humilis*) nest on the surface of the ground (Prŷs-Jones and Corbet, 1991). Other species such as *B. hypnorum* usually nest above ground (chapter 5) and some (e.g. *Bombus appositus* and *B. pratorum*) are more generalist

Table 2.4: The position relative to the ground of nests of different bumblebee species founded in artificial domiciles

Species	Underground	False/Semi-underground	Surface	Aerial	Totals	References
B. appositus	53 (23%)	40 (18%)	104 (46%)	31 (14%)	228	(Hobbs et al., 1962; Richards, 1978, 1987)
B. auricomus	2 (67%)	1 (33%)			3	(Frison, 1926; Hobbs et al., 1962)
B. bifarius	64 (56%)	21 (18%)	19 (17%)	11 (10%)	115	(Hobbs et al., 1962; Richards, 1978, 1987)
B. bimaculatus	2 (100%)				2	(Frison, 1926)
B. borealis	4 (17%)		19 (83%)		23	(Fye and Medler, 1954; Hobbs et al., 1962)
B. californicus	7 (10%)	21 (29%)	27 (38%)	17 (24%)	72	(Hobbs et al., 1962; Richards, 1978)
B. centralis	17 (59%)	1 (3%)	11 (38%)		29	(Hobbs et al., 1962; Richards, 1978, 1987)
B. fervidus	4 (24%)		12 (71%)	1 (6%)	17	(Fye and Medler, 1954; Hobbs et al., 1962; Richards, 1987)
B. flavifrons	7 (50%)	4 (29%)	1 (7%)	2 (14%)	14	(Richards, 1978)
B. frigidus	28 (19%)	17 (11%)	43 (29%)	61 (41%)	149	(Hobbs et al., 1962; Richards, 1978)
B. hortorum	3 (7%)		41 (93%)		44	(Sladen, 1912; Barron et al., 2000; Chapter 3)
B. huntii	60 (87%)	2 (3%)	6 (9%)	1 (1%)	69	(Hobbs et al., 1962; Richards, 1978, 1987)
B. impatiens	1 (100%)				1	(Frison, 1926)
B. lapidarius	35 (100%)				35	(Sladen, 1912; Chapter 3)
B. lucorum	4 (80%)	1 (20%)			5	(Chapter 3)
B. melanopygus			2 (50%)	2 (50%)	4	(Hobbs et al., 1962; Richards, 1978)
B. mixtus		5 (7%)	29 (43%)	33 (49%)	67	(Richards, 1978)
B. nevadensis	121 (72%)	1 (1%)	46 (27%)	1 (1%)	169	(Hobbs et al., 1962; Richards, 1978, 1987)
B. occidentalis	83 (78%)	9 (8%)	10 (9%)	5 (5%)	107	(Hobbs et al., 1962; Richards, 1978, 1987)
B. pascuorum			1 (100%)		1	(Sladen, 1912)
B. pensylvanicus	3 (100%)				3	(Frison, 1926)
B. praticola	1 (100%)				1	(Hobbs et al., 1962)
B. pratorum	2 (100%)				2	(Chapter 3)
B. ruderatus	28 (44%)	8 (12%)	28 (44%)		64	(Sladen, 1912; Palmer, 1968; Pomeroy, 1981; Barron et al., 2000)
B. rufocinctus	40 (15%)	36 (14%)	150 (56%)	40 (15%)	266	(Fye and Medler, 1954; Hobbs et al., 1962; Richards 1978, 1987)

Species	**Underground**	**False/Semi-underground**	**Surface**	**Aerial**	**Totals**	**References**
B. separatus	1 (25%)		3 (75%)		4	(Frison, 1926; Fye and Medler, 1954)
B. subterraneus	6 (100%)				6	(Sladen, 1912)
B. sylvarum	2 (100%)				2	(Sladen, 1912)
B. ternarius	10 (59%)	4 (24%)	2 (12%)	1 (6%)	17	(Hobbs et al., 1962; Richards, 1978, 1987)
B. terrestris	17 (46%)		20 (54%)		37	(Sladen, 1912; Palmer, 1968; Pomeroy, 1981; Barron et al., 2000; Chapter 3)
B. vagans	1 (8%)	1 (8%)	10 (84%)		12	(Fye and Medler, 1954; Hobbs et al., 1962; Richards 1978)

in their preferences (Richards, 1978; Alford, 1975).

Occupancy rates of domiciles in different positions by each bumblebee species combined across all studies are presented in table 2.4. It is not possible to combine the data from artificial domicile studies without bias, as different numbers and styles of domicile were used in each, so these data must be treated with caution and in many cases, uptake rates may over- or under-estimate the propensity of each species to nest at each position. However, these data do provide an idea of how specialised each species is in terms of its preference for domicile positioning and which species might be expected to occupy each domicile style.

Some studies suggest that it may be possible to provide conditions that will attract both surface and subterranean nesting species. For example, Hobbs (1967a) found that a false underground domicile design with an upward tilting entrance tunnel attracted all species of bumblebee in the area, regardless of their positional preference.

2.5.3 Landmarks

Bumblebees are known to use visual landmarks in navigation (Collet and Ziel, 1996), so domiciles positioned in the vicinity of landmark features may have a higher attractiveness to bumblebee queens as they are easily re-located. The zig-zag flight of bumblebee queens searching for nest sites is reminiscent of that of a bumblebee navigating by landmarks (Wellington, 1974), suggesting that visual cues are important in nest site location.

In practice, the effectiveness of the use of landmarks to enhance uptake of artificial domiciles seems to be variable and may depend on the homogeneity of the landscape into which the domicile is placed as well as the species to be attracted. Fye and Medler (1954) found that domiciles placed by landmarks such as fence posts, rocks, trees and stumps were occupied more frequently than those placed in the open and Barron et al. (2000) specifically placed domiciles next to landmarks to aid location by queens. However, Hobbs, et al. (1962) found no evidence of an increase in uptake rate as a result of proximity to landmark features. In this case, domiciles placed beside rocks, fence posts and shrubs were occupied as often as those placed in homogenous grassland.

Whether or not landmarks aid uptake by bumblebees, if artificial domiciles are to be placed in close proximity to one another, it is important that there are recognisable differences between the domiciles themselves or in their immediate surroundings in order to prevent queens entering the wrong domicile. If this occurs and another queen is present, a fight will always take place resulting in the death of either the resident or the invading queen (Hobbs et al., 1962). Painting domiciles different colours can be used as an aid to recognition and it is also helpful to have entrances to proximate domiciles facing in different directions (Hobbs et al., 1962).

Since bumblebees have good vision and may use visual cues to locate suitable nest sites, it has been hypothesised that certain colours or colour contrasts may be more attractive to nest site searching bumblebee queens than others. For example, since many nests are found in holes in the earth or in grass, it has been suggested that a dark hole on a green or brown background might provide a stimulus that would encourage investigation of a site, or, since bumblebees are known to show an innate preference for investigating blue objects, that painting domiciles blue may encourage exploration by nest site searching queens (Donovan and Weir, 1978). This hypothesis has not been well tested, but where artificial domiciles have been painted, there was no evidence for any obvious effect on uptake rates (Donovan and Weir, 1978). However, Pomeroy (1981) found that replacing translucent nest entrance tunnels with otherwise identical black entrance tunnels increased the frequency of exploration by bumblebee queens, suggesting that dark entrance holes are more attractive to nest site searching bumblebee queens.

2.5.4 Timing of placement - emergence time related to nest box uptake

Timing of emergence after diapause is very variable from species to species. Some (such as *B. bimaculatus, B. perplexus, B. impatiens* and *B. terrestris*) emerge very early in the spring and others (such as *B. appositus, B. rufocinctus, B. californicus* and *B. sylvarum*) emerge much later (Hobbs, 1967a; Goulson et al, 2005). In theory, it should be possible to place artificial domiciles to coincide with the phenology of the specific species required to try to enhance the likelihood of attracting it and this has been done with some success by Hobbs (1967a), who required later emerging species for pollination of legume crops. This may be particularly useful in conservation, as most species of conservation concern are later emerging species (Goulson et al., 2005; Fitzpatrick et al., 2007; Williams et al., 2009). These species could be targeted by placing boxes out when these species commence nest site searching, after many of the earlier emerging species have already established colonies.

Although conservation efforts for bumblebees tend to focus on the provision of suitable forage, nest sites may also limit bumblebee populations. The fact that queens may take several weeks to locate a suitable nest site (Alford, 1975), that high levels of conspecific usurpation takes place (see section 2.6.8) and that there is a high overlap of nest site preference between species (Richards, 1978) suggests that this may well be the case. Interestingly, it is later emerging species (such as *B. appositus, B. rufocinctus* and *B. ruderatus*) that often show the highest rates of domicile colonisation, regardless of their relative abundance at the location of the artificial domiciles (Sladen, 1912; Hobbs et al., 1962), providing more evidence to support this hypothesis. In Canada, the latest emerging bumblebee species tend to be much less specialist in nest site preference than those that emerge early on in the season (Richards, 1978).

2.5.5 Nesting material

Nesting material for bumblebees should be fine, absorbent and easily manipulated by the queen, and should provide good insulation for the brood (Fussell and Corbet, 1992). It should not contain synthetic fibres, as these can tangle around the feet of the bees, causing mortality (Intenthron and Gerrard, 1999). A variety of different nest materials have been trialled in artificial domiciles including old mouse nests, carpet underlay, upholsterers' cotton and shredded moss (Sladen, 1912; Fye and Medler, 1954; Hobbs et al., 1960, 1962; Donovan and Weir, 1978; Richards, 1978; 1987; Pomeroy, 1981; Intenthron and Gerrard, 1999), but there is no evidence to suggest that one is preferable to another. All materials trialled have proved functional, and bumblebees appear to be very generalist in the types of nesting materials that they will accept.

However, all of the materials used in these studies were considered carefully by the authors and it is certain that wholly inappropriate nest material would dramatically reduce uptake rates. Sladen (1912) suggests that his early attempts at attracting queens to domiciles may have been limited by the suitability of his nest material (which consisted of grass that he had cut into short lengths, torn moss or lengths of tow, cut into pieces), as it was coarser than the material usually found to constitute nest material in wild bumblebee nests. He solved this problem in latter years by using grass that had been scratched up by chickens or by raking up grass himself.

It is likely that the straw provided with many commercially available artificial domiciles is unsuitable for colonisation by bumblebees. It is notable that when commercially available domiciles are occupied, this tends to occur several years after initial placement (pers. obs.) and it is possible that their success depends on the importation of more suitable nest material by other animals such as mice.

2.5.6 Baiting domiciles – are uptake rates increased by the presence of mouse nests?

Since bumblebees nest in pre-existing cavities and rely on the presence of insulating material for their brood, small mammals may be of vital importance in the provision of natural nest sites for bumblebees. Many nests are discovered in the old, abandoned homes of such species (Svensson and Lundberg, 1977; Donovan and

Weir, 1978) and it has been suggested that bumblebees may use specific cues associated with these types of nest sites, particularly odour cues, in order to find nesting sites. This hypothesis is consistent with the nest site searching behaviour displayed by bumblebees. A nest site searching queen will fly very slowly and close to the ground, adopting a zig-zag flight path which would certainly allow detection of short-range olfactory cues and may serve a purpose similar to that of a moth locating an odour plume.

If odour cues are used by nest-site searching queens, baiting artificial domiciles with old mouse nests or odours associated with old mouse nests should improve occupancy rates. However, this does not seem to be the case. Fye and Medler (1954) baited their domiciles with flax straw and grain in order to attract mice to nest over autumn and winter and then vacated the mice in spring, adding a mouse excluder to prevent the mice reinvading the domiciles. Where no mice nested, they replaced the bedding with an old mouse nest from elsewhere or with some felt, rug matting. This methodology was repeated by Hobbs et al. (1960), who found that bumblebees would use fresh upholsterers' cotton just as readily as old mice nests. In the UK, domiciles have been baited with upholsterers cotton that had previously been used as bedding by domestic mice, and granules of acetamide have also been used with clean bedding to mimic the odour of small mammal urine, but in both cases, no uptake was achieved (Carvell, 2000). Some studies carried out over several years record mouse occupancy from year to year, and whilst levels of mouse occupancy are usually very high, bumblebees nests are not founded more often in those domiciles that have previously been occupied compared to those that have not (Fussell and Corbet, 1992; Barron et al., 2000). In addition to this, Pomeroy (1981) found that *B. hortorum* accepted clean nest material more readily than the same type of material that had been previously nested in by mice.

It is possible that, if such cues are used, these are species-specific, such that nest foundation by a queen of a particular bumblebee species will be influenced only by odours associated with small mammals with similar nesting ecology. For example, some evidence suggests that nest foundation in *B. terrestris* may be facilitated by odours associated with old vole nest material. Djegham et al. (1994) found that odours associated with the common vole, *Microtus arvalis*, stimulated colony initiation by *B. terrestris* queens whilst Lye found that *B. terrestris* queens caught whilst nest site searching were attracted to odours associated with aged nest material of the bank vole, *Clethrionomys glareolus* (chapter 4).

Whilst odour cues may play a role in the location of nest sites by bumblebees, it is clear that this is not the only mechanism used. Colonies are presumably founded in abandoned small mammal nests because they provide conditions under which a queen can found a successful colony (i.e. a dry cavity containing suitable nest material) and there is evidence to suggest that bumblebees will use the abandoned homes of a wide range of small animals including birds (Rasmont et al., 2008), squirrels (Sladen, 1912) and hedgehogs (chapter 5). Although isolating odour cues to which nest site searching bumblebee queens are attracted may provide a method of boosting exploration rates of domiciles, suitable artificial domiciles should provide conditions conducive to colony foundation without prior occupation by a small animal such that provision of small mammal nesting material is probably not required to achieve favourable results. However, it is probable that where unsuitable nesting material is provided, occupation by small mammals will increase the likelihood of inhabitancy by bumblebees by improving conditions within the domicile.

2.5.7 Exploitation of consecutive occupancy

It has been suggested that reusing artificial domiciles over several years can increase the likelihood of occupancy by bumblebees and several studies provide evidence to suggest that this is the case (Hobbs et al., 1962; Donovan and Weir, 1978; Barron et al., 2000). Various hypotheses have been put forward to explain this phenomenon, the first of which is that new queens will return to the site of their maternal nest site in order to try to found a new colony either in the same location or close by (Donovan and Weir, 1978). The reasoning behind this is that if a colony succeeds in producing new queens in one year, there is a high chance that this site will still be suitable in the subsequent year. Some evidence for this behaviour was reported by Pomeroy (1981) who marked queens leaving their nests at the end of the season and found that at least one marked queen returned the following year and entered the same domicile, although the individual did not exhibit any nesting behaviour once inside the domicile. Barron et al. (2000) also found that if a colony was founded in a domicile one year, the domicile was more likely to be occupied the subsequent year than would be expected by chance.

However, increasing occupancy rates appear to occur even when domiciles are moved from year to year (Donovan and Weir, 1978). One explanation put forward to explain this is that when new adult queens reside in their maternal nest, a kind of imprinting process might be taking place in which the queens learn cues associated with the appearance of their nest and will preferentially explore sites with a similar appearance the following year (Donovan and Weir, 1978). Contrary to this argument, Hobbs et al. (1962) found increasing occupancy in boxes left out across subsequent years despite the removal of colonies to a distant crop site each year. In this case it seems unlikely that the new founding queens contributing to occupancy belonged to the original colonies that had been founded in the previous year.

A second hypothesis for consecutive occupancy is that bumblebee queens are able to detect domiciles that have been used in previous years (the most likely mechanism for this being via olfactory cues) and will preferentially nest in these boxes (Barron et al., 2000). Again, the explanation put forward for such a phenomenon is that if a site has proved to be suitable in previous years, it is likely that it will remain a good site in subsequent years.

There are several other factors that might lead to increasing occupancy over successive years and these are also likely to play a role in such observations. The first is that newly built artificial domiciles are likely to seem alien in the landscape context into which they are placed, but as the domiciles remain in the environment they will lose unnatural odours and take on those around them, and will also become more camouflaged and sheltered as vegetation grows up around them (Barron et al., 2000). Camouflage of nest entrances may be an important factor with regard to colony survival (Richards, 1978) therefore nest site searching queens may actively search for cavities that are inconspicuous. Many species are known to actively camouflage their nest entrances with vegetation (Hobbs, 1966, 1967b, 1968; Richards, 1978) and it is believed that this results in alteration of the volatile and/or visual profile of the nest entrance, protecting it against predators and other nest enemies (Richards, 1978). If this is the case, then placing artificial domiciles far in advance of the onset of colony foundation may be a useful method of enhancing the likelihood of occupancy. However, if this is done, it will be important to check the serviceability of access points into the domicile and check that bedding is still present and dry when the first bumblebee queens are observed.

The short duration of most studies (generally two or three years) means that patterns observed in occupancy rates may often be due to unrelated factors such as the size of the bumblebee population (which will fluctuate from year to year), the number of natural nest sites available (perhaps linked to the small mammal population from the previous year) and the weather conditions. Not all studies report increasing occupancy. MacFarlane et al. (1983) report consistent occupancy rates throughout their trial, Richards (1987) reports very variable rates of uptake over the 6 years of his study and no increase for any species, and Hobbs (1967a) reports a decrease in occupancy rates over two years.

2.5.8 Confinement of queens in domiciles

Many authors have attempted to obtain bumblebee colonies by confining queens in the spring either to encourage nest foundation in an artificial domicile or to induce colony initiation in the laboratory for later relocation to the field (Frison, 1927; Hasselrot, 1952, 1960; Holm, 1960; Intenthron and Gerrard, 1999). Queen confinement is sometimes effective for increasing colony foundation within a domicile and a method for doing this is described by Intenthron and Gerrard (1999). It is important to ensure that queens introduced into domiciles do not already have a nest and this can be done by catching nest site searching queens only. These should be confined in a small box supplied with nest material and nectar (or 50% sugar solution) until they no longer display signs of stress. Following this, a queen should be introduced to each domicile and confined with a feeder for approximately 48 hours. After this time, the entrance can be unblocked and the queens may accept the domiciles.

2.6 Maximising colony success - factors affecting nest survival and mitigation methods

Several artificial domicile studies report detailed descriptions of the fate of colonies founded within them and these provide valuable insights into factors affecting the survival of bumblebee colonies over time and methods by which colony success can be promoted.

2.6.1 Forage availability

Few artificial domicile studies report on the positioning of domiciles in relation to spring forage, but some of the highest levels of uptake have been achieved in areas that are likely to contain plentiful spring forage such as botanical gardens, meadows and low intensity agricultural environments (Sladen, 1912; Barron et al., 2000; chapter 3; L. Pelletier and R. Cartar, pers. comm.). The presence of spring forage is likely to attract spring queens, increasing local bumblebee abundance and perhaps giving rise to higher uptake rates, although Donovan and Weir (1978) found that the number of queens feeding on a nearby source of spring forage did not predict the occupancy rate of adjacent boxes. Queens may have an active preference for nesting in the vicinity of spring forage since local availability of spring forage is likely to have a large impact on the success of a newly established colony. Colony failure seems to occur particularly often in the early stages of colony development. Success of the first brood relies on adequate nutrition being available and effective incubation by the queen as well as avoidance of predation. Bumblebee first broods often suffer attacks by ants or small mammals and this usually occurs whilst the queen is absent from the nest (Sladen, 1912). The proximity of good sources of spring forage reduces the amount of time during which the queen is absent from the brood, allowing effective incubation of the brood and affording greater protection against natural enemies.

If colonies are to thrive, a succession of forage throughout the season is required. This can be achieved by careful positioning of domiciles in high quality habitat, but can also be achieved by planting suitable flowers in the vicinity of the colonies or by the provision of resources through artificial feeders (MacFarlane et al., 1983). Repeated relocation of domiciles to areas of plentiful forage through the year is another possible method of ensuring adequate provisioning of colonies and may be appropriate where domiciles are being moved around for crop pollination.

2.6.2 Unfavourable weather

For most domicile styles, poor weather increases the likelihood of domicile nest chambers becoming damp, a common cause of queen desertion in the early stages of colony foundation (Richards, 1987). Even if the queen does not abandon the nest, a bumblebee colony will fail to thrive in excessively damp conditions and the comb often succumbs to attack by fungus (Sladen, 1912). Thus a successful artificial domicile for bumblebees must be weather-proof.

In poor weather, workers become lethargic and forego their duties until conditions improve. One or two days of bad weather have no long-term negative effects on bumblebee colonies, but prolonged periods often lead to the death of the colony as a result of brood neglect (Sladen, 1912). Sladen protected colonies against this fate by providing a solution consisting of two parts honey to one part water which he injected directly into the cells using a syringe (Naphthol-beta was added to the solution to prevent fermentation). Such methodology could be valuable, particularly if artificial domiciles are to be used for conservation purposes, although sugar solution would provide a preferable alternative to honey solution since this would eliminate any risk of disease transmission and/or spread of harmful chemicals that might be associated with the introduction of honey into the nest.

2.6.3 Conditions within the nest chamber

Moisture levels are difficult to control within artificial domiciles and excessive moisture is a problem associated with almost all domicile styles, particularly those designed for subterranean use (Sladen, 1912; Frison, 1926; Fye and Medler, 1954; Intenthron and Gerrard, 1999). Queens do not appear to be repelled by moist nest sites and have often been discovered attempting to found nests in domiciles containing damp nest material but in these cases, the brood often succumbed to fungal attack causing the queen to desert (Sladen, 1912; Pomeroy, 1981). Moisture also attracts invertebrates such as centipedes and slugs, both of which may cause harm to the colony either directly, by eating the contents of the nest (Sladen, 1912), or indirectly, for example by a slug blocking the entrance hole and preventing bees moving in and out of the colony (Intenthron and Gerrard, 1999).

Domiciles in which nest material comes into direct contact with the earth should be positioned in well-drained ground and should be protected from rain water from above. A plate of tin or plastic can also be placed at the base of the nest cavity to form a barrier between the nest material and the earth (Sladen, 1912). Closed domiciles made of non-porous material such as the Frison tin domiciles are also prone to excess moisture as a result of condensation forming on the inside and collecting in the bottom of the cavity. This problem can be solved by opening the bottom up and placing a mesh between the soil and the domicile base so that the nest was still protected within the domicile but excess water could drain away (Frison, 1926). Such a strategy also allows drainage of faeces which otherwise collects in the bottom of the nest and may reduce colony success (Donovan and Weir, 1978).

Although bumblebees will nest in domiciles made from most materials, porous materials such as wood or concrete may provide more favourable nesting conditions than materials such as tin, plastic and polystyrene which do not allow moisture to escape. If ventilation holes are incorporated into artificial domicile design, this will allow airflow through the domicile and should also help to keep moisture levels down (Donovan and Weir, 1978; Intenthron and Gerrard, 1999). However, it has been suggested that domiciles that keep the nest too dry may also be unsuitable for successful colonisation by bumblebees (Fussell and Corbet, 1992). This may well be the case as successful laboratory rearing of bumblebee colonies requires the brood to be maintained at humidity levels of around 50% in the early stages (Manino et al., 1994; Kwon et al., 2006), suggesting that successful brood development or at least the optimal conditions under which a queen will commence colony initiation does rely on relatively high humidity.

Temperature within artificial domiciles is also an important factor for consideration. Hobbs et al. (1962) found that in warm regions, temperatures within their domiciles became extremely high and all workers and even the queen commenced fanning behaviour so that no foraging took place. This problem was solved by shading the domiciles. Donovan and Weir (1978) also found that their black polystyrene domiciles reached very high temperatures, and this was solved by painting the exterior of the domiciles white and in later designs, by the addition of ventilation holes at the top of the boxes. Ideally domiciles should be sited out of direct sunlight to prevent the build up of heat within. Dark colours should also be avoided and again, ventilation holes will allow airflow and help to keep the interior cool.

2.6.4 Natural enemies - Ants

Ants are commonly found either inhabiting artificial domiciles or raiding bumblebee colonies founded within them (Sladen, 1912; Fye and Medler, 1954;

Intenthron and Gerrard, 1999). Species of the genera *Myrmica* and *Lasius* (*L. niger* and *M. rubra* in Europe and *L. alienus* and *M. lobicornis* in North America) seem to be a particular problem and are reported to steal eggs and provisions from newly founded bumblebee colonies in the absence of the queen, causing her to desert on her return. However, once the first batch of workers has emerged, it seems that ants and bumblebees are able to live side by side with little interference (Sladen, 1912; Hobbs et al., 1962) and other species of ant, such as *Formica fusca,* have also been observed to live alongside bumblebees in domiciles in complete harmony (Hobbs et al., 1962).

In some studies, early stage colonies were protected from invasion by ants using noxious chemicals or insecticides placed in rings around potential areas of invasion. These are placed at such a distance as to avoid contact with the queen when she alights to enter the domicile (Sladen, 1912; Hobbs et al, 1962). Aerial domiciles can also be protected by the use of sticky substances such as ‘tanglefoot’, applied to the object supporting the domicile (L. Pelletier and R. Cartar, pers. comm.).

2.6.5 Natural enemies – large mammals

One of the most voracious predators of bumblebee nests in North America is the skunk (*Mephitis mephitis*). These animals are able to attack mature colonies, and are reported to destroy workers one by one as they emerge to defend their nests (Plath, 1934). Even if skunks are unable to access artificial domiciles they can still cause considerable disturbance in attempting to. They will topple domiciles, often causing spillage of honey from the honey pots, and this can then attract ants which result in the demise of the colony (Hobbs et al., 1960). Rocks can be placed on domiciles in an attempt to protect against attack by skunks (Fye and Medler, 1954). However they will sometimes dig underneath the boxes, so that the most successful method of protection is to wire the domiciles to a secure feature such as a tree trunk or post and ensure secure fastening of the lid (Hobbs et al., 1962).

In Europe, the European badger (*Meles meles*) is well-known to depredate the nests of bumblebees (Cumber, 1953) and might be expected to behave similarly. Badger attacks on colonies in artificial domiciles in Europe are not reported in the literature although Goulson et al. (2002) reported the destruction of two commercially available bumblebee colonies by badgers and the overturning of another, suggesting that if artificial domiciles are to be used in Europe, similar considerations should be made.

2.6.6 Natural enemies – small mammals

Since the time of Charles Darwin, it has been known that small mammals can act as predators of bumblebee nests. Darwin believed that field mice were important predators of bumblebee nests and that bumblebee populations in England were limited by mouse predation. He cites a Mr Newman as suspecting that in excess of two thirds of all bumblebee nests are destroyed by mice in England (Darwin, 1906). However, since that time the effect of small mammal predation on bumblebee populations has never been satisfactorily established.

Artificial domicile studies have provided some evidence for bumblebee nest predation by small mammals. Sladen (1912) reports several colonies in the advanced stage of first brood development having been destroyed by an unknown invader. He set up traps in the location of the destroyed nests and caught shrews, which, being insectivorous, are likely candidates for bumblebee nest predation. He also listed the field mouse and house mouse as likely candidates and often found mice nesting in the cavities subsequent to such an event. Interestingly, no such destruction occurred once the first workers emerged and Sladen believed that if small mammals do depredate bumblebee nests, they will only do so when no adult bees are present within the nest.

Frison (1926) reports small mammal predation of 6% of colonies established in his artificial domicile study and Donovan and Weir (1978) report predation rates of 13%, although in 2% of cases, colonies had progressed to queen production prior to mouse invasion. Richards (1987) and Barron et al. (2000) also list rodents as a reason for colony demise.

Fye and Medler (1954) found that mice are increasingly likely to invade domiciles toward the end of the season and suggest that mice may be important predators at the end, as well as at the beginning of the colony cycle, as the colony will be significantly weakened at this time. They hypothesise that mice may be a significant cause of mortality of new queens.

It is relatively simple to exclude potential small mammal predators from artificial domiciles using mouse excluders. Mouse excluders tend to consist of a sheet or block of a material with a hole cut into it (~1.6cm in diameter) designed to be fitted onto the entrance of an artificial domicile such that a bee can readily pass but mice cannot. Some authors have used two-dimensional excluders of sheet metal with small holes cut into them (Fye and Medler, 1954; Hobbs et al., 1960; MacFarlane et al., 1983) but these have been found to be ineffective in some instances (Fye and Medler, 1954; Hobbs et al., 1960). Creating a mouse excluder from a material with some depth (such as a block of wood) appears to be a more effective method of excluding small mammals (Sladen, 1912; Hobbs et al., 1960).

Sladen (1912) describes another form of mouse excluder which may be even more successful. This consists of a cylinder of tin, which is pressed into the ground to surround the entrance hole to the domicile once a queen has been seen to inhabit it. This provides a tin barricade around the entrance, within which the queen soon learns to alight, but which effectively excludes mice and most other non-flying natural enemies (Sladen, 1912).

2.6.7 Natural enemies – invertebrates

Several invertebrate species can cause harm to bumblebee colonies, perhaps the most important of these, in Europe at least, being bumblebee wax moths (*Aphomia sociella* in Europe and *Vitula edmandsae* in North America). In Europe, bumblebee wax moths are known to be extremely prevalent (Goulson et al., 2002a) and are believed to cause the premature demise of many colonies (Alford, 1975; Intenthron and Gerrard, 1999). Sladen (1912) protected his domiciles from *A. sociella* (and from the parasitoid fly, *Brachycoma devia*) by placing balls of naphthalene around points of access to the domicile.

In contrast, the North American bumblebee wax moth (*V. edmandsae*) is reported in several artificial domicile studies (Hobbs, et al, 1960, 1962), but does not appear to have any deleterious effects on colonies, failing to thrive until reproductive production had ceased. Hobbs et al. (1960) found no difference in the number of cocoons produced by infested colonies versus non-infested colonies after colony termination.

Other invertebrates reported to have caused bumblebee mortality in artificial domicile studies include the conopid flies, *Physocephala texana* and *P. sagittaria* (Hobbs et al., 1960, 1962), larvae of the checkered beetle, *Trichodes ornatus,* (Hobbs et al., 1962) and potentially centipedes, earwigs and mites also (Sladen, 1912; Donovan and Weir, 1978).

2.6.8 Usurpation

Bumblebees of the sub-genus *Psithyrus* are kleptoparasitic species that emerge slightly later than true bumblebees (sub-genus *Bombus*) and invade their nests, killing the queen and enslaving the worker force to rear their own offspring. The host workers care entirely for the offspring of the kleptoparasitic bees, so there is no worker caste in any species of the sub-genus *Psithyrus* and the only offspring that are produced are males and new females.

Psithyrus are present throughout the native range of true bumblebees but they appear surprisingly uncommonly in studies using artificial domiciles. Only one study (Hobbs et al., 1962) has found that *Psithyrus* species were a common reason for colony failure: 106 *Psithyrus* of the species *B.(Ps).insularis*, *B.(Ps.).suckleyi*, and *B.(Ps.) fernaldae* invaded colonies established in domiciles over two years of study, with as many as eight individuals found to invade the same colony. The investigators often managed to locate and remove these individuals before the death of the foundress queen but despite their intervention, the colonies generally did not develop any further.

Psithyrus invasions can be prevented by using a queen excluder, placed over the nest entrance once the queen has ceased foraging. These are similar to mouse excluders but the aperture is smaller (usually approximately 0.8cm in diameter). These have been used in several artificial domicile studies (MacFarlane et al., 1983; Hobbs et al, 1962). However, again, care must be taken in designing such an excluder. Hobbs et al. (1962) report an attempt by a *Psithyrus* queen to invade a colony protected by such an excluder which resulted in the invader becoming lodged in the hole, preventing traffic in and out, and consequently causing the death of the colony. In some species, such as *B. nevadensis*, workers frequently reach a similar size to queens rendering queen excluders unsuitable (Hobbs, 1967a)

The addition of a moat of foul-smelling chemicals such as oil of cloves or butyric acid around artificial domiciles has been trialled as a method of masking the odour of bumblebee colonies to protect against *Psithyrus* species but this was not effective (Hobbs, 1967a).

Usurpation or attempted usurpation within true *Bombus* is also common and is reported many times in artificial domicile studies (Barron et al., 2000; Palmer, 1968; Donovan and Weir, 1978; Richards, 1978). This behaviour could be interpreted as later emerging individuals attempting to catch up on lost time, but Richards (1978) found that wing wear was approximately equal between host queens and usurpers suggesting that the queens had been on the wing for approximately the same length of time.

Most common species have been observed to demonstrate usurpation behaviour, although rates differ between species. For example, *B. bifarius*, *B. occidentalis* and *B. rufocinctus* show particularly high usurpation rates (Richards, 1978). Usurpation rates also vary between species from year to year (Richards, 1978), providing support for the hypothesis that nest sites may limit bumblebee populations. Varying rates of usurpation may reflect variation in nest site availability for a given species from year to year. Most usurpation attempts are between conspecifics and the success of each queen in these interactions appears to be determined by her size (Richards, 1978). However, interspecific, and even intersubgeneric interactions have been recorded, although these have never been observed to result in a successful usurpation (Richards, 1978).

Again, colonies can be protected from invasion by conspecific usurpers using a queen excluder, although it is interesting to note that Richards (1978) found that colonies that had been usurped tended to achieve higher rates of reproduction than those that were not. This may suggest that usurpation in some way provides the colony with extra vigour, although it may simply be a reflection that usurpation is a risk that is only worth taking for the highest quality nest sites.

2.6.9 Further considerations

If domiciles are to be placed where they may come into contact with the public, vandalism is also an important concern. Considerable losses can result from damage in this way (Frison, 1926; pers. obs.). If possible, domiciles should be well camouflaged and kept away from main thoroughfares.

Damage by animals is also an important cause of domicile loss. If domiciles are placed out in grazing land, precautions must be taken to protect the domiciles against trampling or other damage by curious animals. Porcupines are also reported as a major cause of damage to wooden boxes as they will chew on the wood (Elliot, 2008; L. Pelletier and R. Cartar, pers. comm.).

Inability to locate domiciles as a result of vegetation growing up around them is also a problem in artificial domicile studies (Carvell, 2000). Careful notes should be made as to the positions of all artificial domiciles and suitable markers may also be used to mark the position (Sladen, 1912).

2.7 Monitoring artificial domiciles and relocation of established colonies

It is well known that queens often desert their colonies before the first brood of workers emerge and for this reason, disturbance to domiciles containing a newly established queen may be detrimental to colony development. However, many artificial domicile studies involve regular monitoring of activity throughout the nest founding period, and this inevitably involves some degree of disturbance to newly established queens. For example, MacFarlane et al. (1983) recommend checking domiciles four to six times at 15 day intervals during the period of nest founding, Sladen (1912) checked his domiciles every ten days or so and Richards (1978) checked his domiciles 2-3 times a week. None of these authors record losses of queens as a result of disturbance early in nest founding.

Hobbs et al. (1962) do report abandonment of nests following investigator disturbance, but this was either after direct interference with the queen in order to mark her or when they rearranged nest material that the queen was in the process of arranging. Sladen (1912) carried out considerable manipulations to newly founded nests in his artificial domiciles, even changing the nesting material completely. He achieved this by ensuring that the foundress queen was away from the nest and by frightening her off should she try to re-enter whilst he was in the process of manipulating the nest.

Vibration is also believed to greatly reduce the likelihood of a queen to settle in laboratory rearing, but this does not seem to be the case for nests in the field. Some authors recommend rapping on the lid of domiciles in order to ascertain whether or not queens are present (Sladen, 1912; Intenthron and Gerrard, 1999). This usually elicits a protective response and the queen buzzes, confirming inhabitation, although Sladen (1912) notes that queens can become accustomed to rapping such that it elicits little or no response. Overall, it seems that regular monitoring and even manipulation of newly founded colonies does not affect the propensity of the queen to desert if carried out carefully.

For colony observations, viewing panes can be incorporated into domicile designs. In some of his later designs, Frison (1926) placed a red coloured viewing pane beneath the lid of his domiciles and since bumblebees cannot detect red light, this allowed him to observe his colonies with minimal disturbance to the bees themselves. Similarly, if there is no viewing pane, checking boxes at dusk ensures that there is not enough light for the bees in the colony to fly when the domicile is opened. Sladen (1912) reports that opening the domicile causes some disturbance at first but that the bees will soon settle down.

Some colonies grow too large for their domiciles and require moving to a larger domicile. Donovan and Weir (1978) recommend that domiciles should be at least 25cm x 24cm x 12cm in size since this was the maximum size of field colony found by them, but if a colony outgrows its domicile, it is relatively simple to move them to a larger one (Hobbs, 1967a; Intenthron and Gerrard, 1999).

In order to relocate colonies, an insert such as a sheet of plastic or tin can be placed within the nest box prior to occupation (as in Sladen, 1912) such that the brood can be moved from the field domicile to another location, allowing recolonisation of the nest box (as in Pomeroy, 1981).

If colonies are relocated subsequent to emergence of the first brood, care should be taken that all workers are collected. Foragers have often been noted to stay outside the nest over night and it is believed that this may occur mainly as a result of rapidly dropping light levels to the point where they can no longer fly (Hobbs et al., 1962). The proportion of bees that stay out overnight has been reported to be as high as 22.5% (Free, 1955). If the colony is to be moved to another field site early in colony development it is particularly important that as many workers as possible are collected otherwise the queen may recommence foraging and become susceptible to attack by parasitoids and other natural enemies. This can be prevented by the placement of false domiciles on the site of the original domicile on the morning after removal. These false domiciles have trapdoors such that workers can enter but not leave. These workers can then be relocated to the new site (Hobbs et al., 1962). Queen excluders may also be used to prevent the queen from leaving the nest again after movement. Waiting until the second batch of workers has emerged is also an effective method of ensuring that the queen will remain within the nest.

2.8 Occupancy versus colony success and success relative to wild nests

Colony success rates within domiciles are much lower than colony foundation rates. Richards (1987) found that of colonies founded by common species in artificial domiciles, 45% were abandoned by the queen before the emergence of the first brood as a result of poor weather conditions, parasites and predator pressures, or lack of food. Donovan and Weir (1978) found a comparable pattern for colonies established in artificial domiciles in New Zealand. In their study, 52.4% of colonies founded were terminated because of the death of the founding queen or as a result of her failure to return to the nest. Only 38.1% of the colonies produced reproductive individuals and of these, only 15.5% produced a substantial number of new queens.

It is worth noting that where high occupancy rates are recorded, occupancy is defined as anything from a queen manipulating the nest material within a domicile. Since queen abandonment is so common in the early stages of nest foundation it is possible that where occupancy is reported to be low (such as in Fussell and Corbet, 1992 and Gaston et al., 2005b) less regular monitoring meant that such early stage occupancies were not observed and therefore not recorded. In future studies, the stage at which a colonisation is described as occupancy should be clearly defined and, if possible, data should be provided regarding queen presence within domiciles, the initiation of colony foundation (the building of a honeypot and accumulation of the pollen lump into which the first brood will be laid), survival of first brood and progression to reproductive production.

There is no evidence to suggest that the fates of colonies established within artificial domiciles are very different to those of 'wild' colonies. Donovan and Weir (1978) found that colonies of *B. hortorum* established in artificial nest boxes produced on average fewer queens than wild colonies but the greatest number of queens produced by any colony studied was from a colony established in an artificial domicile, and it is likely that the sample of wild nests was biased since only larger colonies are likely to have been discovered and used in the study. Similarly they report that a colony of *B. terrestris* established within a domicile produced an equivalent number of new queens to that of naturally occurring colonies. Cumber (1953) found that of 80 wild colonies of *B. pascuorum* monitored, 23 produced queens. This proportion is actually much lower than those observed in most domicile studies (data presented in table 2.1), suggesting that artificial domiciles can provide suitable sites for bumblebee nest establishment.

2.9 Conclusions

Several aspects of domicile design appear to be surprisingly unimportant in terms of attracting bumblebee queens to found nests. For example, whilst wooden box domiciles have often yielded acceptable occupancy rates (>30%) in the US, Canada, Europe (though notably not the UK) and New Zealand, some of the highest occupancy rates reported are of domiciles built from metal and plastic, and favourable results have also been achieved with polystyrene and concrete domiciles. This suggests that domicile material plays little part in acceptability to bumblebee queens. The most important factors appear to be optimisation of conditions within the nest chamber and appropriate positioning of the domiciles. The habitat type into which domiciles are placed and their positioning relative to the ground are important in determining the species that are likely to be attracted, and there is also evidence to suggest that placing domiciles where nests have been abundant in previous years may increase the chances of high uptake rates. Cues used by queens to locate nest sites are as yet unclear but it is likely that natural looking domiciles are more attractive to nest site searching queens. The length of time that a domicile remains in the natural environment is likely to influence attractiveness as unnatural odours are lost and vegetation grows up to camouflage the domicile giving rise to more favourable conditions. Conditions within the nest chamber may strongly influence uptake rates and will certainly affect the subsequent survival of colonies founded within domiciles. In particular, a domicile for bumblebees should be weather-proof and have adequate ventilation and drainage for excess water and faeces. It is also necessary to provide a source of fine, insulating nest material that can easily be manipulated by a bumblebee queen.

Domicile success cannot only be determined by domicile design and placement but is also likely to be strongly influenced by external factors such as the abundance of bumblebees at the trial site and the availability of natural nest sites in the area.

Artificial domicile designs that can attract bumblebee queens and which subsequently promote colony survival and reproduction could provide several benefits for study, conservation, recreation and agriculture. However, occupancy levels are rarely high and many occupied domiciles do not give rise to successful colonies. The establishment of colonies that progress to worker production is unlikely to exceed 20% and external influences will have a large influence on success rates, so that large numbers of artificial domiciles are likely to be required if this technique is to be used. Studies comparing queen investigation rates with uptake rates would provide information as to which of these factors limit occupancy rates (initial attraction of queens or the perceived suitability of the chamber for nest foundation), providing valuable information for developing more effective domicile designs and/or optimising domicile positioning. However, it may be that the recent trend towards lower occupancy in the use of artificial domiciles for bumblebees is a reflection of a general decline of bumblebees across their native range. These findings are concerning since they indicate that in addition to well-documented declines of rare bumblebee species, common species may also now be lower in density than they once were.

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Appendix 2.1: Domicile designs trialled for use with bumblebees in different artificial domicile studies

Author	Design type	Description
Sladen, 1912	Sladen basic	Nest chamber: 10cm diameter cavity dug into the soil and roofed with a tile, on top of which was placed the original sod of earth removed from the spot. Entrance: two-foot long tunnel made with a metal pole driven into the earth.
Sladen, 1912	Sladen cover	As above but roofed with a 'Sladen cover' - circular piece of wood with a band of metal protruding downwards such that it could cut into turf and form a seal between the ground and the lid. Cover with handle for easy removal for colony observation. Tin plate sometimes placed at the base preventing direct contact with the earth.
Sladen, 1912	Sladen tin	Nest chamber: cylinder of tin, 13cm in diameter and 13-15cm high, placed into the ground with the base open and a lid placed over the top (with felt underneath to form a seal). Entrance: tunnel produced as above.
Sladen, 1912	Sladen terracotta	Nest chamber: an upturned terracotta flower pot inserted into the ground as in the tin domicile. Entrance: tunnel produced as above.
Frison, 1926	Frison large tin	Nest chamber: metal can (13cm x 15cm) buried so that the top of the can is 7.6cm below the surface of the ground. Entrance: tunnel created from spouts of diameter 3.5cm at the base and 2.5cm at the mouth and 33cm in length, with paint and sawdust poured down to provide grip and at a 35º angle.
Frison, 1926	Frison small tin	Nest chamber: as above but of a smaller size and with the addition of a glass viewing lid beneath the lid. Red glass sometimes used to reduce colony disturbance. Entrance: as Frison large tin domicile.
Frison, 1926	Frison small tin ('improved')	Nest chamber: as above but with the base of the can removed and replaced with fine copper mesh. Entrance: as Frison large tin domicile.
Frison, 1926	Wooden box underground	Nest chamber: square wooden box with hinged lid and removable glass lid beneath it. Base open and covered with fine copper mesh. This was buried into the ground. Entrance: tunnel consisting either of a tin spout as above, or rubber hosing.
Wilcke, 1953	Wooden aerial box	Details of design unknown
Wilcke, 1953	Wooden box surface or semi-underground	Details of design unknown
Wilcke, 1953	Wooden box underground	Details of design unknown

Author	Design type	Description
Fye and Medler, 1954	Fye and Medler flower pot	Nest chamber: flower pot with wooden lid covered in roofing paper, placed on the surface of the ground. Entrance: hole bored into side of flower pot.
Fye and Medler, 1954	Fye and Medler metal can	Nest chamber: metal cans buried ~30cm below ground with wooden lids beneath the original sod (as Sladen tin). Entrance: tunnel consisting of rubber hosepipe.
Fye and Medler, 1954	Fye and Medler tiles	Nest chamber: clay tiles (20cm x 30.5cm) buried upright into the soil to produce a cavity between them which is half-filled with sand. Entrance: tunnel consisting of rubber hose.
Fye and Medler, 1954	Fye and Medler cones	Nest chamber: cones made of 30.5cm diameter circles of roofing paper placed on the surface of the ground. Entrance: access under the edges of the cones.
Fye and Medler, 1954	Wooden box surface	Nest chamber: wooden box (volume ~6-7 litres) of stock lumber with lids covered with roofing paper placed on the surface of the ground. Entrance: 3.2cm dimater hole bored into the centre of one side of the box. (Metal plate with 1.6cm aperture was placed over the entrance in the spring to act as a mouse excluder.)
Fye and Medler, 1954	Wooden box aerial	Nest chamber: wooden box (3.5 litre volume) placed above the ground on buildings. Entrance: as Fye and Medler surface box.
Hobbs et al, 1960	Wooden box surface	Nest chamber: as Fye and Medler surface. Entrance: initially as Fye and Medler surface but later mouse excluder created from 1.9cm thick plywood instead of metal sheet.
Hobbs et al, 1962	Wooden box surface	As Hobbs et al., 1960
Hobbs et al, 1962	Wooden box underground	Nest chamber: as Fye and Medler surface but buried so that the lid is approximately 10cm underground. Entrance: black plastic hosing (~46cm long, 2.5cm diameter) with a v-shaped valley excavated around the tube entrance. Entrance end cut diagonally to prevent access by rain water.
Hobbs et al, 1962	Wooden box semi-underground	As Hobbs et al., 1960 but buried into banks so that only the lid and front of the boxes are exposed.
Hobbs, 1967	Wooden box surface	As Hobbs et al., 1960 but with 5 cm tall wooden runners nailed to the base of the domicile to raise it off the ground. (Box dimensions given as ~15cm x 15cm x 15cm.)
Hobbs, 1967	Wooden box underground	As Hobbs et al., 1962 but with 5 cm tall wooden runners nailed to the base of the domicile to raise it off the ground. (Box dimensions given as ~15cm x 15cm x 15cm.)

Author	Design type	Description
Hobbs, 1967	False underground hive	Nest chamber: as Hobbs et al., 1960. (Box dimensions given as ~15cm x 15cm x 15cm.) Entrance: ~30 cm tunnel made from black plastic hosepipe and attached to an aperture in the centre of the base of one side of the domicile. Sod was placed over the entrance to mimic a subterranean nest entrance.
Hobbs, 1967	Wooden box aerial	As Hobbs et al., 1960 but secured onto a steel post at a height of ~1.8m. (Box dimensions given as ~15cm x 15cm x 15 cm)
Palmer, 1968	Wooden box surface	Nest chamber: wooden box of approximate dimensions 20cm x 15cm x 10cm with removable lid placed on the surface of the ground. Entrance: aperture of approximately 2cm.
Richards, 1978; Richards, 1987	Wooden box surface	Nest chamber: 15cm cube plywood box placed on the surface of the ground. Entrance: 1.6cm diameter entrance hole in the centre of one side. (As Hobbs, 1967)
Richards, 1978; Richards, 1987	Wooden box underground	Nest chamber: 15cm cube plywood box buried with the lid approximately 10cm below the surface of the ground. Entrance: 30.5cm long black plastic hosepipe (2.5cm diameter), with v-shaped valleys constructed to funnel towards the entrance. (As Hobbs, 1967)
Richards, 1978	False underground hive	Nest chamber: 15cm cube plywood box placed on the surface of the ground. Entrance: as Richards, 1978 underground domicile. Sod is placed over the entrance to mimic a subterranean nest entrance. (As Hobbs, 1967)
Richards, 1978	Wooden box aerial	Nest chamber: 15 cm cube box either wired to a tree at chest height or mounted on steel posts. Entrance: as in Richards, 1978 surface domicile. (As Hobbs, 1967)
Donovan and Weir, 1978	Wooden surface	Nest chamber: as Palmer, 1968. Entrance: as Palmer 1968 but enlarged to 25mm diameter.
Donovan and Weir, 1978	Wooden false underground	Nest chamber: As Palmer, 1968. Entrance: two lengths of wood nailed to form a v-shape and used to create a tunnel (30.5cm long, 3cm high and 7cm across at base) leading to the domicile entrance (25mm hole).
Donovan and Weir, 1978	Polystyrene surface domicile	Nest chamber: polystyrene box (30cm x 21cm x 28cm) painted black on all surfaces and placed on the surface of the ground. (The outside of these were latterly painted white.) Entrance: 25mm hole drilled into one side, just above the floor of the domicile.
Donovan and Weir, 1978	Polystyrene false underground domicile	Nest chamber: polystyrene boxes painted black and placed on the surface of the ground. (The outside of these were latterly painted white.) Entrance: two lengths of wood nailed to form a v-shape and used to create a tunnel (30.5cm long, 3cm high and 7cm across at base) leading to the domicile entrance (25mm hole).
Donovan and Weir, 1978	Donovan and Weir polystyrene 'hives'	Nest chamber: polystyrene box (30cm x 30cm x 21cm) painted black on the inside with drainage holes (7mm diameter) punched into the base and ventilation holes (7mm diamater) punched into the top, placed on the surface of the ground. Entrance: 25mm hole drilled towards the base of one side of the box.

Author	Design type	Description
Pomeroy, 1981	Pomeroy plastic underground	Nest chamber: upturned plastic bowl (32cm diameter, 20cm high) buried under the ground. Entrance: black PVC hosepipe. A wooden slab cut to look like a mouse hole in a skirting board was placed over the hosepipe entrance.
Pomeroy, 1981	Pomeroy brick surface	Nest chamber: eight bricks cemented together in a square to form a cavity of 17cm x 17cm and 20cm in height, with a wad of newspaper or strip of felt and a polystyrene cover placed on top and held in place with additional bricks. Entrance: one of the lower bricks was displaced to create a gap to provide access.
Pomeroy, 1981	Pomeroy pumice-concrete surface	Nest chamber: cylindrical concrete structure with conical concrete lid painted with silver paint and placed on the surface of the ground. (For details of concrete components see Pomeroy, 1981).
Pomeroy, 1981	Pomeroy semi-underground	Nest chamber: as in the Pomeroy pumice-concrete surface domicile but partially buried to form a chamber similar to that of Pomeroy plastic underground. Entrance: as in the Pomeroy plastic underground domicile.
MacFarlane et al, 1983	Wooden surface	Nest chamber: wooden box (30cm x 29cm x 29cm) raised by runners and placed on the surface of the ground. Entrance: 25mm diameter hole with 11mm diameter mouse excluder and 7-8mm queen excluder placed just above the base of the domicile with wooden landing platform below the entrance.
MacFarlane et al, 1983	Wooden underground	Nest chamber: wooden box (as MacFarlane wooden surface domicile) semi-submerged in the ground. Entrance: 60cm long triangular tunnel, 4cm high and 5cm at the base, made from wood. Excluders as in MacFarlane wooden surface domicile.
Fussell and Corbet, 1992	Small wooden box surface	Nest chamber: as Richards, 1978 surface domicile with a hinged lid and covered by an inverted plastic tray. Entrance: as Richards, 1978 surface domicile.
Fussell and Corbet, 1992	Small wooden box aerial	As Fussell and Corbet, 1992 small surface domicile but mounted on a metal pole to give an aerial position.
Fussell and Corbet, 1992	Large wooden box surface	Nest chamber: as MacFarlane et al., 1983 surface domicile with a hinged lid and covered by an inverted plastic tray. Entrance: as MacFarlane et al., 1983 surface domicile.
Fussell and Corbet, 1992	Large wooden box aerial	As Fussel and Corbet, 1992 large surface (above) but mounted on a metal pole to give an aerial position.
Fussell and Corbet, 1992	Fussel and Corbet brick domiciles	Nest chamber: four bricks arranged in a square to form a cavity and covered with a concrete roofing tile. Entrance: a gap between the bricks. Other designs based on this principle were also trialled (see Fussell and Corbet 1992 for details).
Intenthron and Gerrard, 1999	Domicile design 1	Nest chamber: a bottomless upturned flower pot in a concrete base with a second, complete flower pot placed over the top as a weather-proof lid. A wire mesh cradle is included for the nesting material and drainage holes and a recess for a feeder are incorporated into the base. Entrance: various entrances including holes and tubes built into the concrete base.

Author	Design type	Description
Intenthron and Gerrard, 1999	Domicile design 2	Nest chamber: two flower pots adjoined mouth-to-mouth and containing a wire cradle for the nest material and pebbles in the bottom for drainage. Entrance: a hole in the base of one flower pot, or a tube if the domicile is to be used underground. A similar opening is positioned on the opposite corner of the domicile to provide ventilation.
Barron et al, 2000	Wooden surface 'hotels'	Nest chamber: plywood box (110cm x 31cm x 30cm) split into four compartments with plywood dividers to provide four possible nest chambers with runners attached to the bottom of the box and a lid covered with aluminium-painted rubber sheeting. Boxes placed on the surface of the ground. Entrance: a single 25mm circular hole on one side of each chamber.
Carvell, 2000	Carvell flower pot surface	Nest chamber: A large upturned flowerpot, lined and covered with wire mesh and placed on the surface of the ground.
Carvell, 2000	Carvell flower pot subterranean	Nest chamber: As Carvell 2000 surface domicile. Entrance: A small entrance tunnel leading to the nesting chamber.
Gaston et al, 2005	Gaston terracotta pot	Nest chamber: upturned terracotta flower pot with the drainage hole sealed, placed on a tile and put on the surface of the ground. Entrance: the lip of the pot overhangs the tile to provide access into the nest chamber.
Gaston et al, 2005	Gaston buried terracotta pots	Nest chamber: upturned terracotta flower pot with the drainage hole sealed, placed on a tile and buried so that the top of the flower pot is level with the ground. Entrance: the drainage hole in the top of the flower pot provided access to the chamber within.
Gaston et al, 2005	Two-chamber wooden surface boxes	Nest chamber: plywood, two-chambered boxes with the second compartment designated as the nesting compartment, incorporating ventilation holes and with a sloping lid to allow drainage of rainwater, and raised off the ground on runners. Entrance: access hole.
Cartar, pers. comm.	Surface, aerial and subterranean wooden domiciles	As Richards, 1978.
Carvell, pers.comm.	Wooden surface	Nest chamber: wooden box with mesh ventilation on all sides, placed on the surface of the ground.
Elliot, 2008	Wooden surface	Nest chamber: 15 or 20 cm cube wooden boxes placed on the surface of the ground.
Chapter 3	Complex underground	Nest chamber: two flower pots (16cm diameter at widest point) joined mouth to mouth with an inner perforated plastic lining to allow air circulation around the nest, buried ~5cm below the surface of the ground. (See chapter 3 for details) Entrance: as Intenthron and Gerrard, 1999 'domicile style 2', but a roof tile is placed overhanging the entrance to create a more natural looking crevice.

Author	Design type	Description
Chapter 3	Roosting pocket	Nest chamber: an ovoid wicker basket ~12cm diameter. Some include a 20cm diameter piece of roofing felt folded to form a cone and placed over the top of the domiciles as a weatherproof roof. Entrance: a hole in the front of diameter ~4cm. (Nest is a commercially available wicker basket designed to attract small garden birds.)
Chapter 3	Subterranean slab domicile	Nest chamber: a cavity of ~25cm x 25cm x 20cm dug into the ground with a concrete slab of dimensions 45cm x 45cm placed over the top. A wire cradle keeps the nest material away from the earth. Entrance: a 30cm length of 2.5cm diameter black hosepipe from the middle of the wire cradle to the soil surface, where a v-shaped valley forms a funnel towards the tunnel entrance.
Chapter 3	Semi-subterranean flowerpot domicile	Nest chamber: a bottomless upturned flower pot (23cm diameter at widest point) half sunk into the ground with a complete flower pot and lid placed over the top. A wire cradle keeps the nest material away from the earth. Entrance: as subterranean slab domicile (chapter 3).
Chapter 3	Wooden semi-underground	Nest chamber: a wooden box (17x26x15cm) half sunk into the ground. A wire mesh cradle holds the nest material just above the base of the box to protect from moisture. Entrance: a 2.5cm diameter hole towards the base of the box to which is attached a 30cm piece of 2.5cm diameter black hosepipe which extends to the dsurfaceof the ground. A v-shaped valley is excavated as above. (This design is a modification of a commercially available bumblebee nesting box.)
Chapter 3	Wooden surface	Nest chamber: a wooden box (17x26x15cm). Entrance: a 2.5cm diameter hole towards the base of the box (Nest is a commercially available bumblebee nesting box.)

Chapter 3

Assessing the efficacy of artificial domiciles for bumblebees in the UK

3.1. Abstract

Bumblebees have suffered declines in the UK as a result of a reduction in habitat availability associated with agricultural intensification. Although several conservation strategies for bumblebees address forage availability, other aspects of bumblebee ecology are often overlooked. The availability of sufficient nest sites is a key requirement of bumblebee populations and since nesting habitat is likely to have become more scarce on intensively farmed land, reduced nest site availability may contribute to bumblebee declines. The use of artificial bumblebee domiciles have been proposed as a potentially useful conservation tool, providing a way of boosting nest sites where they are otherwise limiting. An effective domicile would also have valuable commercial and research applications. Here, six different artificial domiciles for bumblebees are trialled in different habitats in southern England and central Scotland. Of these, only one domicile design at one particular site achieved acceptable uptake rates, with all other combinations of domicile and site trialled achieving low success. This study suggests that the effective use of artificial domiciles for bumblebees may be possible in the UK but that further research into factors determining uptake rates is required. Based on current knowledge, attempts to use domiciles for conservation or research in the UK are likely to be ineffective. Commercially available domiciles for bumblebees performed poorly in these trials and the implications of these findings for manufacturers are discussed.

3.2 Introduction

Bumblebees have suffered severe declines in the UK and it is generally agreed that this is a result of the reduction in habitat availability associated with agricultural intensification (Williams, 1986; Goulson et al., 2008a). Loss of flower-rich hay meadows and clover leys have resulted in dramatic declines of bumblebee forage plants in the agricultural environment (Carvell et al., 2006a) and this is likely to have had a huge impact on British bumblebee populations. However, agricultural intensification has also resulted in the loss of vast areas of other natural and semi-natural habitat types including hedgerows, woodland and unimproved grassland (Fuller, 1987; Robinson and Sutherland, 2002). These areas would have provided ideal nesting habitat for bumblebees, which generally nest in tussocky grasses or in the abandoned subterranean homes of small mammals, and it is possible that availability of suitable nesting habitat is also limiting bumblebee populations in the UK (Kells and Goulson, 2003).

Several aspects of bumblebee ecology suggest that nest sites may limit bumblebee populations. Bumblebees often spend several weeks searching for nest sites and this would not be expected if nest sites were abundant (Richards, 1978). High rates of nest usurpation are also observed among conspecifics (Palmer, 1968; Donovan and Weir, 1978; Richards, 1978; Barron et al., 2000) and evidence suggests that usurpers emerge at roughly the same time as host queens, demonstrating that this is not simply the result of later emerging queens attempting to ‘catch up’ (Richards, 1978).

There is evidence to suggest that nest site availability may limit bumblebee populations in North America (McFrederick and LeBuhn, 2006; Greenleaf and Kremen, 2006), but whether or not this is the case in the UK is as yet unknown. Certainly the bumblebee species that have shown the greatest declines in the UK tend to be those that emerge from hibernation later in the year (Goulson et al., 2005) and it is possible that this pattern is at least partly explained by an increase in competition for nest sites as a result of habitat loss associated with agricultural intensification. Little is known about differences in nest site preferences between British bumblebee species but it has been shown that there are often large overlaps in the nesting habitat utilized by different bumblebee species in Canada (Richards, 1978). From our limited understanding of the nest site preferences of British bumblebees, it seems likely that early emerging species such as the surface-nesting *B. pascuorum* and the subterranean nesting *B. terrestris* may be competitors for nest sites with later emerging species with superficially similar nesting ecology such as the threatened *B. humilis* and *B. soroeensis* respectively (Sladen, 1912). If this is the case, a general decrease in nest site availability is likely to have had negative consequences for these later emerging species.

The development of effective artificial domiciles for bumblebees would have several benefits for bumblebee conservation and research. If nest sites do limit British bumblebee populations, the provision of artificial domiciles to coincide with the emergence of declining species could significantly boost their population sizes where nest sites are scarce. The use of artificial domiciles would also allow monitoring and management of bumblebee colonies founded within them, facilitating detailed observation of the fate of colonies of both common and rare bees thus providing new insights into the colony dynamics and colony-level responses of wild bumblebees. The use of protective devices such as mouse excluders or queen excluders or of chemicals to deter insect pests (Fye and Medler, 1954; Hobbs et al., 1960; 1962) would afford protection against natural enemies, and supplementary food resources could be provided in poor years (Sladen, 1912; MacFarlane et al., 1983). These measures could significantly enhance the success rates of colonies founded within artificial domiciles and might be particularly important where low population sizes result in high susceptibility to stochastic events. Additionally, artificial domiciles could be used as a method of procuring wild bumblebee colonies for experimental studies.

Artificial domiciles have been used with some success in Canada, New Zealand and the US (Frison, 1926; Fye and Medler, 1954; Donovan and Weir, 1978; Richards, 1978, 1987; Pomeroy, 1981). The most commonly used of these consists of a wooden box containing fine, insulating nest material, either placed on the surface of the ground, wired to a tree or mounted on a post above the ground, or buried underground with a piece of hosing used as an entrance tunnel (Hobbs et al., 1962). However, many other designs have been trialled including constructions of plastic, concrete, wood and polystyrene in a range of different shapes and configurations and these often achieve comparable occupancy rates (Sladen, 1912; Frison, 1926; Donovan and Weir, 1978; Pomeroy, 1981). A successful artificial domicile must provide a cavity containing suitable nest material and be free from excess moisture. The habitat into which it is placed, the position relative to the ground and the timing of placement are all important with respect to which species are most likely to inhabit the domiciles. It seems that there is little effect of factors such as domicile constituent material, shape and size, and aspect on the attractiveness of domiciles to bumblebee queens (Richards, 1978).

Similar artificial domicile trials have been repeated in the UK, but success has been very limited (Fussell and Corbet, 1992; Carvell, 2000; Gaston, 2005). This may be because nest sites do not limit British bumblebee populations, because British bumblebee species are less disposed to colonise artificial domiciles, or because British bumblebee populations are much smaller than those elsewhere. However, some authors have achieved favourable results (Sladen, 1912; Intenthron and Gerrard, 1999). Success of domiciles can vary hugely from year to year (Richards, 1987) and site selection for domiciles is likely to be of vital importance in influencing uptake rates. Since most British trials were only run for a single year, it is possible that experimenter inexperience may be partially responsible for the comparatively low occupancy rates achieved in the UK. If domicile design and placement were optimized, it is possible that artificial domiciles could provide a useful tool for the conservation and study of bumblebees in the UK.

In addition to their potential use in conservation, effective artificial domicile designs would be of great commercial value. Bumblebees are charismatic insects and well-loved by the British public. Their role as pollinators of crops, garden plants and wildflowers (Osborne and Williams, 1996) is well known and there is an understanding that the presence and well-being of garden bumblebee populations is beneficial. This public awareness has been exploited by retailers and artificial domiciles for bumblebees are available for the public to buy for use in allotments and gardens as a method of supporting bumblebee populations. There are a range of different artificial domicile designs available for bumblebees currently on sale for the public, the most common being a design similar to that used by Hobbs et al. (1962) described above, and these tend to retail at somewhere between £18-£25 per unit.

Little is known as to the success of these commercial boxes and anecdotal evidence suggests that uptake rates are low (pers. obs.). Since urban gardens support strong bumblebee populations (Goulson et al., 2002a; Osborne et al., 2007) a successful domicile design should produce good results in this environment. However, commercially available domicile designs sold for use in the UK often seem to be unsuitable for the purpose for which they are sold. Artificial domicile studies advocate the use of soft, fine material such as upholsterers' cotton or finely shredded moss as nesting material since these provide good insulation and are easily manipulated by the queen (Fussell and Corbet, 1992; Intenthron and Gerrard, 1999) but the nest material provided with commercial domiciles is usually coarse, roughly cut straw which is unsuitable as nest material. Commercially available domiciles also tend to be designed for placement on the surface of the ground but many of the most abundant bumblebee species in British gardens (e.g. *Bombus terrestris*, *B. lucorum* and *B. lapidarius*) prefer to nest underground (Fussell and Corbet, 1992). Surface nesters (such as *B. pascuorum*) or more generalist nesters (such as *B. pratorum*) may make use of these boxes, but domiciles designed for underground use would target many other species and might produce better results.

The wide range of different domicile designs found to be successful in attracting nesting bumblebees in previous studies suggests that it may be possible to develop a range of cheap and simple ‘home-made’ domicile designs that could be built by members of the public, and could potentially yield better results than those achieved with current commercially available domiciles. If such designs could produce reliably high uptake rates, they might also be used in agriculture alongside, or even as a replacement for commercially reared bumblebee colonies currently used for crop pollination.

The following study assesses the efficacy of different artificial domicile designs trialled in a range of different habitat types in the south of England and central Scotland.

3.3 Methods

3.3.1 Study sites

1) The Sir Harold Hillier Gardens, Romsey. This site is a botanical garden situated in the south of England, covering 180 acres of land and incorporating a wide range of native and non-native plant species with a broad range of flowering periods. Domiciles were placed in an area of mixed woodland containing a high density of rhododendron plants.

2) Grounds of the University of Stirling, Scotland. This site consists of 300 acres of land including grassland, woodland, lakes and gardens. Domiciles were placed in woodland or woodland edge habitats and were distributed across the extent of the campus.

3) Agricultural land in central Scotland. Domiciles were placed out on ten arable or mixed farms across central Scotland. Five of these were participants of the Scottish Rural Stewardship scheme, an agri-environment scheme designed to enhance and protect habitat features by encouraging the implementation of environmentally sensitive land-management practices. Rural Stewardship farms were selected based on their implementation of three management prescriptions that could benefit spring bumblebees and were therefore hoped to attract nest site searching bumblebee queens. These were a hedgerow management prescription, a field margin management prescription and a species-rich grassland prescription, all of which were developed to promote the build up of complex vegetation structure and increase floral abundance. The remaining five farms were chosen as pairs for the five Rural Stewardship participant farms based on location and farm type (see Lye et al., 2009).

4) Garden habitat in central Scotland. Domiciles were placed in 13 suburban gardens belonging to staff of the University of Stirling in central Scotland.

3.3.2 Domicile designs

1) Commercially available bumblebee nest box (supplied by RSPB). The design used consisted of a wooden box of dimensions 17cm x 26cm x 15cm with runners on the lower surface which raise the domicile above the earth providing protection from moisture. A hinged wooden lid covering a Plexiglas window allowed

Figure 3.1: Commercially available domicile

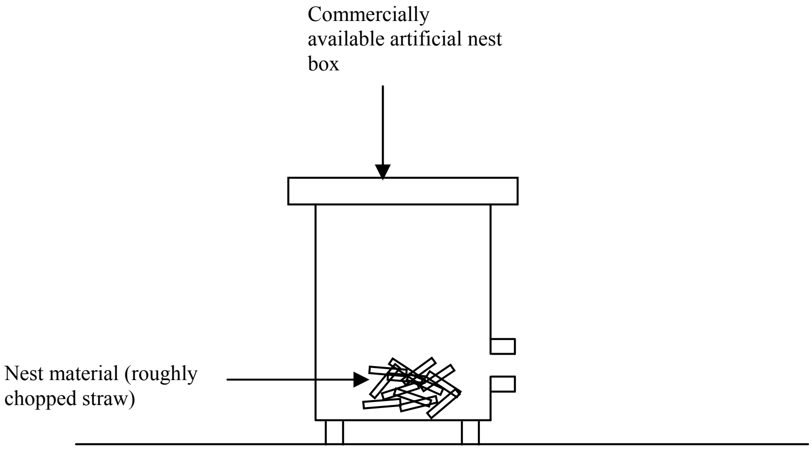


Figure 3.2: Roosting pocket domicile

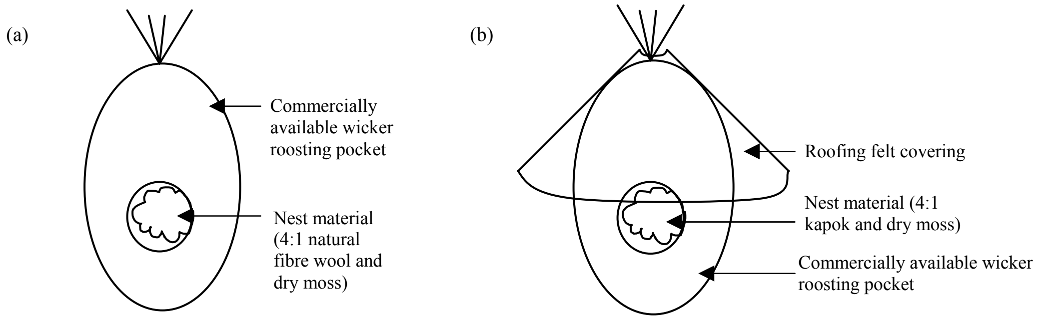


Figure 3.3: Slab domicile

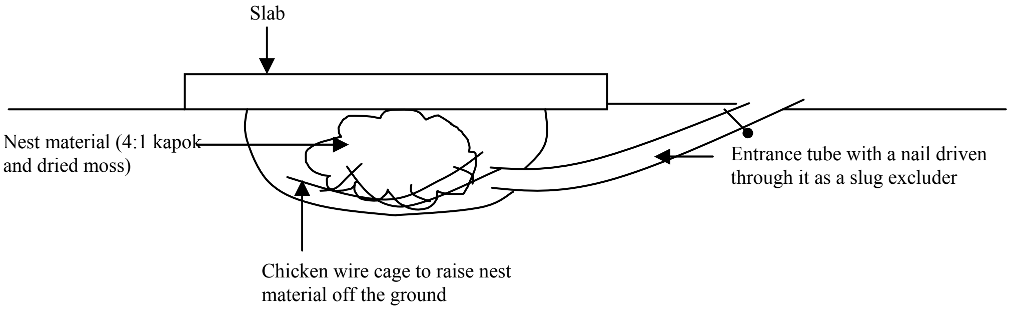


Figure 3.4: Flower pot domicile

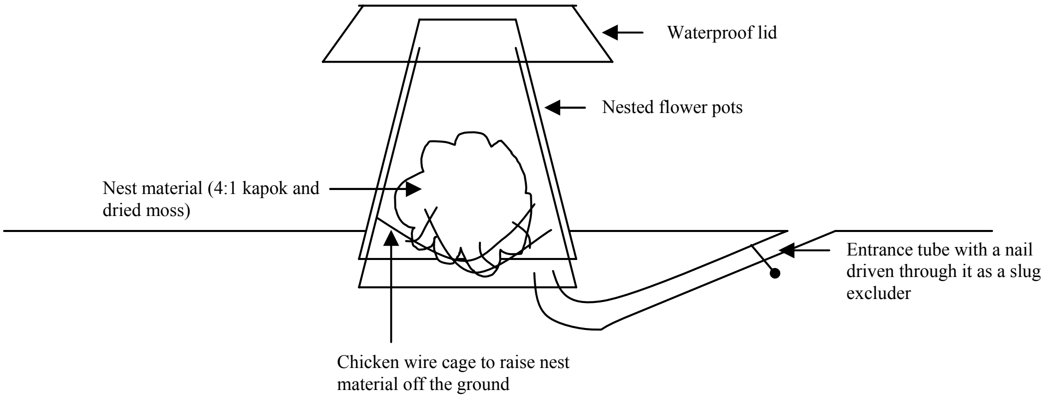


Figure 3.5: Wooden semi-subterranean domicile

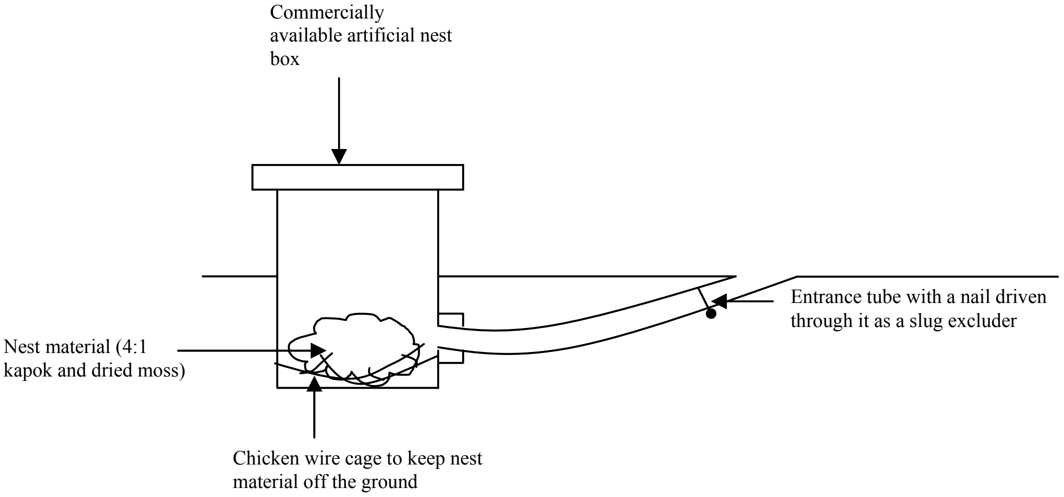
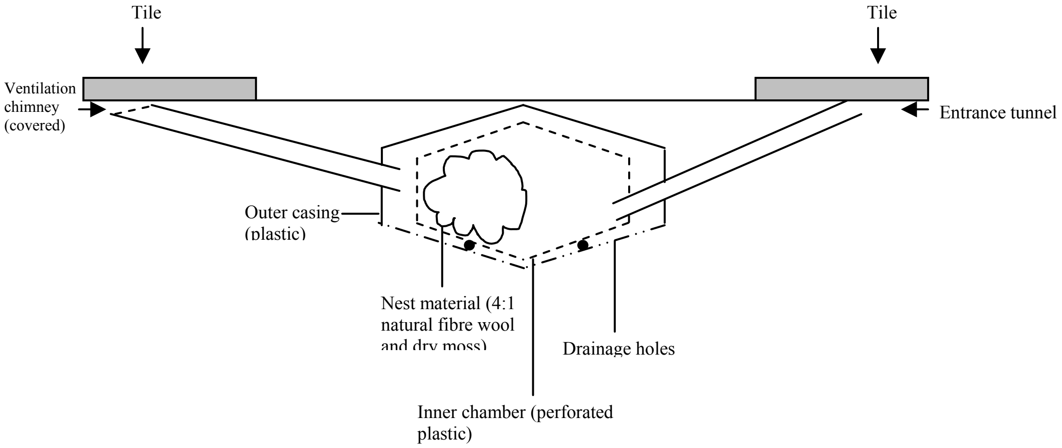


Figure 3.6: Complex subterranean domicile



observations within the domicile. The entrance consisted of a 1.8cm diameter hole on the bottom of one side of the box. The nest material provided inside the domicile consisted of approximately 2g roughly cut straw (figure 3.1).

2) Roosting pocket domicile. This design comprised a commercially available roosting pocket (supplied by RSPB), marketed as a method for the provision of over-wintering refuges for garden birds. These consist of a 12cm diameter ovoid wicker basket with an entrance hole of 4cm in diameter on one side (figure 3.2a). Approximately 2g nesting material consisting of 1 part dried and shredded moss to 4 parts natural-fibre viscose wool or kapok was placed within each domicile. These were then hung on upright features (e.g. trees, fence posts etc.) at a height of between 1.5m and 2m above ground level. In some cases, these were provided with a protective covering made from a circle of roofing felt (20cm in diameter) made into a cone and placed over the top of the domicile (figure 3.2b).

3) Slab domicile. A cavity of approximately 25cm x 25cm x 20cm was excavated in the earth and into this, approximately 2g nesting material consisting of 1 part dried and shredded moss to 4 parts kapok was inserted. The nest material was suspended slightly above the nest chamber floor in a wire mesh cradle, designed to keep the nest material from absorbing moisture from the soil. A 30cm length of 2cm diameter black PVC tubing was then inserted into the ground such that one end protruded into the cradle at the base of the cavity and the other provided an opening at ground level. A nail was hammered across the external entrance of the tubing to prevent the passage of slugs that might otherwise block the entrance (after Intenthron and Gerrard, 1999). The cavity was then covered by a concrete slab of

45cm x 45cm x 4cm and a v-shaped valley was created around the mouth of the tube with the aim of guiding nest site searching bumblebee queens towards the domicile entrance (figure 3.3).

4) Flower pot domicile. A cavity of approximately 25cm x 25cm x 10cm was excavated in the earth and a wire mesh cradle and nest material inserted as above. An entrance tunnel was also constructed as above. The base was then removed from a plastic flower pot (diameter 23cm at the widest end) and this was upturned and placed over the cavity. A second, intact flower pot of the same size was placed on the top of the first as a lid, and a plastic dish (45cm in diameter) was secured on top of this to act as a rain-proof roof (figure 3.4).

5) Wooden semi-subterranean domicile. This design was based around a commercially available bumblebee nesting box (described above). The nest material provided with the domicile was removed and replaced by approximately 2g of moss and kapok held in a wire cradle as above. The domicile was then converted for underground use by the attachment of a 30cm piece of black PVC tubing (2cm diameter) fixed to the entrance hole by means of a connector consisting of a short length of rigid plastic piping of 1.8cm diameter. A slug excluder was incorporated as above. Domiciles were then half buried into the ground such that the entrance tunnels would emerge from the earth creating the impression of a subterranean cavity (figure 3.5).

6) Complex subterranean domicile (after Intenthron and Gerrard, 1999). Two perforated black plastic flower pots (diameter 13cm at the widest point) were

secured end-to-end to form the inner nest chamber. A weather-proof outer casing was created by securing two larger regular flower pots (16cm in diameter at the widest point) end-to-end around the inner chamber. The inner and outer chambers were held apart with four beads secured to the bottom of the inside of the weather-proof casing, allowing ventilation around the inner nest chamber and drainage of excess water and faeces from the nest, and 5mm drainage holes were punched along the bottom of the outside casing of the domicile. Two lengths of 30cm of black PVC tubing (2cm diameter) were attached to the domicile, one at each end, to act as an entrance tunnel and a ventilation chimney. The entrance tunnel penetrated both the inner and outer walls of the domicile and was positioned towards the bottom of the nesting cavity whilst the ventilation chimney only passed through the outer casing and was positioned towards the top of the domicile (figure 3.6). The drainage holes and the mouth of the ventilation chimney were covered with fine nylon mesh to prevent access by ants and other natural enemies of bumblebee nests. Two grams of nest material consisting of 1 part dried and shredded moss to 4 parts natural-fibre viscose wool or kapok was placed within each domicile. These were then dug into the ground so that the upper surface of the domicile was approximately 5cm below the surface of the ground. The ventilation and entrance tunnels were flush with the ground surface and a roof tile was placed over both to provide protection from the weather and to create a more natural looking entrance to the domicile.

3.3.3 Trials

3.3.3.1 Suburban gardens

In order to test the efficacy of commercially available artificial domiciles for bumblebees (domicile style 1), a total of 26 of these domiciles were taken home by staff of the University of Stirling and placed in 13 suburban gardens in Central Scotland in the spring of 2008. These remained in place throughout 2008 and through the summer of 2009. Boxes were placed out exactly as supplied and were located in sheltered locations along linear features in areas thought to be likely bumblebee nesting habitat.

Boxes were checked on a regular basis by participants and details of any occupancy were provided in June of each year.

3.3.3.2 University of Stirling grounds

One hundred blocks consisting of one of each of domicile designs 2b, 3, 4 and 5 were placed out in the grounds of the University of Stirling between 26/03/07 and 01/04/07, approximately the time of commencement of nest site searching behaviour in bumblebees. Each domicile was positioned at least 1m from neighbouring domiciles and domicile blocks were positioned at least 10m away from neighbouring blocks. Blocks were sited along linear features in areas believed to be good bumblebee nest site searching habitat based on the experience of the investigators.

Domiciles were checked at fortnightly intervals through April and May and the entrance holes were cleared of any obstructions. If evidence of occupancy was observed, domiciles were checked weekly until 20/08/07, at which time all of the domiciles were examined thoroughly and any evidence of occupancy by bumblebees or other animals was recorded.

3.3.3.3 Sir Harold Hillier Gardens

20 blocks consisting of one of each of domicile designs 2a and 6 were placed out in the Sir Harold Hillier Gardens between 26/02/07 and 28/02/07, coinciding with the emergence of *B. terrestris* from hibernation. Each block was placed at least 10m away from any other block and all were placed in locations where nest site searching queens had been observed in abundance the previous year.

The domiciles were checked once a month from March until May and the entrance holes were cleared of any obstructions. On 03/07/07 the boxes were collected and frozen at -20°C for at least 24 hours, and the contents examined for any signs of inhabitancy by bumblebees or other animals.

3.3.3.4 Agricultural land in central Scotland

150 complex subterranean domiciles, design 6, were installed on agricultural land in central Scotland between 24/03/2008 and 11/04/2008, approximately the time of commencement of nest site searching behaviour in bumblebees. Fifteen domiciles were placed out per farm, five each in a grassland, hedgerow and field margin habitat context. Each domicile was positioned at least 3m away from the adjacent domiciles and domiciles on each farm pair were installed on the same day or on consecutive days.

Domiciles were checked weekly between 26/05/2008 and 08/06/2008 and were removed at the end of August 2008. The contents of the domiciles were then examined for evidence of activity by bumblebees or other animals.

3.4 Results

Table 3.1: Overall fate of each domicile style combined across trials (percentages in brackets)

Domicile design	Bumblebees	Bird/ small mammal	Other insects	Damaged/ missing	Unoccupied	Total
Commercially available domicile	0 (0%)	unknown	unknown	unknown	unknown	26
Roosting pocket domicile	3* (3%)	32 (27%)	0 (0%)	28 (23%)	57 (48%)	120
Flower pot domicile	0 (0%)	8 (8%)	1 (1%)	9 (9%)	82 (82%)	100
Slab domicile	2* (2%)	22 (22%)	7 (7%)	6 (6%)	63 (63%)	100
Wooden semi-subterranean domicile	1* (1%)	0 (0%)	2 (2%)	3 (3%)	94 (94%)	100
Complex subterranean domicile	13* (8%)	7 (4%)	3 (2%)	6 (4%)	141 (83%)	170

* For details see text

3.4.1 Suburban gardens

None of the 26 commercially available wooden domiciles placed out in urban gardens were occupied by bumblebees in 2008 or 2009.

3.4.2 University of Stirling grounds

Domiciles trialled in the grounds of the University of Stirling received low occupancy rates. No bumblebee colonies were founded within flower pot domiciles.

Eight percent showed evidence of occupancy by small mammals (probably wood mice) and one contained a large colony of vespid wasps. Similarly, no successful colonies were founded within wooden semi-subterranean domiciles. A queen of *B. lucorum* was found within one box, but there was no evidence of an attempt at nest founding. It is likely that, having explored the domicile, the queen was unable to get out again, perhaps as a result of a tube blockage or simply of being unable to relocate the entrance tunnel. These domiciles also appeared to be unattractive to other organisms, with the majority of boxes remaining unoccupied (table 3.1). Two slab domiciles were occupied by bumblebees, one by *B. lucorum* and one by *B. lapidarius*. The former was first observed on April 26th and the latter on May 24th. Both colonies thrived and the colony of *B. lucorum* progressed to queen production. The colony of *B. lapidarius* was observed to be producing males on July 12th but at the next visitation, one week later, the nest was dead and the comb was some way outside the nest entrance. It is believed that this colony may have been attacked by a mammalian predator. No queen cells were evident in the comb that was retrieved, but this may not be representative of the state of the colony at termination. Small mammals and ants were also regular occupants of slab domiciles (22% and 7% respectively), and the slab domicile containing the colony of *B. lapidarius* contained nest material other than that provided by the experimenters suggesting occupancy by small mammals prior to colonisation by bumblebees. One roosting pocket was occupied by a bumblebee colony and this belonged to *B. pratorum*. The queen was seen to enter the roosting pocket on 12th April and queens and males were observed leaving the nest shortly before its expiration in the middle of June. Roosting pockets were also often occupied by vertebrates (31%), but in most cases, it was unknown whether this was by birds or by small mammals (which often make use of these domiciles – D. Beaumont, pers. comm.).

3.4.3 Sir Harold Hillier Gardens

At the Sir Harold Hillier Gardens, high occupancy rates were recorded for the complex subterranean domicile design with nine out of the twenty domiciles (45%) showing evidence of bumblebee activity. Four of these (two *B. terrestris*, one *B. lucorum* and one *B. hortorum*) were still active at the time of collection and of these, two had commenced queen production. One domicile contained the intact remains of a colony of *B. terrestris* but no queen cells were present. There was no evidence to suggest the reason for the demise of the colony, but it is likely to have happened very close to the time of collection as other previously occupied boxes contained material in advanced stages of decay. Three other domiciles showed evidence of reasonably large colonies, but the remains were highly degraded and it was only possible to deduce that the colonies had belonged to *B. terrestris* or *B. lucorum*. A further domicile contained nest material that had been shaped in such a way as to suggest manipulation by a queen bumblebee as well as the yellow faeces associated with bumblebee activity, but no cells were present, indicating that the queen either deserted the domicile or perished very early on in colony foundation. Of the remaining complex subterranean domiciles, two were flooded, one contained a large and thriving colony of vespid wasps, one was inhabited by ants and a further one showed evidence of occupancy by mice.

Lower occupancy rates were observed for the roosting pocket domiciles with only two (10%) showing evidence of bumblebee activity. One of these was occupied by a colony of *B. pratorum*, which progressed to queen production. The other contained around 6 cells but no bodies were found so the identity of the species that the comb belonged to could not be ascertained. No queen cells were present. One of the roosting pockets also showed evidence of occupancy by birds.

3.4.4 Agricultural land in central Scotland

Occupancy of artificial domiciles placed on agricultural land was low and there was no evidence for a preference of either bumblebees or small mammals for any particular habitat type (grassland, hedgerow or field margin) or land management type (Rural Stewardship vs. conventional). Six (4%) of the domiciles showed evidence of inhabitation by small mammals and 1 (<1%) was colonized by vespid wasps. A further two were flooded and two were accidentally destroyed by farm machinery but showed no evidence to suggest occupancy prior to their destruction.

Only 4 (3%) of the domiciles showed any evidence of bumblebee activity. One of these, on a conventionally-managed hedgerow, did not contain any cells, but the nest material had been rearranged in the characteristic manner that provides evidence of manipulation by a queen bumblebee. A further domicile, destroyed by farm machinery, contained a small comb (~eight cells and a honeypot), however no bees were present at the time of discovery so it was impossible to ascertain which species this colony had belonged to. This domicile was located on a Rural Stewardship field margin. The remaining two colonies belonged to *B. lucorum* and both thrived and progressed to queen production. The first, located on a Rural Stewardship managed grassland site, had completed the colony cycle by the time of domicile removal in late August, but the second, located on a conventionally managed grassland site, was still very active. With such low rates of occupancy it is not possible to ascertain whether domicile uptake should be expected to differ between farms deploying agri-environment schemes and conventional farms (3% in each case), or between grassland, field margin and hedgerow habitats (4%, 2% and 2% respectively).

3.5 Discussion

Past studies conducted in the US, Canada and New Zealand report relatively high uptake rates by bumblebee queens (often of between 30 to 50%) when trialling wooden boxes similar to the commercially available domicile used in this study (Fye and Medler, 1954; Hobbs et al., 1962; Hobbs 1967; Richards, 1978; MacFarlane et al., 1983). However, similar trials conducted more recently in the UK have failed to replicate these rates of success (Fussell and Corbet, 1992; Gaston et al., 2005b). The results of this study are consistent with those of the latter, demonstrating low uptake rates of wooden commercially available domiciles, whether used according to the manufacturer's guidelines or with the addition of more suitable nest material and modified for underground use.

In the early 20th century, Sladen (1912) developed the 'Sladen cover' domicile design, which consisted of a hole in the ground supplied with suitable nesting material and covered by a wooden lid (see chapter 2 for details). When trialled in the UK, these achieved an overall uptake rate of 30% and 6 different bumblebee species were represented (Sladen, 1912). The slab domicile design used in this study was very similar to that of the Sladen cover domicile but occupancy rates achieved were much lower (2%). However, slab domiciles were commonly occupied by mice, and since bumblebees are often found nesting in the abandoned homes of mice (Svensson and Lundberg, 1977; Donovan and Weir, 1978), it is possible that these domiciles might have been occupied more readily by bumblebees in subsequent years. Increasing occupancy across years is common in artificial domicile trials but the explanation for this is generally unclear (Hobbs et al., 1962; Donovan and Weir, 1978; Barron et al., 2000). The slab domiciles occupied in this study were colonized by the subterranean nesting species *B. lucorum* and *B. lapidarius*. *Bombus lapidarius* was the most commonly recorded occupant of artificial domiciles trialled by Sladen (1912) suggesting that this species may have a propensity to nest in manmade sites. This species has a high temperature threshold for activity compared to other British species (Corbet et al., 1993) and is believed to prefer to nest in association with stone because of the heat reservoir effect that is provided (Fussell and Corbet, 1992). This may explain why the slab domicile was chosen by this species. Since *B. lapidarius* is known to make use of artificial domiciles, it is possible that creating domiciles tailored to the preferences of this species may provide a method of increasing the probability of domicile occupancy.

Roosting pockets were the only aerial design trialled in this study. In past studies carried out in the US, Canada and the Netherlands, aerial designs have achieved occupancy rates of between 33 and 43% (Wilcke, 1953; Fye and Medler, 1954; Hobbs, 1967a; Richards, 1978) and it has been suggested that the number of bumblebee colonies founded above ground may often be underestimated (Richards, 1978), perhaps because such colonies are less likely to be observed. Certainly rates of bumblebee occupancy of bird boxes appear to be high (Fussell and Corbet, 1992) and some species such as *B. hypnorum*, seem to nest almost exclusively above ground (Hasselrot, 1960). However, in the present study, roosting pockets yielded low occupancy rates. In the two cases of bumblebee occupancy of roosting pockets to which a species could be attributed, both colonies belonged to *B. pratorum*. This species is known to make use of a diverse range of nesting sites and is sometimes found nesting in old bird nests (Sladen, 1912; Alford, 1975). Fussell and Corbet (1992) found that nests of *B. pratorum* were commonly discovered in bird boxes suggesting that aerial positioning may be attractive to this species. As with the slab domiciles, roosting pockets were frequently occupied by birds or small mammals so it is possible that uptake rates would have increased if the domiciles were left out over subsequent years.

Flower pot domiciles were never occupied by bumblebees and did not appear to be particularly attractive to small mammals. This type of domicile was also fragile and although most survived the first summer, many succumbed to bad weather and/or vandalism over the winter.

Overall uptake rates were low for the complex subterranean domicile but this design did show potential as a method of providing suitable nest sites for bumblebees. At the botanical garden site, uptake rates were comparable with those achieved by Richards (1978, 1987) and Hobbs et al. (1960, 1962) in Canada and three different bumblebee species were represented, suggesting that this domicile style could provide benefits for multiple species. However, in the agricultural environment, the domiciles performed poorly and uptake rates were closer to those achieved with other domicile designs and in other British studies (Fussell and Corbet, 1992; Gaston et al., 2005b). Since other designs were not trialled in the botanical garden site, the effects of location and domicile design cannot be disentangled, thus there is no evidence that this design is more effective than other designs trialled in this study. However, the success of colonies founded within the domiciles demonstrates that this design can provide suitable nest sites for bumblebees.

The domiciles trialled in this study were based on designs that have previously been used with some success (Sladen, 1912; Hobbs et al., 1962; Hobbs, 1967a; Intenthron and Gerrard, 1999) yet low occupancy rates were achieved for all. These discrepancies can be explained in a number of ways.

Most successful artificial domicile studies were not carried out in the UK so it is possible that low occupancy rates achieved in the UK reflect a lower propensity of British bumblebee species to nest in artificial domiciles than species present in other parts of the world. However, both Sladen (1912) and Wilcke (1953) achieved relatively high uptake rates by European bumblebee species. A more likely explanation for the discrepancies observed between this and other studies is that many of the successful artificial domicile studies carried out previously were conducted several decades ago. Given ongoing declines of bumblebee populations throughout most of their range (Williams and Osborne, 2009) the lower occupancy rates observed here may be a direct reflection of lower bumblebee population sizes.

Domicile occupancy is also likely to relate to the abundance of natural nest sites available. If the availability of natural nest sites is high, it might be expected that domicile occupancy would be low since nest site availability is less likely to be limiting rates of bumblebee colony foundation. Domiciles installed in the grounds of the University of Stirling were generally placed in woodland edge habitat along banks or bushes. Within these areas there was a great deal of evidence for activity of small mammals and two of the domicile designs trialled at this site were commonly occupied by mice and birds, suggesting a high density of animals that create preferred nest sites for bumblebees. Several natural nests were also discovered in close proximity to the sites of domicile placement both in 2008 and 2009 (S. O'Connor, pers. comm.) suggesting that these sites do provide suitable nesting habitat for bumblebees. Therefore it seems likely that nest site availability was not limiting at this site.

The location of domiciles placed in the botanical garden site were selected based on the presence of an abundance of nest site searching queens in the previous year, a method of site selection which has also often yielded successful results in past studies (Sladen, 1912; Frison, 1926; Pomeroy, 1981). It is possible that an abundance of nest site searching queens may reflect a deficit of nest sites relative to local bumblebee population sizes. This might be expected at the botanical garden site since it provides a succession of flowers throughout the spring and summer which is likely to promote bumblebee colony survival and reproduction. This would lead to high local abundances of bumblebees and could potentially cause nest sites to become a limiting resource. Conversely, uptake rates might be expected to be higher in areas that provide many natural nesting sites since a greater number of queens would be likely to come into contact with the domiciles. As nest site searching queens were abundant in the localities of the botanical garden domiciles, this provides an alternative explanation for the relatively high occupancy observed at this site.

Visual cues are sometimes suggested to be important in nest site location by bumblebee queens (Fye and Medler, 1954) and it is possible that the entrances of the majority of domiciles used in this study were not conspicuous enough to be readily observed by nest site searching queens. Most designs were also such that the entrances were readily blocked by leaves and other debris, which could have prevented discovery by queens. If this is the case, this may provide an additional explanation for the relative success of the complex subterranean domicile. This design incorporated a tile overhang which gave rise to a more natural looking entrance compared to the v-shaped valley excavation used with the other designs. The tile overhang may not have been conspicuous in the agricultural sites since these often became overgrown with grasses and other vegetation but at the botanic garden site, most domiciles were buried in bare earth such that the entrances remained visible.

That artificial domiciles were unsuccessful in the agricultural landscape is perhaps unsurprising. Bumblebee declines have been linked to the simplification of farmland and there is evidence to suggest that bumblebee abundance is lower in the rural environment than the urban environment (Goulson et al., 2002a; Osborne, et al., 2007). This is presumably because open spaces managed by man for recreation or aesthetic value generally contain an abundance and diversity of flowers that can provide forage resources throughout the period of bumblebee activity, whilst floral abundance and diversity within the agricultural environment is generally very poor. Spring forage availability in the agricultural sites used in this study was generally low and it is notable that two out of the three nests founded in domiciles placed on agricultural land were adjacent to patches of early flowering *Lamium* spp. Proximity of spring forage to a potential nest site may be an important consideration for bumblebees. A spring bumblebee queen must feed, incubate and defend her first brood unaided (Goulson, 2003) thus the need for extended foraging trips would be likely to be detrimental to colony survival.

Urban gardens are known to support strong populations of bumblebees (Goulson et al., 2002a; Osborne, et al., 2007) and the floral abundance and diversity present at the garden sites represented in this study are likely to have been similar to those within the botanical garden site. As a result, it might have been predicted that the commercially available domiciles placed in gardens should also have achieved some success. However, the lack of occupancy achieved with unmodified commercial domiciles is perhaps unsurprising since the straw provided as nesting material with commercially available domiciles is coarse and would not easily be manipulated by a bumblebee queen. The suitability of nest material is believed to be very important in attracting bumblebee queens to nest (Sladen, 1912) and the inclusion of more suitable nest material within commercially available domiciles could increase the likelihood of occupancy. Similarly, a domicile design targeting subterranean nesters such as *B. lapidarius*, *B. terrestris* and *B. lucorum* which are common in the UK and may be more likely to occupy artificial domiciles might also yield more satisfactory results. Identifying modifications that might increase the success of commercially available domiciles for bumblebees should be a key priority for those that manufacture and market these since at present they appear to provide very poor value to customers.

It is possible that discrepancies between rates of occupancy reported in this study and those of many other published studies may be due to underreporting of unsuccessful domicile trials as a result of a bias toward the publication of positive results. The success of the trial using complex subterranean domiciles in the botanical garden site demonstrates that it is possible to attain uptake rates that are comparable to those of the majority of published studies, but the results of the other trials demonstrate that in this case at least, these rates do not provide a good representation of effort versus reward.

3.6 Conclusions

Attempts at attracting bumblebees to nest in artificial domiciles generally yield very poor results in the UK. Here, it is shown that artificial domiciles can achieve high uptake rates, but that this appears to be uncommon. One domicile design at one site yielded levels of occupancy that were comparable to those reported in studies in which domiciles were considered to be successful but the factors determining this success are unclear. Factors influencing the likelihood of success of artificial domiciles are probably numerous and may include domicile design, local bumblebee abundance, nest site preferences of bumblebee species present, availability of natural nest sites, weather conditions and availability of local forage.

Findings presented here suggest that based on current knowledge, attempts to use artificial domiciles for obtaining colonies of wild British bumblebees or as a tool for bumblebee conservation are likely to be unproductive. It is also demonstrated that commercially available domiciles for bumblebees are often ineffective and it is recommended that manufacturers should replace the nest material provided with a more suitable material and consider investing further research towards the development of a more effective product.

3.7 Acknowledgements

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Chapter 4

A possible role of odour cues in nest site location by bumblebees

4.1 Abstract

Nest site selection is an important aspect of bumblebee ecology yet little is known regarding the factors involved in this process. Nest site searching bumblebee queens demonstrate a characteristic zigzagging flight consistent with the detection of olfactory cues and it has been hypothesised that odour cues may play a role in nest site location. Specifically, it has been proposed that bumblebee queens may target odours associated with old nest material belonging to small mammals or bumblebee colonies. Here sticky box traps baited with nest material of the field vole, *Microtus agrestis*, the bank vole, *Clethrionomys glareolus* and the bumblebee, *B. terrestris* were used to test the responses of bumblebee queens to these odours. A complementary experiment was also conducted in the laboratory using a y-tube olfactometer to assess choices made by *B. terrestris* and *B. pascuorum* queens when presented with an odour associated with one of the trial materials or clean air. No differences were found between queens caught in baited traps and control traps in the field, but laboratory bioassays demonstrated that queens can detect and respond to odours associated with vole nest material. Queens of *B. terrestris* were found to avoid odours associated with fresh *M. agrestis* nest material but were attracted to air containing odours associated with aged *C. glareolus* nest material. These findings are discussed with a particular focus on the likely importance of species-specific nesting ecology of bumblebees in determining responses to different odour cues and the potential role of age of odorous material in determining attractiveness to bumblebee queens.

4.2 Introduction

Odour cues play an important role in the behavioural ecology of bumblebees. They are used in nest entrance recognition (Pouvreau, 1996), nest-mate identification (Gamboa et al., 1987) and avoidance of depleted forage resources (Stout et al., 1998) and are also implicated in the location of host colonies by usurping conspecific bumblebee queens and kleptoparasitic species (Frison, 1930; Fisher, 1983; Fisher et al., 1992).

Nest site selection is an important aspect of bumblebee ecology as colony survival and success is dependent on the suitability of the location of the nest (Sladen, 1912). However, the mechanisms of nest site selection by bumblebee queens are poorly understood. Nest site searching bumblebee queens display a highly characteristic behaviour, adopting a zigzag flight trajectory, flying close to the ground and landing frequently in order to investigate potential nest sites on foot (Kells and Goulson, 2003). This behaviour is consistent with the detection of short-range olfactory cues and two hypotheses have been put forward in relation to a possible role of odour cues in nest site location by bumblebee queens:

1) Nest-searching bumblebee queens use odour cues to identify old nests of small mammals:
Bumblebees often nest in the abandoned homes of field mice, voles or other small animals (Sladen, 1912; Svensson and Lundberg, 1977; Donovan and Weir, 1978; Fussell and Corbet, 1992; Rasmont et al., 2008). Such sites are probably favourable as they provide a sheltered cavity and a suitable source of insulating nest material.

Trials with artificial domiciles have gone some way towards testing whether the presence of small mammal nest material can influence nest site selection by bumblebee queens but have found no evidence to support this. For example, a study in Canada found that bumblebee queens were equally likely to nest in domiciles containing fresh bedding or those that had been baited with mouse nests (Hobbs et al., 1960) and in New Zealand, *B. hortorum* was found to show an active preference for founding colonies in clean nest material over the same type of material after previous use by mice (Pomeroy, 1981). However, such studies are not able to detect differences in queen investigation rates thus do not allow the examination of behavioural responses to these odours. (Even if odour cues were important for initiating the investigation of a potential nest site, secondary cues encountered within the nest chamber are likely to be more important in determining the likelihood of the queen to settle.) Also, little attention has been paid to relating the ecology of the small mammal to that of bumblebee species targeted. Certain small mammals are more likely to provide suitable nest sites for certain bumblebee species than others depending on species-specific differences in the nesting preferences of each.

To my knowledge, all studies previously carried out have used nest material from unidentified species of mouse, and since mice are commonly reported to depredate bumblebee nests (Darwin, 1906; Donovan and Weir, 1978; Richards, 1987), it is perhaps unlikely that bumblebees should be attracted to odours associated with these animals. It has been suggested that lower occupancy of artificial domiciles in the UK compared to New Zealand (where some of the same bumblebee species are present) is a result of lower nest site availability in New Zealand due to a paucity of small mammals (Fussell and Corbet, 1992). However, house mice and rats are abundant in New Zealand (King, 1990), so that if this is the case, other species of small mammal must be more important in the adequate provision of nest sites for bumblebees in the UK.

In the UK, both the field vole (*Microtus agrestis*) and the bank vole (*Clethrionomys glareolus)* are likely to provide suitable nest sites for bumblebees, but each has very different nesting ecology. The bank vole nests underground and probably provides suitable nest sites for subterranean nesting bumblebee species such as *B. terrestris*, whereas the field vole nests on the surface of the ground so may be more likely to provide suitable nesting sites for surface nesting species such as *B. pascuorum*. Therefore it might be predicted that odour cues associated with bank voles would be more likely to be attractive to nest site searching queens of *B. terrestris* than *B. pascuorum* but that the reverse should be true for odours associated with field voles. Djegham et al. (1994) report that queens of *B. terrestris* are more likely to initiate colony foundation in the presence of odours associated with the common vole, *Microtus arvalis*, which is known to excavate subterranean nest tunnels of approximately 30-40cm in length, fitting well with the known nesting preferences of *B. terrestris* (Sladen, 1912).

Since small mammals are known to depredate bumblebee nests (Sladen, 1912), it seems likely that the age of the small mammal nest material might be important. If the nest material is very fresh, this might be an indication that the occupant is likely to return and this would be a danger to a newly founded bumblebee colony. The age of small mammal nest material is probably readily detectable since the odour profile of the material is likely to change significantly as the various volatile chemicals break down or disperse. Therefore age of material may also play an important role in the likelihood of attracting nest site searching bumblebee queens. It is possible that studies to date have used material that is too fresh such that the odour profile is not indicative of a suitable nest site for bumblebees.

2) Nest-searching queens use odour cues to identify old bumblebee nests: Bumblebees often nest where there have been bumblebee colonies in previous years and this is evidenced by the fact that occupancy rates of artificial domiciles for bumblebees often increase over consecutive years (Donovan and Weir, 1978; Pomeroy, 1981; Barron et al., 2000). Two hypotheses have been suggested to explain the trend for bumblebee queens to found nests at the same sites in consecutive years. The first is that after the diapause, a bumblebee queen will return to the site of her maternal colony in order to found a nest as close as possible to this site (Donovan and Weir, 1978, Pomeroy, 1981). As this site was able to support a colony that progressed to queen production in the previous year, there is presumably a good chance that it will be a suitable site for a nest in subsequent years. An alternative hypothesis is that queens are in some way able to detect sites which contain old bumblebee nest material, again possibly via olfactory cues, and presume this to be a good site as it has previously been used successfully by another colony. Little evidence has been produced to support either of these hypotheses but ‘enhanced [bumblebee queen] attraction and nest founding behaviour in the presence of bumblebee nest odour’ was reported in a personal communication to Barron et al. (2000).

If odour cues are used by bumblebee queens to locate suitable nest sites, the identification of these cues could confer many benefits. Primarily, knowledge of such cues would provide a method of attracting queens to investigate artificial domiciles. Such an attractant would have commercial applications for sale with artificial domiciles and to promote colony foundation adjacent to flowering crops to boost pollinator abundance, as well as providing a potential method of obtaining colonies for research and conservation.

This study investigates the responses of nest site searching bumblebee queens to odours associated with nest material used by two species of vole (*C. glareolus* and *M. agrestis*), and nest material of the bumblebee, *B. terrestris*. The aims were to establish whether or not nest site searching bumblebee queens respond to these odour cues, to assess the effect of the age of the materials on responses observed and to look for evidence of species-specific differences in responses relating to the known differences in nesting ecology among the bumblebee and small mammal species studied.

4.3 Methods

4.3.1 Materials used

4.3.1a Bumblebee nest material

All experiments were carried out using frozen nest material from colonies of *Bombus terrestris* which had been reared by Koppert Biological Systems UK, placed out in the field and then later killed by freezing at -18˚C.

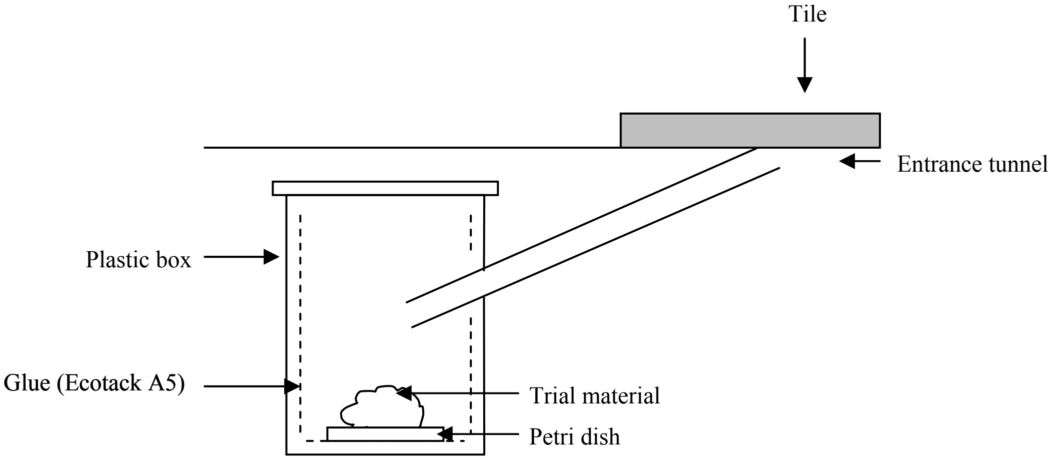
4.3.1b Vole nest material

Nest material from the field vole, *M. agrestis*, and from the bank vole, *C. glareolus,* was obtained from laboratory populations that had recently been captured from the wild, and consisted of shredded absorbent paper. Material was collected over a period of two weeks and stored in a freezer at -18°C.

4.3.2 Field trials

Traps were designed to mimic the types of site that bumblebee queens might investigate when searching for a suitable nest site. These were constructed from 15cm x 15cm x 15cm plastic boxes, coated inside with approximately 15mls Ecotack A5. A 2cm diameter hole was cut into the centre of one face to which a 35cm length of black plastic conduit (diameter 20mm) was attached, so that 5cm of the conduit protruded into the box. One of four treatments was placed into a 5.5cm diameter petri dish in the bottom of each trap. Treatments consisted of bank vole nest material (2g), field vole nest material (2g), bumblebee nest material (4g) and no material (control). Boxes were buried approximately 10cm beneath the soil surface and positioned such that the entrance holes were flush with the ground. A tile was used to cover the entrance of each trap to keep it clear and to protect against weather (figure 4.1).

Figure 4.1: Trap designed to assess responses of nest site searching bumblebee queens to different potential odour cues. Traps were baited with old bank vole, field vole or bumblebee nest material or left empty (control treatment).



A total of 75 blocks of 4 traps (one of each treatment) were installed in the Sir Harold Hillier Gardens, Romsey between 21/03/06 and 23/03/06 along linear features such as fences, banks and borders that were considered to represent suitable bumblebee nesting habitat. Each trap was separated from the next by a distance of 1m and each block of four was placed at least 10m away from any other block of four.

Traps were checked every four weeks and the entrance holes cleared of any obstructions. They were retrieved at the beginning of July 2006. Bees caught were soaked in kerosene to remove any Ecotack and identified to species level. The level of decomposition of the bees caught made distinction between the very similar *B. terrestris* and *B. lucorum* impossible, so these species were pooled for analysis. This is common practice since these species share very similar ecology and are extremely difficult to distinguish in the field.

A chi-square test was carried out to test for an effect of treatment on total number of bumblebees caught. This analysis was repeated using only the combined data for *B. terrestris* and *B. lucorum* (which share similar nest site preferences) to control for behavioural differences between species. Catch rates were such that numbers of other species were too low for individual statistical analysis. All analyses were carried out using SPSS version 16.0.

4.3.3 Laboratory bioassays

In 2006, 50 nest site searching queens each of *B. terrestris* and *B. pascuorum* were caught from the wild. These were kept five to a cage in 15cm x 15cm x 15cm wire cages with a Tubigauze covering. Reinforced tape was used to strengthen the corners of the cages. The bumblebees were kept in a well-ventilated room at a temperature of 21˚C with a lighting regime of 16 hours of light followed by 8 hours of darkness. The queens had continuous access to feeding tubes containing a 1:1 mixture of honey and water in a feeding tube.

A y-tube olfactometer was used to examine any behavioural responses to the three different treatments trialled in this study. The olfactometer consisted of a 25x15x10cm arena connected by a 25mm diameter glass tube to a 20mm diameter y-tube (figure 4.2). Clean air (passed through a charcoal filter to remove any volatiles present) was pumped through two sample chambers and then down each arm at a flow rate of 0.25L/min. One sample chamber remained empty, whilst the other contained the treatment, so that air passing through one arm of the y-tube remained clean whilst air passing down the other arm contained odours released from the treatment material. Treatments consisted of either 0.5g bumblebee nest material, 0.1g field vole nest material or 0.1g bank vole nest material. The bioassays were carried out in red light as these were the conditions under which queens were found to explore the tube leading from the arena most readily.

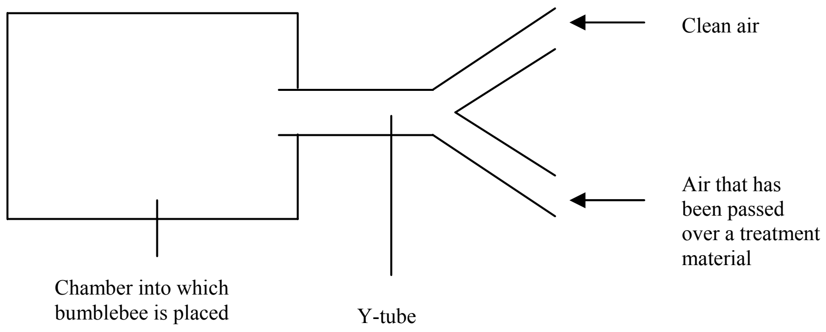


Figure 4.2: Plan view of a y-tube olfactometer designed to test the response of nest site searching bumblebee queens to odour cues released from old bank vole, field vole and bumblebee nest material.

Individual queens were placed into the arena and observed until the y-tube had been explored, at which point the choice of arm was recorded. A choice was defined as the point at which the bee had travelled at least two centimetres up an arm. Only the first choice of each bee was recorded. To control for an innate preference for choosing one direction over the other, or for choosing one arm over the other, the orientation of the y-tube and the arm through which the treatment passed were selected randomly for each trial. Components of the y-tube setup were cleaned with detergent (Decon 75), rinsed with solvent (acetone) and then baked in an oven at 180°C for at least two hours after every 10 replicates, and the y-tube section was cleaned with detergent and solvent after each replicate in order to remove any footprint odours left by the bumblebees. Each bee was trialled with each of the three treatments, but the order in which the treatments were presented was randomized. No bee was used more than once in a 48 hour period. Several bees died during the course of the experiment so that sample sizes varied. The cause of the deaths is unknown but bumblebees can sometimes be slow to learn to use feeding tubes (D. Goulson, pers. com.) and it is possible that at least some of the deaths could be attributed to starvation.

The materials used for the extraction of volatiles were stored in a sealed container at -18°C throughout 2006 and early 2007, and the experiment was repeated with 15 individuals of *B. terrestris* in the spring of 2007. The odour associated with material stored in this way was very different to the human nose (being noticeably less pungent) and this was believed to be a result of the dispersal of many of the smaller volatile chemicals such as ammonia. As a result of this presumed alteration in the chemical profile of the materials, odours associated with these were assumed to represent aged material more closely than fresh material.

Data were analysed using chi-square tests with Yates' corrections.

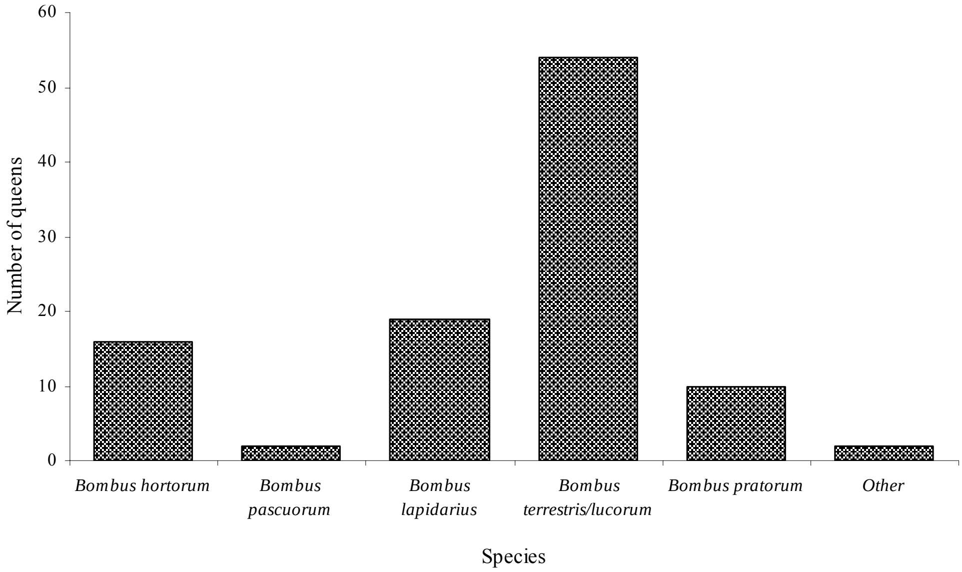
4.4 Results

4.4.1 Field trials

In total, 103 bumblebee queens were caught in field traps and these were found in 49 out of the 300 boxes (16%). *Bombus terrestris* and *B. lucorum* (pooled) made the greatest contribution to the total catch, accounting for 52% of all bees caught.

Bombus lapidarius and *B. hortorum* were also frequently caught, accounting for 18% and 16% of catches respectively. *Bombus pratorum* made up 10% of the total catches whilst *B. pascuorum* contributed only 2% (figure 4.3).

Figure 4.3: Species composition of nest site searching bumblebee queens caught in subterranean sticky box traps at the Sir Harold Hillier Gardens, Romsey. (‘Other’ category consists of two individuals, one of which was so badly decomposed that identification was impossible and one belonged to *B. jonellus*)



No effect of odour was observed during the field trials. Boxes containing all three treatments (bumblebee nest material, field vole nest material and bank vole nest material) attracted the same number of queens as the control boxes ($\chi^2_3 = 3.36$, p = 0.34, figure 4.4a). This was also true when considering only *B. terrestris/lucorum* ($\chi^2_3 = 0.82$, p = 0.85, figure 4.4b).

4.4.2 Laboratory bioassays - results

In 2006, there was no evidence for an effect of the odour of bank vole nest material on the arm choices made by queens of either *B. terrestris* or *B. pascuorum*, and there was also no effect of the odour of bumblebee nest material on *B. terrestris* queens (table 4.1, figure 4.5a and b). Seventy three percent of *B. pascuorum* chose clean air over air that had been passed over nest material belonging to *B. terrestris*, but this difference was not significant (table 4.1). Odours emitted by field vole nest material also had no effect on queens of *B. pascuorum*, but *B. terrestris* showed a strong avoidance response to this odour (table 4.1, figure 4.5c).

Table 4.1: Summary of chi-square results for choices made by *B. terrestris* and *B. pascuorum* queens when presented with odours associated with bank vole, field vole or bumblebee nest material versus clean air. (Results from 2006)

Treatment	Species	Chi-square	Degrees of freedom	P-value
Bank vole	*B. terrestris*	2.45	1	0.12
	B. pascuorum	0.24	1	0.62
Field vole	*B. terrestris*	15.43	1	**<0.01**
	B. pascuorum	0.84	1	0.36
Bumblebee nest	*B. terrestris*	0.04	1	0.84
	B. pascuorum	3.68	1	0.06

In 2007, only *B. terrestris* queens were used and these showed very different responses to the odours of the (now aged) materials presented. The odour of old bumblebee nest still had no significant effect on the choice made by *B. terrestris* queens ($\chi^2_1 = 0.27$, p = 0.60, figure 4.6). However, odours associated with field vole nest material no longer had an effect on arm choice ($\chi^2_1 = 0.067$, p = 0.80) whilst odours associated with bank vole nest material were found to be attractive to *B.*

terrestris queens ($\chi^2_1 = 4.27$, $p = 0.04$), with 80% of queens choosing the arm with air passed over bank vole nest material (figure 4.6).

Figure 4.4a: Average number of bumblebee queens caught per trap for subterranean sticky box traps baited with bumblebee, bank vole or field vole nest material or left empty (control) (±standard error).

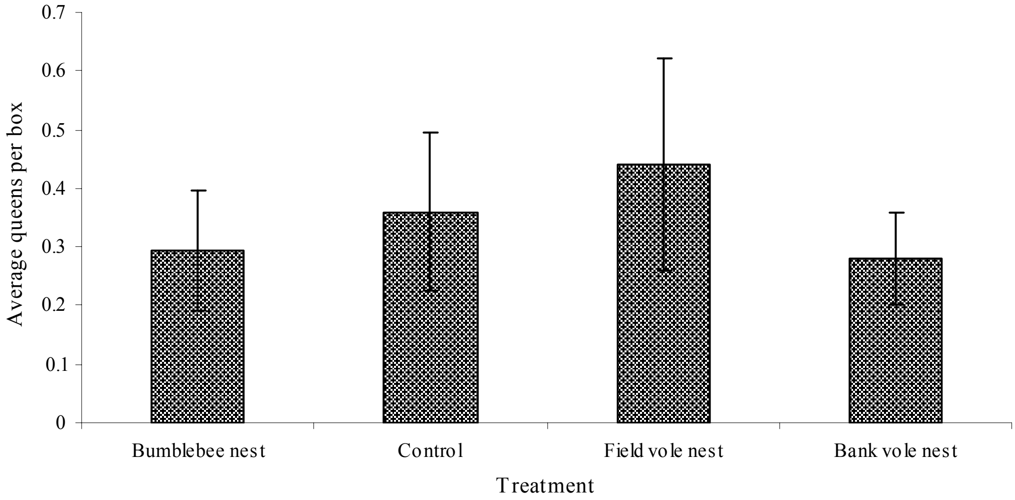


Figure 4.4b: Average number of *Bombus terrestris*/*lucorum* queens caught per trap for subterranean sticky box traps baited with bumblebee, bank vole or field vole nest material or nothing (control) (±standard error).

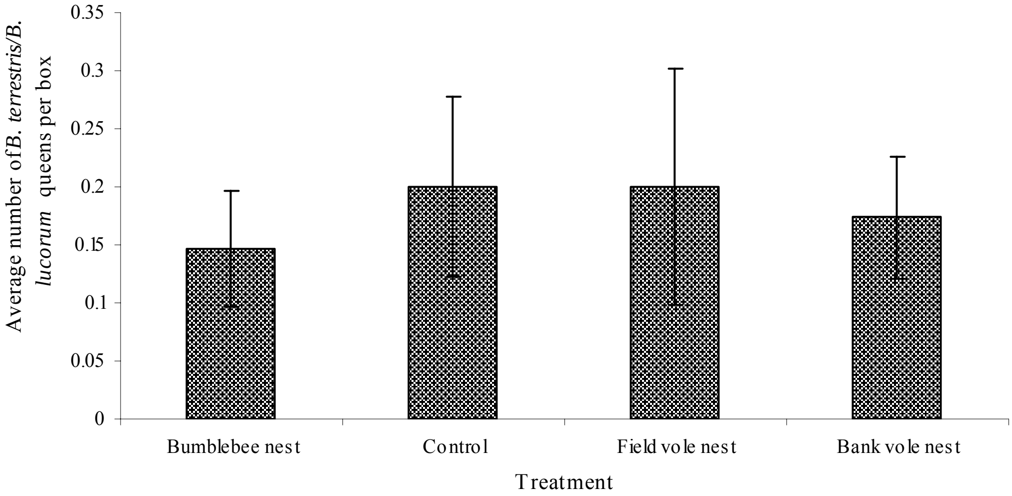
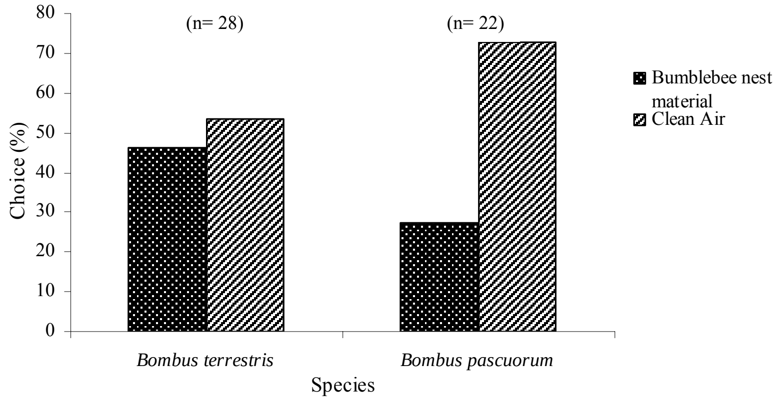
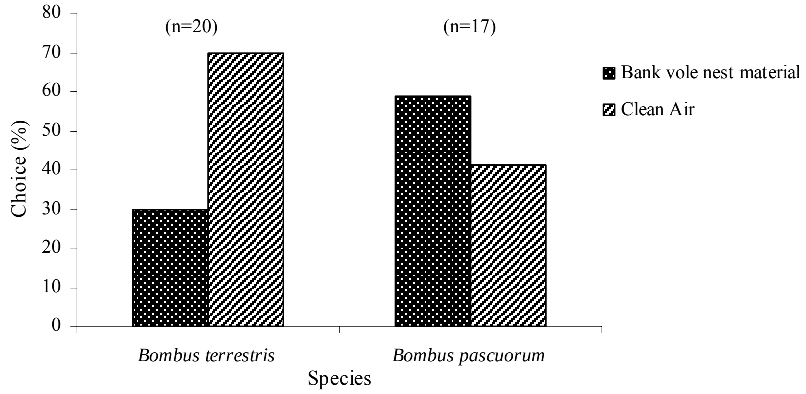


Figure 4.5a: Arm choice of bumblebee queens of *B. pascuorum* and *B. terrestris* when presented with a y-tube with one arm containing odours associated with (a) bumblebee nest material, (b) bank vole nest material or (c) field vole nest material and the other containing clean air. (Data from 2006)

(a)



(b)



(c)

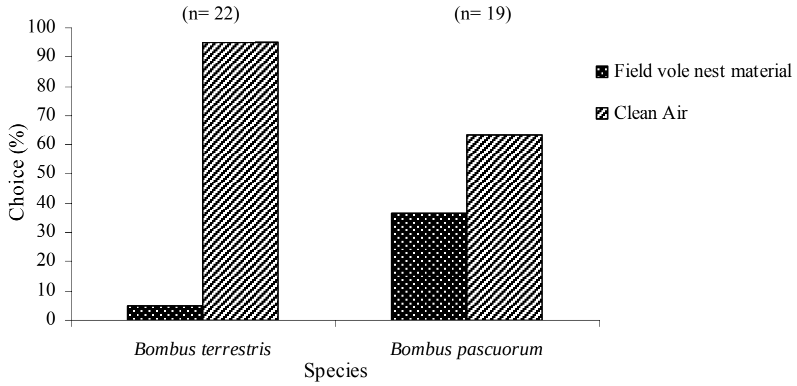
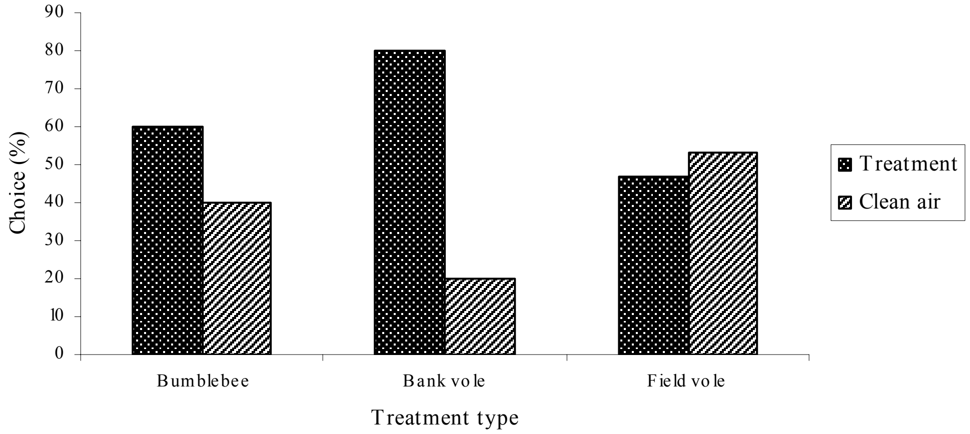


Figure 4.6: Arm choice of bumblebee queens of *B. terrestris* when presented with a y-tube with one arm containing odours associated with aged bumblebee, bank vole or field vole nest material and the other containing clean air. (Data from 2007)



4.5 Discussion

4.5.1 Species caught in field trials

The species composition of bees caught within the traps was consistent with the known ecology of the common British bumblebee species. *Bombus terrestris, B. lucorum* and *B. lapidarius* are all known to have a preference for nesting underground (Sladen, 1912) and these three species comprised the majority of individuals caught. As expected, *B. pascuorum* was rarely caught since it generally nests on the surface of the ground (Fussell and Corbet, 1992). *Bombus pratorum* and *B. hortorum* are known to be very generalist in their nest site preferences which explains the relatively high representation of these species in this study.

One queen of *B. jonellus* was also caught in one of the traps. This species has a patchy distribution and is uncommon in many areas of the UK but tends to be abundant in patches of suitable habitat (Edwards and Jenner, 2005). This species had previously been observed at the experimental site (J. Ellis, pers. comm.). It is also known to be generalist in its nest site requirements and has been known to nest underground, so it is not unexpected that this species should have been represented.

4.5.2 Responses to bumblebee nest material

If bumblebee queens use the odour of old bumblebee nest as a cue for the location of suitable nest sites, it might be predicted that queens would only identify and/or respond to odours associated with nests of their own species since bumblebee species differ in their nest site preferences. The bumblebee nest material used in this study belonged to *B. terrestris*, so it might be hypothesised that only this species should be attracted. However, there was no difference in the numbers of queens of any species, including *B. terrestris*/*B. lucorum*, caught in traps baited with old bumblebee nest material and control traps nor was there any significant effect of the odour of the old bumblebee nest material on *B. terrestris* in the laboratory.

The bumblebee nest material used in this trial belonged to commercially reared colonies of *B. terrestris* which belong to a different sub-species of *B. terrestris* from those found in the UK (Ings et al., 2006). These colonies are also bred in confinement and artificially fed, so that the odour profiles associated with their nests may differ from those that would be experienced by British *B. terrestris* queens in the field. However, it is also possible that bumblebee queens simply do not use the odour of old nest material as a cue when nest site searching. Nest odours may indicate sites that had provided suitable conditions for nest development the previous year, but they may also indicate sites which contain parasites or pathogens surviving from the previous year, in which case these odours would not be expected to be used as a positive cue in nest site selection.

The observation that nests are often founded in similar places in subsequent years is also explicable by the hypothesis that new queens return to their maternal nest site in subsequent years, or that nest site characteristics are learned by new queens in a form of imprinting upon leaving their maternal nest as a template for a suitable nest site the following year (Donovan and Weir, 1978). Fussell and Corbet (1992) found that of six conspecific nests recorded in similar places in subsequent years, only one was found in exactly the same position as one from the previous year, and this was an old nest box from which the bumblebee nest material had been removed, and replaced with upholsterers’ stuffing, suggesting that it was not the odour of the old nest that had attracted the queens to found new nests in those locations. It seems a sensible strategy for a bumblebee queen to return to a similar site to her maternal nest site since only very successful colonies progress to reproductive production. It is therefore likely that local resources are abundant and that a nest in a subsequent year may also be successful.

4.5.3 Responses to field vole nest material

Baiting traps with field vole nest material had no effect on the number of bumblebee queens caught. Field vole nesting ecology closely resembles that of *B. pascuorum* in that they tend to nest on the surface of the ground, often in tussocks in open grassland, and their abandoned homes probably provide good nest sites for this species. However, since *B. pascuorum* rarely nests underground, the traps used in this study are unlikely to attract this species and this was reflected by the species composition of the catches. Species such as *B. terrestris, B. lucorum* and *B. lapidarius* tend to nest underground and this is reflected by their higher representation in the total individuals caught. However, these species would be unlikely to use the odour of field vole as a cue to find a suitable nest site since their nesting ecology differs from that of the field vole.

Interesting effects of small mammal odours were observed in the laboratory bioassays. In the first year *B. terrestris* demonstrated a strong avoidance response to the odour of field vole nest material. The material itself had a very strong and repellent ammonia smell to the human nose. Since small mammals are believed to depredate bumblebee colonies, this avoidance response might have been predicted. However, no such response was evident for *B. pascuorum*, as might be expected if *B. pascuorum* do indeed use abandoned field vole nests for colony foundation. Since *B. terrestris* tends to nest underground, this species is unlikely to come across the scent of field vole in anything other than a threatening context. These fundamental differences in the ecology of the two species may explain the differences in the responses observed.

It is worth noting that *B. terrestris* did not show an avoidance response to field vole nest material in the field trials. This is probably due to the fact that volatiles from material placed out in the field had the chance to disperse, so that the volatile profile emanating from the traps would not have been identical to that presented in the laboratory.

This is supported by the fact that in the second year of laboratory trials, *B. terrestris* showed no response to the odour of year old field vole nest material. This suggests that the response previously observed was stimulated by small, highly volatile chemicals such as ammonia which disperse very quickly and that the change in the profile of the chemical components of the field vole nest material over the course of a year rendered the material innocuous to this species.

4.5.4 Responses to bank vole nest material

Bank voles are associated with sheltered areas such as woodland, banks and hedges and tend to create their nests beneath the surface of the ground, lining the cavity with grass, moss and feathers. An abandoned bank vole nest would provide suitable conditions for nest founding for those bumblebee species that prefer to nest underground (such as *B. terrestris*, *B. lapidarius* and *B. lucorum*). Since the trap design and locations used in this trial should also have favoured these species, a response would have been expected if bumblebee queens are using odour cues associated with small mammal nest material to aid nest site location. However no difference in catch rates were observed for traps baited with bank vole material versus control traps. This can be partly explained by the results of the laboratory bioassays. In the first year of trials, neither species showed any significant response to bank vole nest material. No positive response was expected for *B. pascuorum*, since it is a surface nesting species and is therefore unlikely to use cues associated with bank voles in nest site location. However, in the second year of laboratory trials, *B. terrestris* showed a significant attraction to the odour of bank vole nest material. This suggests that *B. terrestris* may use cues from bank vole nest material for nest site location, but that the material must be sufficiently old in order to attract this species, presumably as bumblebees are unlikely to use current vole nests in which to found a colony. It is probable that the bouquet of volatiles released from old nest material is very different to that released from fresh nest material and it would be expected that the cues used by bumblebee queens should be associated with older material. It should be borne in mind that the conditions of storage of this material over the year were not reflective of the conditions to which these materials would be exposed in the field. However, the difference in odour to the human nose coupled with the altered response of the bumblebee queens suggest that alterations in odour profile did take place. It seems likely that the majority of these differences (for example the drop in pungency suggesting dispersal of small, highly volatile components) would also occur under natural conditions (though aging of materials under field conditions would be required to confirm that this is the case).

The observed response of *B. terrestris* queens to aged bank vole nest material fits well with previous observation that nesting and egg-laying in *B. terrestris* can be facilitated by the presence of common vole nest material (Djegham et al, 1994), and demonstrates that species and age of material provided must be considered when testing hypotheses relating to the use of odour cues by nest site searching bumblebees. The differences in responses observed in the laboratory trials conclusively demonstrate that bumblebees are capable of detecting and responding to volatiles released by vole nest material but further work is required to identify specific odour cues used and to confirm the ecological context of these responses.

4.6 Acknowledgements

I would like to thank Allen Coombes, Fran Zaenglein and all staff at the Sir Harold Hillier Gardens for access to sites and assistance with my trials and to Dr. Richard Birtles and group at the University of Liverpool for the provision of vole nest material. Thanks also to Alex Stewart-Jones, Ben Darvill, Ken MacDonald, Craig Rogers and Steph O’Connor for general support and assistance. I would also like to thank the University of Southampton for use of facilities and the Natural Environment Research Council and the Game and Wildlife Conservation Trust for financial support.

Chapter 5

Replication of a public bumblebee nest survey as a method for monitoring responses of bumblebee populations to environmental change

5.1 Abstract

Several of Britain's bumblebee species have undergone declines in recent years, but since the reproductive unit of social insects is the colony and bumblebee nests are difficult to locate, quantification of the extent of these declines is difficult. Here, a public survey was conducted in which participants were asked to record attributes of bumblebee nests discovered in their gardens and data collected were compared to those of similar studies conducted in 2004 and 1989-1991. Nest site choice by different bumblebee species were consistent with those reported elsewhere. Small mammals and birds were found to provide nest sites for bumblebees although several participants reported that nests were founded at sites that had not been previously occupied by another animal. As in previous studies, little evidence was found to support the hypothesis that bumblebees tend to nest in the same site in consecutive years. Changes in the relative contributions made by the species represented in the surveys to the overall nests discovered suggested that the common bumblebee species *B. pascuorum* may have undergone declines over the past 20 years. The 'browns' division of the colour group system used in previous studies (for which most observations represent *B. pascuorum*) constituted 21% of colonies discovered in 1989-1991, but just 8% of colonies in 2004 and 11% in 2007-2008. This was accompanied by a reduction in the proportion of nests of this colour group discovered on the ground surface (the preferred position of this species) and it is hypothesized that this may be due to adverse effects of increased rainfall on survival of nests built on the surface of the ground. If this is the case, *B. pascuorum* may be ill-equipped to face ongoing changes in climatic conditions

predicted for the UK. Since this species is the only medium-tongued species to remain common in Britain the impacts of its decline could be severe.

5.2 Introduction

Bumblebees are important pollinators of crops and wildflowers (Osborne and Williams, 1996) but many species have shown declines in recent years (Williams et al., 2009). These have been driven, at least in part, by changes in land management practices associated with agricultural intensification which have led to reductions in forage availability and preferred nesting habitat for bumblebees (Kosier et al., 2007; Goulson et al., 2008a; Williams and Osborne, 2009). There is evidence to suggest that urban parks and gardens act as refuges for bumblebees in a poor quality environment (Goulson et al., 2002a; Osborne et al., 2007) by providing flowering plants throughout the year on which bumblebees can forage. Urban areas also provide an abundance of varied nesting habitats for bumblebees, many species of which have been found to make use of man-made features such as buildings, decking, bird boxes, compost bins, walls and hedgerows (Donovan and Weir, 1978; Fussell and Corbet, 1992; Osborne et al., 2007).

Bumblebee forage requirements are simple to establish and are consequently very well studied (e.g. Carvell, 2002; Pywell et al., 2005; Goulson et al., 2005) but the nesting ecology of bumblebees is less well understood. This is largely because bumblebee nests are inconspicuous and as a result, it is difficult to collect a large and unbiased sample of nest records (Kells and Goulson, 2003). Nest density in urban and suburban gardens is probably high (Goulson et al., 2002a) and since members of the public often spend large amounts of time in their gardens, the likelihood of discovery of bumblebee colonies is improved. This provides an opportunity to study nest site choice by bumblebees in the urban environment by means of a public survey.

Public surveys can be a useful tool for accumulating large datasets of ecological information in situations where these would otherwise be difficult to collect and allow simultaneous data collection across a wide geographic range (Silvertown, 2009). They also provide a mechanism for boosting public awareness of important issues in conservation and of promoting ecologically sensitive attitudes and behaviour (Cooper et al., 2007).

Fussell and Corbet exploited this opportunity in 1992, carrying out a survey in which members of the British public were asked to report any bumblebee nests discovered and to describe the sites of these nests. The survey gave rise to a large data set (432 records of nests) collected over a three year period, although the distribution of the locations of the participants of the survey across the UK was unreported. The majority of records were from garden habitats and these data were used to make inferences regarding the species-specific nest site preferences of common British bumblebees with particular reference to the urban environment (Fussell and Corbet, 1992).

In eusocial species such as bumblebees, the effective population size should be measured in numbers of colonies rather than of individuals as the colony is the reproductive unit (Wilson, 1975). However, since bumblebee nests are so difficult to locate, it is very difficult to monitor the dynamics of bumblebee populations. Conducting nest surveys according to a standard protocol such as that of Fussell and

Corbet allows a comparison of the proportional contribution of each species to total nests found across years. This will provide an indication of any changes in the relative abundance of different species over time at the colony level, a measure which is extremely difficult to quantify in any other way.

Osborne et al. (2007) used similar methodology to that of Fussell and Corbet to provide data for the estimation of bumblebee nest density and comparison of bumblebee nesting ecology in the urban versus the rural environment. In this study, the locality of participants spanned all of England, Scotland and Wales, but the majority of participants were based in England with participant density increasing towards London. Although many of the results showed strikingly similar patterns to those reported by Fussell and Corbet, some notable differences were observed (Osborne et al, 2007). However, the records collected were fewer (just 232 nest records) and the methodology too dissimilar (participants were required to intensively survey a prescribed area of land rather than simply to report nests discovered as in Fussell and Corbet) to draw any firm conclusions based on these differences.

Both Fussell and Corbet (1992) and Osborne et al. (2007) divided bumblebee species by colour-group in order to aid identification by untrained individuals. These colour groups are designed to include the six most common species in the UK, but do not allow differentiation between these and rarer species if they are present (table 5.1). However, this limitation can be eliminated since it is now easy to take digital photographs of bumblebees and nests and simple to send them via the internet to allow expert identification of species. This allows more sensitive sampling, and in particular, could show important differences between nest site preferences of *B. terrestris* and *B. lucorum,* two morphologically similar species which are combined in the colour group approach. These two species are rarely separated in ecological studies and very few data are available on differences between them. However, the ranges of these two species are different, with *B. lucorum* dominating in northern locations and *B. terrestris* dominating in the south (Sladen, 1912; Williams, 1982) suggesting that they have different environmental tolerances and ecological traits.

Table 5.1: Bumblebee colour groupings used in public surveys conducted by Fussell and Corbet, 1992 and Osborne et al., 2007 in order to aid identification, and the species which are encompassed by each.

Colour group	Common species	Rare species
Two-banded white tail	*B. lucorum, B. terrestris*	*B. soroeensis, B. magnus, B. cryptarum*
Three-banded white tail	*B. hortorum*	*B. ruderatus, B. jonellus, B. (Ps.) barbutellus*
Black-bodied red tail	*B. lapidarius*	*B. ruderarius, B. (Ps.) rupestris*
Banded red tail	*B. pratorum*	*B. monticola*
Brown	*B. pascuorum*	*B. muscorum, B. humilis*

Fussell and Corbet (1992) attempted to use their survey as a method of collecting data regarding consecutive occupancy in bumblebees. Several authors have noted that bumblebees will often nest where there have been nests in previous years (Hobbs et al., 1962; Barron et al., 2000) and it is hypothesised that queens will actively seek a site that has previously hosted a successfully bumblebee colony, either by returning to their maternal nest sites or by using cues to locate the remains of old bumblebee colonies (Donovan and Weir, 1978). Fussell and Corbet asked participants reporting a bumblebee nest to report whether or not the same nest was occupied by bumblebees the following year but few responses were received. This problem may be solved by means of the internet, which can now be used to aid data collection from the public, providing a quick and easy method of contacting participants and potentially generating higher response rates than previously achieved. Use of the internet for public surveys also allows more general advertisement of the project and easier access to materials required for participation.

Here, the results of a public bumblebee nest survey conducted in 2007 and 2008 are presented and compared to those of similar surveys conducted in 2004 (Osborne et al., 2007) and 1989-1991 (Fussell and Corbet, 1992). This comparison allows assessment of changes in species' relative contributions to nest records and species-specific differences in the positioning of nests, providing an indication of changes in the composition and nesting ecology of bumblebee populations in the urban environment over the past 20 years.

5.3 Methods

5.3.1 Survey Methods

In 2007, members of the public were asked to send bumblebee records to the Bumblebee Conservation Trust as part of the BeeWatch 2007 recording scheme. As a result of this appeal, 165 bumblebee nests were reported from urban and suburban gardens. Recorders reporting nests were asked to provide information regarding the species of bumblebee present and the type of nest site being used.

As a result of the success of the 2007 survey, a specific nest survey was run through the Bumblebee Conservation Trust in 2008. A nest survey form (appendix 1) was provided online or by post on request. Participants were asked to record the identity of the bumblebee colony to species level. If unsure, they were directed to identification guides provided on the Bumblebee Conservation Trust website and were encouraged to provide a photograph so that identification could be verified.

In 2008, all participants that had reported a nest in the previous year were asked to report on the status (occupied/unoccupied/damaged) of the nest site that year. If another colony was discovered in the same location, participants were asked to report the species that the new colony belonged to.

5.3.2 Statistical analysis

Wherever the observed numbers of nests allowed, Pearson's chi-square tests were used to compare species-specific differences in the sites in which nests were found and also to compare the results of the current survey with those of Fussell and Corbet (1992) and Osborne et al. (2007). In order to allow comparison between the three studies, sites in which nests were found were grouped into five different 'nest environment' categories based on those used by Fussell and Corbet (1992). These were grasses (defined as any nest reported to be discovered either in grass or in a hole in grass), stone (defined as any nest reported to be associated with stone or stone structures not directly part of buildings), wood (defined as any site described to be in association with trees or in a cavity constructed from wood such as a bird box), buildings (any site directly associated with human occupancy such as houses, garages or sheds) and other (any sites that did not fall into the previous categories). Since records from these previous studies were collected primarily in South East

England, all comparisons among studies were carried out using only data collected from this region in order to eliminate any effects of geographic location. *Bombus hypnorum, B. muscorum, B. jonellus* and *B. humilis* were excluded from all analyses due to low representation of these species. Where there was one degree of freedom, Yates' continuity correction was applied. Where data were available (species representation and nest environment), data were combined across 2007 and 2008 but for all other analyses, data referred to were collected in 2008 alone.

5.4 Results

5.4.1 Differences in species representation

In 2007, 165 nests were reported by 157 independent recorders distributed across the United Kingdom. One hundred and twelve of these were identified to species level and a further 14 were identified as two-banded white tails (most likely to be either *B. lucorum* or *B. terrestris*). In 2008, 354 nests were reported by 327 independent recorders. Of these 349 were recorded to species level and 5 were recorded as two-banded white-tails. The distribution of the records among major regions of the UK are presented in table 5.2. Sixty percent of records across the two years were of nests belonging to either *B. lucorum* or *B. terrestris*. *Bombus lapidarius* and *B. pascuorum* made up 11% of records each and *B. hortorum*, *B. pratorum* and *B. hypnorum* made up 9%, 7% and 3% of observations respectively. Three of the nests recorded in 2008 belonged to species that are uncommon in the UK urban environment (*B. muscorum*, *B. jonellus* and *B. humilis*).

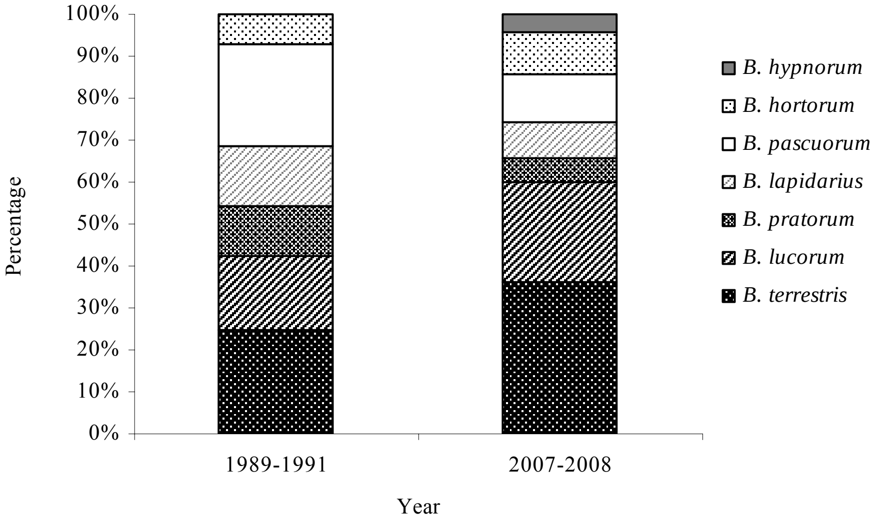
Table 5.2: Percentage of bumblebee nest records reported by members of the British public in 2007 and 2008 located in each of the major regions of Great Britain

Location	British National Grid Letters	Percentage of nest records in:	
		2007	2008
South-east England	SK, SP, SU, SZ, TF, TG, TL, TM, TQ, TR, TV	57.0	56.1
South-west England and Wales	SH, SJ, SM, SN, SO, SR, SS, ST, SV, SW, SX, SY	18.3	21.6
Northern England	NU, NY, NZ, OV, SD, SE, TA	15.1	9.8
Scotland	NA, NB, NC, ND, NF, NG, NH, NJ, NK, NL, NM, NN, NO, NR, NS, NT, NW, NX	9.7	12.5

The contribution of each species to the total number of nests recorded differed between 2007 and 2008 ($\chi^2_5 = 12.87$, $p = 0.03$). *Bombus lucorum*, *B. pratorum* and *B. hortorum* did not differ between the two years, but the proportion of colonies identified as *B. terrestris* increased from 26% of observations to 40% of observations between 2007 and 2008 whilst the proportion of colonies of *B. lapidarius* reported decreased from 18% to 9%.

Fussell and Corbet (1992) received 244 records of bumblebee colonies that were identified to species level. When data for 2007 and 2008 (South East England only) were pooled and compared to these data, a significant difference in species composition was observed between the time periods ($\chi^2_5 = 29.47$, $p < 0.001$). The proportion of *B. pascuorum* and *B. pratorum* nests reported decreased between the two studies whilst the proportion of *B. lucorum* and *B. terrestris* nests increased (figure 5.1). The recently invaded *B. hypnorum* was also represented in the current study although the relative contribution of this species was low.

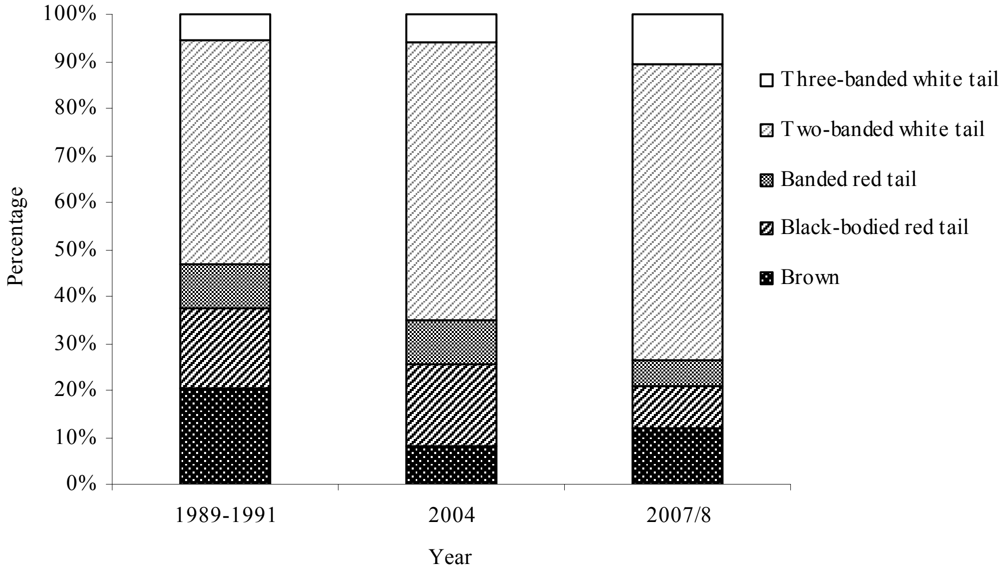
Figure 5.1: Percentage of nests belonging to different bumblebee species discovered by members of the public in 1989-1991 and 2007-2008 (latter includes records collected in South East England only)



Osborne et al. (2007) required only that survey participants identify their colonies to colour-group and several of the 1992 records were also assigned to colour group only. When the results from the three surveys were divided by colour group and compared, there were also significant differences in species composition between the three time periods ($\chi^2_8 = 39.24$, $p < 0.001$).

Browns (mostly *B. pascuorum*) were reported relatively less often in both modern studies whilst two-banded white tails (mostly *B. terrestris* and *B. lucorum*) were reported more often (figure 5.2). Banded red tails (mostly *B. pratorum*) and black-bodied red tails (mostly *B. lapidarius*) were reported relatively less often in the current study than in either of the previous studies whilst three-banded white tails (mostly *B. hortorum*) and two-banded white-tails were reported more commonly (figure 5.2).

Figure 5.2: Percentage of nests belonging to different bumblebee colour groups discovered by members of the public in 1989-1991, 2004 and 2007-2008 (latter includes records collected in South East England only)



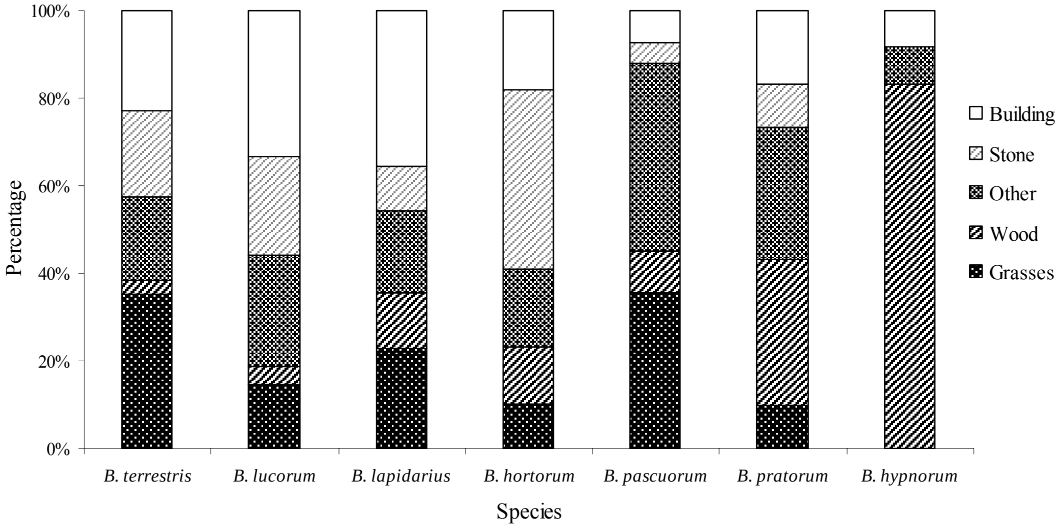
5.4.2 Site type and nest environment

In the 2007-2008 survey bumblebees were reported nesting in a wide range of different sites (table 5.3). When split into the nest environment categories stone, wood, grass, building or other there was evidence for species-specific differences (figure 5.3). *Bombus lapidarius* was often associated with buildings whilst *B. pascuorum* was often found nesting in association with grass, as well as with ‘other’ materials (most commonly rotting vegetation such as compost or grass clippings – table 5.3). *Bombus hortorum* was regularly found in association with stone whilst *B. pratorum* was commonly found in association with wood or ‘other’ materials (usually compost – table 5.3). *Bombus hypnorum* was almost always discovered in association with wood and was frequently discovered nesting in bird boxes (table 5.3).

Table 5.3: The numbers of different bumblebee species found nesting in different site types by members of the public in 2007 and 2008 grouped by the type of environment with which the nest is associated (grasses, stone, wood, buildings or other). Numbers in brackets are percentages of the total nests of each species discovered. (*B. hort* = *B. hortorum*, *B. hyp* = *B. hypnorum*, *B. lap* = *B. lapidarius*, *B. luc* = *B. lucorum*, *B. pasc* = *B. pascuorum*, *B. prat* = *B. pratorum* and *B. terr* = *B. terrestris*)

Nest environment	Site type	*B. hort*	*B. hyp*	*B. lap*	*B. luc*	*B. pasc*	*B. prat*	*B. terr*	Total
Grasses	base of long grass	0 (0)	0 (0)	0 (0)	0 (0)	10 (24)	1 (3)	1 (1)	12 (3)
	hole in long grass	0 (0)	0 (0)	3 (6)	4 (4)	3 (7)	2 (7)	16 (10)	28 (6)
	hole in short grass	4 (10)	0 (0)	8 (17)	11 (11)	2 (5)	0 (0)	40 (25)	65 (15)
Stone	stone	3 (8)	0 (0)	1 (2)	1 (1)	1 (2)	0 (0)	3 (2)	9 (2)
	wall	6 (15)	0 (0)	4 (8)	10 (10)	0 (0)	0 (0)	13 (8)	33 (8)
	concrete slab	7 (18)	0 (0)	0 (0)	12 (12)	1 (2)	3 (10)	16 (10)	39 (9)
Wood	tree stump	1 (3)	0 (0)	0 (0)	2 (2)	1 (2)	0 (0)	0 (0)	4 (1)
	hole in tree	0 (0)	2 (17)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	3 (1)
	bird box	4 (10)	8 (67)	6 (13)	2 (2)	0 (0)	9 (33)	3 (2)	33 (8)
	bumblebee box	0 (0)	0 (0)	0 (0)	0 (0)	3 (7)	0 (0)	1 (1)	4 (1)
Buildings	eves of house	1 (3)	1 (8)	1 (2)	0 (0)	0 (0)	0 (0)	2 (1)	5 (1)
	air brick	0 (0)	0 (0)	5 (10)	2 (2)	0 (0)	0 (0)	2 (1)	9 (2)
	in building	3 (8)	0 (0)	5 (10)	9 (9)	2 (5)	1 (3)	16 (10)	36 (8)
	under building	3 (8)	0 (0)	6 (13)	19 (19)	1 (2)	4 (13)	14 (9)	47 (11)
	wooden decking	0 (0)	0 (0)	0 (0)	4 (4)	0 (0)	0 (0)	3 (2)	7 (2)
Other	compost heap	3 (8)	0 (0)	3 (6)	8 (8)	4 (10)	3 (10)	10 (6)	31 (7)
	flower bed	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	1 (<1)
	grass clippings	0 (0)	0 (0)	1 (2)	2 (2)	4 (10)	0 (0)	1 (1)	8 (2)
	home-made domicile	2 (5)	0 (0)	0 (0)	1 (1)	1 (2)	0 (0)	0 (0)	4 (1)
	leaves	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	3 (2)	4 (1)
	metal	0 (0)	0 (0)	0 (0)	1 (1)	1 (2)	0 (0)	1 (1)	3 (1)
	moss	0 (0)	0 (0)	1 (2)	1 (1)	2 (5)	0 (0)	0 (0)	4 (1)
	pile of turfs	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (<1)
	pine needles	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (<1)
	plastic compost bin	2 (5)	1 (8)	1 (2)	12 (12)	1 (2)	3 (10)	8 (5)	28 (6)
	plastic sheeting	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	2 (1)	3 (1)
	polystyrene	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (<1)
	under bush	0 (0)	0 (0)	2 (4)	1 (1)	3 (7)	1 (3)	4 (2)	11 (3)
	wooden compost bin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	1 (<1)
Total		39	12	48	102	42	30	162	435

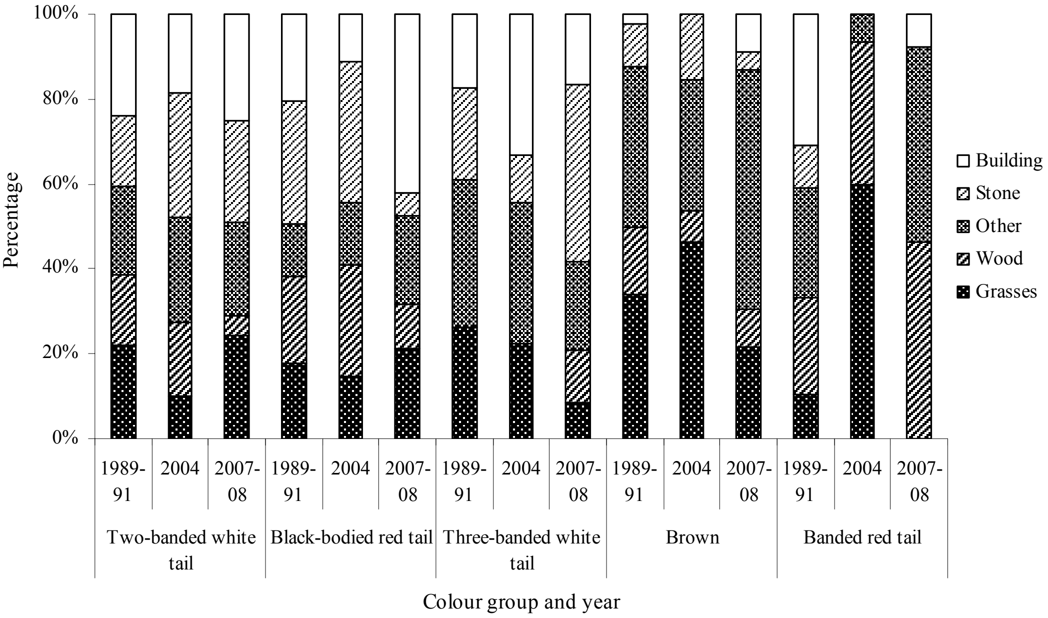
Figure 5.3: Percentages of nests belonging to different bumblebee species discovered in association with different material types by members of the public in 2007 and 2008



The only species that were represented in sufficient numbers to allow statistical comparison of nest environments were *B. terrestris* and *B. lucorum*. Since these species were rarely found nesting in association with wood (3% of the total nests observed for these species), nests records belonging to the ‘wood’ nest # environment category were combined with those belonging to the ‘other’ nest environment category for the analysis. Significant differences were observed in the environments with which the nests of *B. terrestris* and *B. lucorum* were associated ($\chi^2_3 = 13.72$, p = 0.003). *Bombus terrestris* was more commonly found nesting in association with grasses (usually in holes in long or short grass – table 5.3), whilst *B. lucorum* was found more often in association with buildings, and with ‘other’ habitat types (usually compost heaps – table 5.3).

Comparing the current data with that of the previous studies, the distributions of nests of each species between materials were similar (figure 5.4). The distribution of nests of three-banded white tails (most commonly *B. hortorum*) between materials did differ from study to study though low numbers of these species were reported in all. Similarly, banded red tails (mostly *B. pratorum*) differed dramatically in nest association between the studies (figure 5.4). In this study, black-bodied red tails (mostly *B. lapidarius*) were more commonly found in association with buildings and less commonly with stone than the previous two studies.

Figure 5.4: The percentage of nests belonging to each colour group of bumblebee discovered in association with different materials in 1989-1991, 2004 and 2007-2008 (latter includes records collected in South East England only).

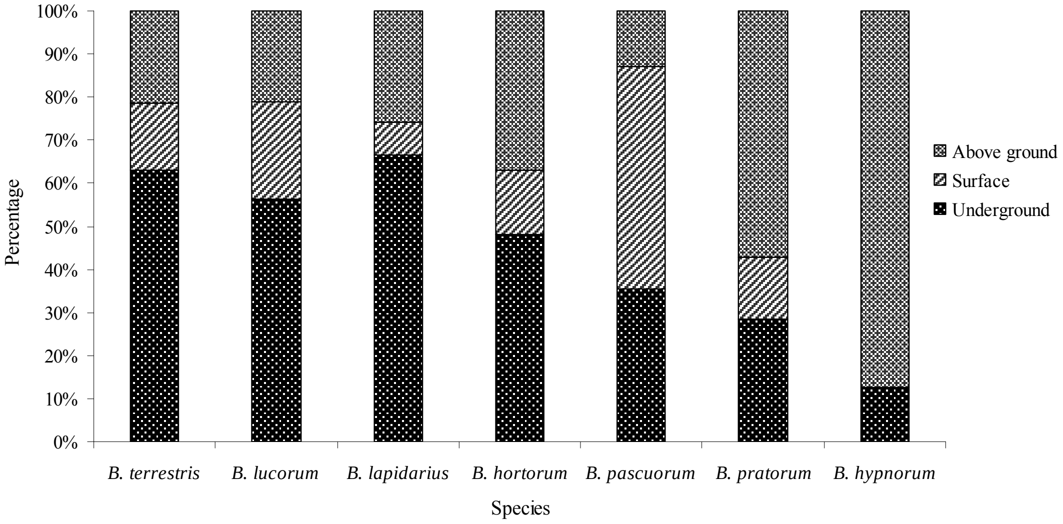


Pooling the data across species, no difference was observed in nest distribution among nest environment types ($\chi^2_8 = 10.67$, p = 0.221).

5.4.3 Nest positioning

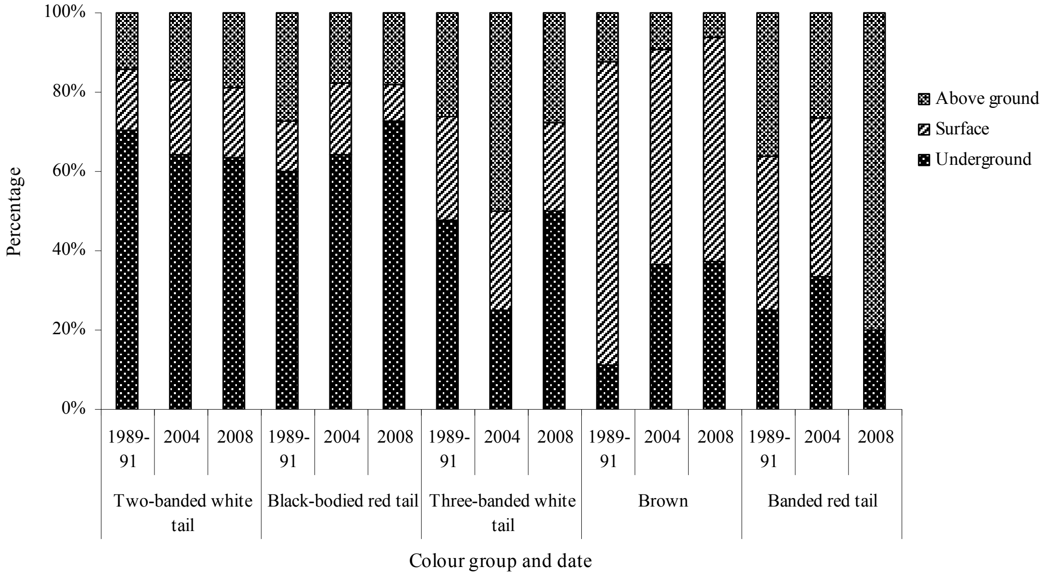
In 2008, species-specific differences were found in the positions in which nests were discovered ($\chi^2_{10} = 40.57$, $p < 0.001$; figure 5.5). The nests of *B. terrestris*, *B. lucorum* and *B. lapidarius* were most commonly underground, whilst nests of *B. pascuorum* were more often on the ground surface and nests of *B. pratorum* were frequently above the ground. *Bombus hypnorum* was almost always discovered in above ground positions (7 out of the 8 nests recorded).

Figure 5.5: Percentages of nests of different bumblebee species discovered above the ground, on the surface of the ground or beneath the ground by members of the public in 2008.



The positions in which different colour groups were found nesting were very similar in this study to previous studies (figure 5.6). However, in this study and that of Osborne et al. (2007), browns (generally *B. pascuorum*) were found nesting under the ground more commonly and on the ground surface less commonly than in 1989-1991 and in this study, black-bodied red tails (generally *B. lapidarius*) were found nesting above ground more regularly and on the surface of the ground less regularly than in the 1989-1991, although this was not found by Osborne et al.

Figure 5.6: The percentage of nests of different colour groups of bumblebee discovered above the ground, on the surface of the ground or beneath the ground in 2008 (including records collected in South East England only), 2004 and 1989-1991



Combining all colour-groups, there was a significant difference in nest position between the three time periods ($\chi^2_4 = 13.85$, $p = 0.008$), but no significant difference between the 2004 and 2008 studies ($\chi^2_2 = 1.94$, $p = 0.380$). In 2004 and 2008, nests were found more commonly in underground locations and less commonly on the ground surface than in the 1989-1991 survey.

5.3.4 Previous occupancy by small mammals

A total of 212 people claimed to know whether or not their nest site had been used by any other animal the previous year, and 40% of these responded positively. Where nest sites were reported as having previously been occupied by another animal, nests of *B. terrestris*, *B. lucorum* and *B. lapidarius* were most often reported to be located where there had been rodents the previous year whilst *B. pratorum* and

B. hypnorum were most often reported to be nesting in old bird nests (table 5.4). Neither *B. pascuorum* nor *B. hortorum* were generally recorded nesting in a site that had previously been occupied by another animal and in one instance in which *B. pascuorum* was reported nesting in an old bird nest, the bird nest material had been relocated to a bumblebee nesting box prior to occupation by the colony. Five participants reporting bumblebee colonies (three *B. pratorum*, one *B. hypnorum* and one *B. terrestris*) in old bird nests reported that the birds had been in the boxes the same year.

Table 5.4: Numbers of nests of different bumblebee species discovered by members of the public in 2008 reported to be nesting in sites that had previously been occupied by other animals. Numbers in brackets are percentages of the total number of nests of each species for which data regarding previous occupancy were provided.

	Bombus terrestris	*Bombus lucorum*	*Bombus pratorum*	*Bombus lapidarius*	*Bombus pascuorum*	*Bombus hortorum*	*Bombus hypnorum*	Total
Bird	1 (1)	2 (5)	7 (39)	1 (5)	2 (11)	2 (13)	5 (83)	20 (9)
Mouse/vole	27 (31)	8 (18)	4 (22)	7 (32)	1 (5)	2 (13)	0 (0)	49 (23)
Rat	1 (1)	4 (9)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)	6 (3)
Rabbit	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)
Hedgehog	3 (3)	2 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (2)
Toad	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (<1)
Wasp	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (<1)
Mole	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (<1)
No animal	54 (61)	25 (57)	7 (39)	14 (64)	15 (79)	11 (73)	1 (17)	127 (60)

5.4.5 Previous occupancy by bumblebees (from questionnaire)

A total of 196 participants reported knowledge of whether or not there had been bumblebees nesting at the site of the current nest in the previous year. Of these, 17 (9%) participants reported that there had been bumblebees in the same site the previous year and one (0.5%) reported that there had been a nest in the same site two years before but not the last year. Two participants (1%) reported that there had been a colony close to the same site but not in exactly the same location.

Seven (41%) of the participants reporting consecutive occupancy identified the species as the same as the year previously and two (12%) identified the species as different. The remaining eight (47%) were unsure as to which species had nested at the site in the previous year. Two reports of consecutive occupancy were of nests of *B. hortorum* (the same, *B. hortorum,* and an unknown species having nested in these sites the year before), one was of a nest of *B. lapidarius* (a nest of *B. terrestris* having been observed at the same site in the previous year), four were of nests of *B. lucorum* (two sites of which had been occupied by unknown species the year before, one by the same species, *B. lucorum*, and one by *B. pratorum*), one was of a nest of *B. pascuorum* and one of a nest of *B. pratorum* (both of which had hosted colonies of an unknown species the year previously) and eight were of nests of *B. terrestris* (five sites of which were known to have hosted the same species the previous year and three of which had hosted colonies of unknown species). The two nests reported to have been close to known nests from the previous year belonged to *B. lapidarius* and *B. hortorum*, but the species of the original colonies were unknown. The nest site reported to have hosted a bumblebee colony two years previously was occupied by *B. terrestris*, but the species identity of the original colony was unknown.

5.4.6 Consecutive occupancy (from follow up survey)

Of the 165 nests reported in 2007, 92 (56%) responses were received regarding the status (occupied/unoccupied/damaged) of the nest site in the following year. Of these, 19 (21%) were reported to be unusable by bees. Five (3%) nest sites were reported as reoccupied by bumblebees, four (2%) participants reported colonies close to the original nest and six participants (4%) reported observing nest site searching bumblebee queens around the entrance to the original nest site. Nine participants (5%) reported that their colonies had died off prior to reproductive production the previous year (five due to flooding, one due to accidental destruction by the participant, two due to infestation by wax moths (*Aphomia sociella*) and one by unknown causes). One out of these nine participants was also one of the participants reporting a colony close to the original nest site.

Of the five nest sites reported to have been reoccupied, one of the original nests belonged to *B. lucorum*, two belonged to *B. terrestris*, one belonged to an unidentified two-banded white tail (probably *B. terrestris* or *B. lucorum*) and one was unidentified. In two cases (the *B. lucorum* nest site and one of the *B. terrestris* nest sites) participants reported re-occupancy by bees of the same species but in the other three cases, the species' identity of the nests in the following year were unknown. Of the four nests founded close to an original nest site, one of the original sites had belonged to *B. terrestris*, two to two-banded white-tails (likely to be *B. terrestris* or *B. lucorum*) and one to an unidentified species. None of these participants reported whether the new nest belonged to the same species as the original colony. The six nest sites near which participants observed nest site searching queens consisted of two nests of unknown species and one each of *B. terrestris*, *B. lucorum*, *B. hypnorum* and *B. pascuorum*. The queens observed searching in the vicinity of the *B. pascuorum*, *B. lucorum* and *B. hypnorum* nest sites were all reported to be the same species as had occupied the sites in the previous year. The species identity of the queen searching close to the site of the *B. terrestris* nest was unknown.

Of the 432 nest records in Fussell and Corbet (1992), information on consecutive occupancy was only available for approximately 7%. Of these, 30 sites were reported to be empty the subsequent year, one was reported to have been re-occupied by the same species (*B. pascuorum*) and five participants reported nests of the same species in close proximity to the original nest site (two two-banded white tails and three *B. pascuorum*). It is notable that the re-occupied site had been cleared and the bedding removed and replaced between the two years.

5.5 Discussion

5.5.1 Species-specific differences in proportionate abundance of nests over time

Between 2007 and 2008, the relative abundance of most species observed remained relatively constant but differences were observed for *B. terrestris* and *B. lapidarius. Bombus lapidarius* showed a decrease in relative abundance from 2007 to 2008. This species is known to be particularly sensitive to bad weather and is generally rarer in wet years (Sladen, 1912). Since rainfall was high in 2008 (http://www.metoffice.gov.uk), this susceptibility might explain the relatively low proportion of nests of this species discovered in 2008. This would also explain the reduction in black-bodied red tails (mostly *B. lapidarius*) reported in this study compared to both the 2004 and the 1992 studies. Conversely, *B. terrestris* increased in relative abundance between 2007 and 2008. *Bombus terrestris* is a very robust species, as is demonstrated by its successful invasion of many parts of the world outside its native range following introduction by man (Hopkins, 1914; Semmens et al., 1993; Inari et al., 2005). The relatively high representation of this species in 2008 may reflect a greater resistance to unfavourable weather compared to that of the other species observed.

The relative abundance of two-banded white tail nests (*B. terrestris* and *B. lucorum*) was higher in both this study and in Osborne et al. (2007) than in Fussell and Corbet (1992), whilst nests belonging to browns (*B. pascuorum*) were reported proportionately less frequently. The sites in which browns were found nesting also varied between the studies with the proportion of nests of *B. pascuorum* found on the ground surface being lower and the proportion of nests found below the ground surface higher in the two recent surveys than in 1989-91. The summers of 1989-91 were considerably drier than those during 2004 and 2007-2008 (http://www.metoffice.gov.uk) so it is possible that this difference represents a greater number of surface nests becoming washed out before they were large enough to be observed by a recorder. This would also explain the generally lower representation of this species in the later studies. It is unclear whether this pattern reflects a general decline in this common species and/or a greater propensity to nest beneath the ground surface, or if these are just coincidental effects of differences in weather patterns during the years in which the studies were carried out.

B. pascuorum belongs to the bumblebee sub-genus Thoracobombus which is represented in the UK by five native species (*B. pascuorum*, *B. muscorum*, *B. humilis*, *B. sylvarum* and *B. ruderarius* – Alford, 1975). Of these, *B. pascuorum* is the only species that has not demonstrated significant reductions in range and abundance in recent years. These species are characterised by mid to long tongue lengths when compared to other bumblebee species, a characteristic which has been linked to proneness to decline in bumblebee species in the UK (Goulson et al., 2005). However, the majority of this sub-genus also build their nests on the ground surface, perhaps making them more susceptible to bad weather, ground disturbance or other environmental perturbations and it is possible that this aspect of their ecology has contributed to their declines. Most British species belonging to the Thoracobombus have always existed in scattered populations and/or had restricted ranges within the UK (Sladen, 1912) but *B. pascuorum* has always been common and ubiquitous throughout the British Isles. It is possible that this species is suffering the same fate as its sister species but that its declines have been masked by its initial high abundance. Even if this is not the case and the patterns observed here are simply the result of poor weather during recent surveys, many climate change models predict alterations in rainfall regimes across the UK in future years (e.g. Jones and Reid, 2001; Ekstrom et al., 2005) so that sensitivity to poor weather may become more important for predicting survival of different species under new climatic regimes.

B. pascuorum is the only common species remaining in the UK with a medium tongue length (Goulson et al., 2005) and for this reason declines of this species are of particular ecological and economic concern. Tongue-length in bumblebees is strongly linked to forage choice, with different species tending to select forage plants with corolla lengths corresponding to that of their tongues (Ranta and Lundberg, 1980; Harder, 1985). In order to ensure effective pollination services, it is therefore important that bumblebee assemblages consist of a range of species of differing tongue-lengths. *Bombus pascuorum* is known to be an important pollinator of crop plants such as field bean (Free and Williams, 1976) and is also likely to be important for the pollination of many wildflower species in the UK.

5.5.2 Species-specific nest site preferences

The sites in which bumblebees were found nesting were consistent with known preferences of different bumblebee species, specifically that *B. terrestris*, *B. lucorum* and *B. lapidarius* tend to nest underground and that *B. pascuorum* often nests on the ground surface in grasses (Sladen, 1912; Cumber, 1953; Alford, 1975; Fussell and Corbet, 1992). It has also previously been reported that *B. lapidarius* shows a tendency toward nesting in association with stone (Fussell and Corbet, 1992). In this study, *B. lapidarius* was found relatively less frequently in association with stone than in the previous surveys, but it was often found in or under buildings, which are likely to create similar environmental conditions. *Bombus lapidarius* is known to have a high temperature threshold for activity compared to other British bumblebee species (Prys-Jones and Corbet, 1991), so that the heat reservoir effect of stone and the warmth associated with many building types probably constitute favourable conditions for this species (Fussell and Corbet, 1992). *B. pratorum* is believed to be very generalist in its nest site choice (Sladen, 1912) and this may explain the large amount of variation in the location of nests of this species across the studies.

B. pratorum and the recently established *B. hypnorum* were very similar in their choices of nest site positioning. Both species were most commonly found nesting in association with wood, in aerial locations, and often in bird boxes. This is consistent with the findings of others relating to *B. pratorum* in the UK (Sladen, 1912; Alford, 1975) and of *B. hypnorum* in Europe (Hasselrot, 1960). Since bird boxes are generally closely monitored, it is possible that these species might be over-represented in public surveys. However, even with this possible bias, the number of *B. hypnorum* nests recorded was low suggesting that, despite its apparent success and rapid spread across the UK since its discovery in 2001 (Goulson and Williams, 2001; Edwards and Jenner, 2005), this species cannot yet be described as common in Britain. *Bombus hypnorum* is not recognised by the colour group system of identification used by Osborne et al. so it is impossible to compare representation of this species across studies. Of the records compiled by Osborne et al., 16% were attributed to unknown colour groups, which was very much higher than the proportion of records attributed to unknown colour groups in Fussell and Corbet (1992). It is possible that many of these modern unidentified records were of nests belonging to *B. hypnorum*.

Differences in the ecology of *B. terrestris* and *B. lucorum* are rarely reported because most studies rely on observations of workers which are extremely difficult to distinguish reliably in the field. Since these two species are closely related, combining them in ecological studies is generally considered to be reasonable, yet it could be argued that by doing this, much useful information is lost. The ability of these two species to coexist suggests there is an ecological mechanism by which competitive interactions between them are reduced. Sladen (1912) observed differences in the nest sites of these two species, noting that *B. terrestris* preferred to nest in subterranean cavities with very long entrance tunnels whilst *B. lucorum* was generally found in cavities accessed by shorter entrance tunnels. Here, both species were recorded in a wide range of site types, but the data suggest that *B. lucorum* may be more flexible in the type of nest site it will accept. *Bombus terrestris* was more commonly discovered in holes in grass, whilst *B. lucorum* was observed as often in other site types and in particularly, was commonly observed nesting in association with buildings. However, it should be noted that recent research has demonstrated that individuals described as *B. lucorum* in Europe can belong to one of three cryptic species (*B. lucorum*, *B. magnus* or *B. cryptarum* – Murray et al., 2008) so that the increased variation in nest location observed in '*B. lucorum*' compared to *B. terrestris* may simply represent differences in nest site preference among these cryptic species.

5.5.3 Previous occupancy by small mammals or birds

It has been suggested that small mammals are important in providing nest sites for bumblebees since nests are often discovered in the abandoned homes of such species (Svensson and Lundberg, 1977; Donovan and Weir, 1978). However, it has also been shown that it is not a requirement as long as the right conditions, such as a sheltered cavity and suitable nest material, are fulfilled (Hobbs et al., 1960). In this study, *B. lapidarius*, *B. terrestris* and *B. lucorum* were all reported nesting where small mammals had nested in previous years, but the majority of nest records for all three species were from sites that had not been previously occupied by mammals. This is surprising since most bumblebee species are unable to gather their own nest material. It is notable that many nests that were reported not to have been home to another animal the previous year were holes in the ground that strongly resembled burrows of small mammals. It is possible that some recorders were simply unaware of the presence of previous occupants since such animals are generally active at night.

In this survey, birds were also found to provide nest sites for bumblebees. It has been suggested that the number of bumblebee nests founded in aerial locations may be underestimated (Richards, 1978) and it is therefore possible that the importance of birds in nest site provision has been underestimated. A recent study of *B. niveatus* behaviour demonstrated that this species will specifically invade nests of the common redstart (*Phoenicurus phoenicurus*), a behaviour that results in the abandonment of the nest by the bird (Rasmont et al., 2008). Similarly, *B. hypnorum* has occasionally been reported ousting tits (*Parus* spp.) from their nests (pers. com. to Rasmont et al., 2008). In this study *B. hypnorum* and, to a lesser extent, *B. pratorum* appear to utilise bird nests on a regular basis, and several other species also occasionally occur where birds have previously nested. Five participants reported bumblebee colonies in previously active bird nests and one participant reported temporary co-existence of wrens and a bumblebee colony until the wrens were ‘seen off’ by the bumblebees. This may suggest that ‘ousting’ behaviour is not specific to *B. niveatus* but might also be demonstrated by other species.

5.5.4 Consecutive occupancy

Although consecutive occupancy has been reported by a number of authors (Hobbs et al., 1962; Donovan and Weir, 1978; Barron et al., 2000) nest survey data do not provide strong evidence to support the theory of preferential reoccupation of nest sites by bumblebees. Reports of consecutive occupancy were relatively rare, both in the current study and in that of Fussell and Corbet (1992).

If consecutive occupancy is due to new queens returning to found a nest near the site of their maternal nest, old and new colonies should belong to the same species and this was generally the case in this study and that of Fussell and Corbet (1992). However, consecutive occupancy may occur because there are a finite number of suitable nest sites available for bumblebees. For example, in a garden habitat, density of bumblebee queens and colonies is likely to be high resulting in a requirement for many nest sites. Thus, colonies founded at the same site or in close proximity in consecutive years would be expected by chance. If this were the case, the presence of the same species in the same location from year to year is likely to be a result of species-specific differences in the effects of microhabitat on bumblebee nest site choice and colony survival. Given the low rates of re-occupancy observed, this seems to be the most plausible explanation.

5.6 Conclusions

Harnessing the enthusiasm of large numbers of amateur volunteers enabled collection of data regarding nest locations, consecutive occupancy of nests, and relative abundance of nests of different bumblebee species in urban sites across the UK. The results largely confirm the known nesting preferences of bumblebees, and demonstrate that man-made structures provide numerous nesting opportunities for bumblebees of a range of species. Data collected here provide little support for the notion that bumblebees preferentially nest in places where bumblebees have previously nested. Comparison of these data with earlier datasets suggest long-term changes in the relative abundance of the common UK bumblebees, with an increase in ‘two-banded white tails’ (*B. terrestris* and *B. lucorum*) and a decrease in ‘browns’ (largely *B. pascuorum*). This is consistent with the well-documented long-term declines of medium and long-tongued bumblebees relative to short-tongued species and is of concern since it suggests that the only remaining widespread bumblebee species of medium tongue length may be in decline.

5.7 Acknowledgements

I would like to thank all members of the public who took part in the survey for their time, enthusiasm and willing cooperation. I would also like to thank staff and volunteers at the Bumblebee Conservation Trust for help with the advertisement and processing of nest surveys. Thanks especially to Bob Dawson, Ben Darvill, Emma Heskey, Natasha Rolf, Linnea Bergstrom, Steph O’Connor and Chrissie Nitsch for their support. Finally, I would like to thank the University of Stirling for access to facilities and the Natural Environment Research Council and the Game and Wildlife Conservation Trust for financial support.

Appendix 5.1: Public survey form used to collect data on attributes of bumblebee nests found in garden habitats

Bumblebee Conservation Trust Nest Survey 2008

Recorder	**Postcode/National Grid Reference**	**Altitude (metres above sea level - if known)**

1. When did you first notice your nest and roughly how frequent was the bee traffic in and out of the nest (e.g. just the queen/a couple of bees in ten minutes/five bees per minute etc.)?

2. Which species does your nest belong to?

Buff-tailed bumblebee (*Bombus terrestris*) ☐
White-tailed bumblebee (*Bombus lucorum*) ☐
Common carder bumblebee (*Bombus pascuorum*) ☐
Early bumblebee (*Bombus pratorum*) ☐
Red-tailed bumblebee (*Bombus lapidarius*) ☐
Garden bumblebee (*Bombus hortorum*) ☐
Tree bumblebee (*Bombus hypnorum*) ☐
Other (please specify)

3. How many entrance holes are being used by the bees (that you know of)?

1 ☐
2 ☐
3 ☐
more than 3 ☐

4. What direction(s) do(es) the entrance to the nest face in?

North facing ☐
North-East facing ☐
East facing ☐
South-East facing ☐
South facing ☐
South-West facing ☐
West facing ☐
North-West facing ☐
Hole faces directly upwards ☐

5. Which of the following best describes the position of your nest?

☐ Under the ground
☐ On the surface of the ground
☐ Raised above the ground

6a. Which of these best describes the site of your nest?
(You may tick more than one box)

☐ In an air brick
☐ In a 'home-made' artificial nest site
☐ At the base of long grass
☐ In a bird box
☐ In a commercial bumblebee box
☐ In a compost heap
☐ In a plastic compost bin
☐ In dry vegetation - moss
☐ In dry vegetation - grass clippings
☐ In dry vegetation - pine needles
☐ In dry vegetation - dry leaves
☐ In the eaves of a house
☐ In a building (e.g. a garden shed/garage)
☐ In a hole in a tree
☐ In a hole in the ground in short grass e.g. a lawn
☐ In a hole in the ground in long grass
☐ In a cavity between pebbles/stones
☐ In a cavity in a wall
☐ Under a tree stump
☐ Under a building (e.g. a garden shed/garage)
☐ Under a bush
☐ Under concrete e.g. a concrete slab
☐ Under plastic sheeting
☐ Under wooden decking

Other (please give details) ☐

6b. Please use this space to include any other details of the nest site which might be useful to us e.g. the types of nest material being used by the bees, if in an artificial nest box, details of the design of the box etc.

7. Would you say that your nest was closely associated with (within 1 foot/30cm of) a linear landscape feature e.g. fence, hedge etc. and if so, what type?

8. Which of the following best describes the amount of shade that your nest receives?

Always shaded from the sun ☐

Shaded only in the morning (afternoon exposure to sun) ☐

Shaded only in the afternoon (morning exposure to sun) ☐

Never shaded from the sun ☐

9. On which sides is of your nest is there shelter from the wind?
(Tick more than one box if necessary)

North ☐

North-East ☐

East ☐

South East ☐

South ☐

South-West ☐

West ☐

North-West ☐

10. Has the cavity being used by the bees previously been occupied by small mammals/birds?
(If yes, please go to question 11. If no, proceed to question 12.)

Yes ☐

No ☐

Don't know ☐

11. What species of small mammal has previously used the cavity (if known)?

12. Was the cavity being used by the bees occupied by bumblebees last year?
(If yes, please go to question 13. If no, proceed to question 14.)

Yes ☐ No ☐ Don't know ☐

13. Which species of bumblebee previously nested in the cavity (if known)?

14. Which of these flowers do you have in your garden?
(Tick as many boxes as needed)

- ☐ Antirrhinum
- ☐ Apple
- ☐ Aquilegia
- ☐ Azalea
- ☐ Birds-foot trefoil
- ☐ Bluebell
- ☐ Broad beans
- ☐ Broom
- ☐ Bugle
- ☐ Bush vetch
- ☐ Camellia
- ☐ Campanula
- ☐ Ceanothus
- ☐ Cherry
- ☐ Chives
- ☐ Clematis
- ☐ Comfrey
- ☐ Cotoneaster
- ☐ Daffodils
- ☐ Escallonia
- ☐ Everlasting pea
- ☐ Everlasting wallflower
- ☐ Flowering currant
- ☐ Foxglove
- ☐ Fuchsia
- ☐ Geranium
- ☐ Green alkanet
- ☐ Hawthorn
- ☐ Heather (Erica)
- ☐ Honeysuckle
- ☐ Iris
- ☐ Kidney vetch
- ☐ Laburnum
- ☐ Lavender
- ☐ Lilac
- ☐ Lily

Lobelia ☐
Lupin ☐
Meadow cranesbill ☐
Monkshood ☐
Pansy ☐
Pear ☐
Penstemon ☐
Peony ☐
Philadelphus ☐
Pieris ☐
Plum ☐
Poppy ☐
Pulmonaria (Lungwort) ☐
Pussy willow ☐
Raspberry ☐
Red campion ☐
Red clover ☐
Red dead-nettle ☐
Rhododendron ☐
Rose (single-flowered varieties) ☐
Rosemary ☐
Sage ☐
Salvia ☐
Skimmia ☐
Snowdrop ☐
Solomon's seal ☐
Thyme ☐
Tufted vetch ☐
Tulip ☐
Viburnum ☐
Weigela ☐
White clover ☐
White dead-nettle ☐
Wisteria ☐
Woundwort ☐

15. Please use this space to list any other Spring flowering plants in your garden that may be attractive to bumblebees.

Thank you very much for taking part in our 2008 bumblebee nest survey!

Please send your completed form to:

beewatch@bumblebeeconservationtrust.co.uk

or

Bumblebee Conservation Trust,
School of Biological and Environmental Sciences,
University of Stirling,
Bridge of Allan,
FK9 4LA.

(These contact details may also be used for any queries regarding this form or if you would like to send us a photograph of one of your bees for identification)

Chapter 6

Assessing the value of Rural Stewardship schemes for providing foraging resources and nesting habitat for bumblebee queens (Hymenoptera: Apidae)

This chapter is an adapted version of ‘Lye, G.C., Park, K., Osborne, J., Holland, J. and Goulson, D. (2009) Assessing the value of Rural Stewardship schemes for providing foraging resources and nesting habitat for bumblebee queens (Hymenoptera: Apidae). *Biological Conservation* **142**, 2023-2032.

6.1 Abstract

Bumblebees (*Bombus* spp.) play a key role within agricultural systems as pollinators of crops and wild flowers. However, this taxon has suffered severe declines as a result of agricultural intensification. Conservation efforts largely focus on providing forage resources for bumblebees through the summer, but providing suitable habitat during the period of nest foundation in early spring could be a more effective method of boosting local bumblebee populations. This study assesses the attractiveness of three different farmland habitat types (hedgerow, field margin and grassland), and the relative merits of respective land management prescriptions under the Scottish Rural Stewardship Scheme to nest site searching and foraging bumblebee queens during the period of queen emergence and colony foundation. Hedgerows were the least attractive habitat type to spring queens. Rural Stewardship species-rich grassland comprised a complex vegetation structure which attracted nest site searching queens, whilst grassland that had been abandoned allowing natural regeneration contained more flowers, which attracted foraging queens. Field margin habitats were the most attractive habitat type, and Rural Stewardship field margins attracted both nest site searching and foraging queens at relatively high densities. This management option consisted of a sown grass mix, giving rise to the complex vegetation structure preferred by nest site searching queens, but regular disturbance allowed invasion by early flowering bumblebee forage plants. These findings suggest that it should be possible to develop simple combined management strategies to provide both suitable nesting sites and spring forage resources on farmland, promoting bumblebee colony foundation and therefore abundance in the agricultural environment.

6.2 Introduction

Agricultural intensification has caused the decline of many native plant and animal species in the UK and western Europe (Wilson et al., 1999; Donald et al., 2001). The drive towards self-sufficiency that followed the World Wars led to the destruction of vast areas of natural and semi-natural habitat to make way for large-scale and more intensively managed farmland. Such changes in countryside management have led to the loss of farmland biodiversity havens such as hedgerows and hay meadows, giving rise instead to a uniform rural landscape of large monocultures divided by simpler field boundary features (Stoate et al., 2001). In the UK, bumblebees (*Bombus* spp.) have suffered severe declines as a result of this agricultural intensification and it is widely accepted that these are directly related to declines in the wild flowers upon which they rely. It has been shown that many of the forage plants that bumblebees prefer have declined disproportionately (Carvell et al., 2006a), and that those species of bumblebee that have suffered the most severe declines tend to be those that display least plasticity in forage plant preferences (Goulson and Darvill, 2004; Goulson et al., 2005).

Bumblebees play a key role within agricultural systems, providing a pollination service that can increase yields of many flowering crops (Corbet et al., 1991). Many of the wildflower species associated with the rural environment also rely on bumblebee populations for survival (reviewed in Osborne and Williams, 1996). The provision of sufficient resources to support large, diverse bumblebee populations is therefore likely to provide both economic advantages and broader conservation benefits.

In recent years, an increasing awareness of the negative effects of intensive farming on native biodiversity has led to the implementation of a number of government-funded agri-environment schemes across Europe (Kleijn and Sutherland, 2003). One of the principal aims of these schemes is to restore and create areas of semi-natural habitat on farmland and thereby increase landscape heterogeneity. The management options presented in these schemes are often designed with target species in mind, and these commonly include game animals, beneficial invertebrates and rare arable plants. However, it is assumed that the improvement of farmland for these species will also provide benefits for a wider range of non-target flora and fauna. The value of these schemes across different taxa is widely debated, but many studies do indicate that certain schemes are of conservation value. For example, agri-environment prescriptions have been shown to benefit many insects, birds, small mammals and wildflowers (e.g. Marshall et al., 2006; MacDonald et al., 2007). One of the most popular forms of conservation management has been arable field margin management, and suitably managed field margins are recognised as havens for biodiversity (Marshall and Moonen, 2002).

The effects of field margin management options on bumblebee communities have been the focus of many studies in recent years, particularly in England, and it has been found that those options involving the sowing of annual or perennial wildflowers or agricultural cultivars of legume species can have positive effects on the abundance and diversity of foraging bumblebees (Carreck and Williams, 2002; Meek et al., 2002; Carvell et al., 2004, 2006b, 2007; Pywell et al., 2005, 2006). It has also been suggested that it may be possible to develop a management strategy that will combine high quality forage with nest site provision for bumblebees (Carvell et al., 2004). However, the suitability of these schemes for providing nesting habitat has not been evaluated, and almost all studies of agri-environment schemes and bumblebees to date have focused on populations of worker bees in the summer. Paradoxically, it is arguable that habitat quality in early spring may be the most important factor determining bumblebee abundance, for at this time of year queens first emerge after diapause and must find a suitable nest site and single-handedly rear the first cohort of workers (Goulson, 2003a).

The availability of sufficient nest sites is vital, yet this requirement is often overlooked. Little is known about bumblebee nest site preferences as nests are inconspicuous although broad species-specific differences are understood. For example in the UK, species such as *B. terrestris* and *B. lucorum* tend to nest under the ground whilst species such as *B. pascuorum* prefer to nest on the ground surface. In both cases there appears to be a strong tendency towards the use of abandoned nests of other small animal species such as small mammals or birds (Rasmont et al., 2008). Nest-searching bumblebees have been found to be associated with linear features such as hedgerows and woodland edges, and also with tall, tussocky grassland (Fussell and Corbet, 1992; Kells and Goulson, 2003). However, these habitat types have declined as a result of agricultural intensification and it is possible that this has resulted in increased competition for nesting sites. It is notable that the bumblebee species that have shown the greatest declines in the UK tend to be those that emerge from hibernation later in the year and their declines may be at least partially accounted for by an increase in competition for nesting sites, with surface nesters such as *B. muscorum* competing with the earlier emerging *B.*

pascuorum and subterranean nesters such as the late emerging *B. soroeensis* competing with earlier emerging *B. terrestris* and *B. lucorum*. Indeed, a recent study in the USA has shown that bumblebee abundance in urban parks is limited by nest site availability (McFrederick and LeBuhn, 2006).

The availability of forage in close proximity to the nest must also be crucial in spring. The bumblebee queen must incubate the brood clump, so it seems unlikely that queens are able to embark on lengthy foraging trips (Cresswell et al., 2000). A recent study in the UK has shown that bumblebee nests appear to be more common in gardens than they are in the countryside (Osborne et al., 2008) and this may reflect a paucity of suitable nesting habitat and/or a shortage of early forage to support nests in the rural environment. Encouraging bumblebees to nest on farmland by offering suitable nesting habitat in combination with plentiful spring forage may help to ensure efficient pollination of crops and also of many wildflowers associated with the farmland environment.

Although most studies of agri-environment scheme suitability for bumblebees have focused on field margin management, other management options are also likely to influence bumblebee populations. For example, the sowing of tussocky grass strips adjacent to, or bisecting crop fields, restoration or creation of hedgerows and wooded areas and restoration or creation of species-rich grasslands are all likely to promote the sorts of vegetation structure generally associated with nesting bumblebees. However, to date there have been few attempts to quantify the value of these schemes for bumblebees.

A paired-farm comparison was used to quantify the relative value of three management options offered as part of the Scottish Rural Stewardship Scheme 2004 for nest site searching and foraging spring bumblebee queens (similar or identical schemes are available in England and Wales). The aim of the study is to assess the potential of these schemes to promote nest foundation and thereby enhance bumblebee abundance in the agricultural environment.

6.3 Methods

6.3.1 Study sites

Ten predominantly arable low lying (0-200m altitude) farms in East and Central Scotland were chosen for inclusion in this study. Five of these were participants of the Scottish Rural Stewardship Scheme (referred to hereafter as RSS) and as such, had signed up to a management plan beginning in 2004. The management plan for each farm consisted of at least one each of the following management prescriptions (adapted from Anon 2006):

1. ‘Management of grass margin or beetle bank in arable fields.’

This prescription involves sowing or maintaining a crop-adjacent strip of land between 1.5 and 6 m wide with a suitable mix of grass species, and is specifically targeted at fields containing an arable crop. The application of fertilisers is forbidden and grazing is not allowed until the crop has been harvested.

The aim of this prescription is to provide a refuge for beneficial insects as well as cover for birds. However, the prescription results in the establishment of large areas of tussocky, undisturbed grassland which may also be of benefit to nesting bumblebees.

2. ‘Management or creation and management of species-rich grassland.’

The former stipulates restrictions on the mowing or grazing of existing areas of unimproved grassland between the months of March and August. The latter involves the removal of existing vegetation cover of an area followed by priming of the land (e.g. by reducing soil fertility and/or removing weed species) and the establishment of a new sward using a low productivity grass and herb mix.

The aim of these prescriptions is to promote the growth and spread of flowering plants and other grassland species. One of the goals was that these should be of conservation value to pollinator species including butterflies and bumblebees, providing a source of wildflowers on which they can feed. The tussocky structure of this grassland may also provide nesting sites for surface-nesting bumblebees as well as attracting small mammals which in turn may provide nest sites for subterranean-nesting species.

3. ‘Management of hedgerows’

This prescription involves managing hedgerows by filling in gaps and limiting cutting to once every three years at most and only in the winter. The hedge-bottom vegetation must not be mown and pesticides must not be applied.

The aim of this prescription is to promote the growth of a diverse hedge-bottom flora as well as to provide shelter for birds, small mammals and invertebrates. Additionally, this scheme may provide a source of bumblebee forage as well as attracting small mammals and birds that will provide nesting sites for bumblebees.

The remaining five farms used in this study were chosen as counterparts for each RSS farm. This was based on three criteria:

1. The paired farm must not be involved in ANY agri-environment scheme.
2. The paired farm must be within 5 km of the corresponding RSS farm.
3. The proportion of the farm dedicated to different land use types must be broadly similar to that of its counterpart.

This design aimed to control variation in bumblebee abundance based on locality and land use.

6.3.2 Sampling methods

On each farm six 100m transects were chosen. On RSS farms, these represented:

FM1. An arable field margin managed according to the grass margin/beetlebank prescription.

FM2. A conventionally managed arable field margin.

G1. An area of grassland managed according to the species-rich grassland prescription.

G2. An area of unfarmed grassland not under any management prescription, referred to from hereon as non-prescription grassland.

H1. A hedgerow managed according to RSS guidelines.

H2. A conventionally managed hedgerow.

On non-stewardship farms, two each of transects FM2, G2 and H2 were chosen to represent the three habitat types (arable field margin, uncultivated grassland and hedgerow). Transects were chosen at random from a farm map prior to visiting the sites themselves. Transects on each pair of farms were matched for aspect and land usage in the adjacent field(s). Grassland transects were set up through the area of grassland rather than at the boundary and when surveying hedgerow transects, bees were only recorded when nest site searching or foraging at the base of the hedge. The edge of the recording area for hedgerow transects was defined by the centre of the hedge, allowing accurate observations of abundances of nest site searching queens.

Non-prescription grassland sites (G2) were areas of land that were largely free from management practices, therefore representing a naturally regenerated grassland habitat. Disturbance to these areas was minimal although vegetation was generally cut back once or twice a year. RSS species-rich grassland (G1) sites used in this study were sown with a wild flower and grass seed mix in 2004, thus allowing three years for the sown mix to become established. Each year, the sites were not mown or grazed from the middle of March to the middle of August to allow season-long flowering, but all were topped at the end of this period to encourage floral diversity.

(Under the RSS management prescription, grazing is suggested as an alternative to topping but this method was not used at any of the study sites.)

The hedgerows surveyed in this study (H1 and H2) consisted predominately of hawthorn (*Crataegus monogyna*) or blackthorn (*Prunus spinosa*) and these did not come in to flower until the very end of the recording period. *Ulex europaeus,* other *Prunus* spp. and *Cytisus scoparius* were also occasional components of the hedgerows themselves. In both RSS and conventionally managed hedgerows, the hedge bottom flora was dominated by grass species. *T. officinale*, *L. album* and *L. purpureum* were minor components of hedge bottom flora in both types of hedgerow.

Each pair of farms was visited once a week over a five week period between 14th April and 16th May 2008. Paired farms were surveyed on the same day so that data collected for each partner on each visit were directly comparable, controlling for differences in weather and date. The order in which the farms were visited and the transects walked was randomised to control for any effect of time of day. Data were collected in dry conditions and temperatures ranged from 5°C at the beginning of the recording period to 25°C later in the season. During each transect walk, the number of bumblebee queens seen within a distance of 3m either side of the transect was recorded. In cases where habitat strips were less than 6m wide, this involved counting any bees observed in the adjacent crop. No fields included in the study contained spring flowering crops. Bees observed were categorised into nest site searching queens (those demonstrating the characteristic slow zigzag flight associated with nest site searching behaviour in bumblebees) and foraging queens and were identified to species level. Each individual was recorded once according to the first behaviour observed. Individuals crawling in vegetation were observed to see whether nest site searching behaviour would commence and if not, the individual was not recorded. The flower on which each foraging queen was found was also recorded.

Abundance of nest site searching queens was used as a measure of the suitability of habitat for nesting bumblebees. It could be argued that numbers of nest site searching queens may not be a good indicator of habitat suitability, as an abundance of nest site searching queens could simply indicate that nest sites are scarce and that the time taken for any individual bumblebee queen to find a suitable nest site is therefore longer. However, this seems unlikely as bumblebee queens should have become adapted to search in those habitat types most likely to yield high quality nest sites (and see discussion).

In addition to the bumblebee counts, the number of individual inflorescences open for each flowering plant species seen in each sampling area was estimated every time a transect was walked. All flowers observed along any given transect walk were recorded, but only those on which bumblebees had been observed to forage were included in data analysis.

On each farm, an additional 30 minute search was made per time point during which time additional areas of suitable habitat were searched and foraging bumblebee queens and flower abundance were recorded as above. These data were used to get a more robust picture of the usage of floral resources by bumblebee queens (for example by revealing whether bees were using flowering trees not present in transects).

A basic vegetation survey was also carried out for each transect in week 2 of the recording period. Margin width and vegetation height were measured and the proportion of land covered by grasses, broad-leaved species, vegetation litter, exposed earth and moss was estimated.

6.3.3 Analysis

All analyses were carried out using SPSS 16.0.

6.3.3.1 Timing of queen activity

A repeated measures analysis of variance was used to investigate species-specific differences in changes in bumblebee abundance over time. Data were combined from transects to give total observations for each species and time point at each farm and were then square root transformed to normalise the data.

6.3.3.2 Queen forage plant usage

A chi-square test of independence was used to examine differences in forage use between species based on all the data collected, both on transect walks and during the additional 30 minute recording period. Only the three most commonly observed bumblebee species (*B. terrestris*, *B. pascuorum* and *B. hortorum*) and the four most popular forage plants (*Prunus* spp., *Lamium album*, *Lamium purpureum* and *Symphytum officinale*) were included in this analysis as inclusion of other species would have resulted in expected frequencies of below 5 rendering the data unsuitable for chi-square analysis.

6.3.3.3 Effects of habitat type and management practice

Two levels of analysis were conducted on bumblebee and flower abundance: the first used only data collected from RSS farms and assessed the effects of habitat type (e.g. field margin) and whether the habitat was prescription or non prescription ('land management type'). The second assessed the effects of habitat type and whether the farm was in a RSS scheme ('farm type') across both RSS and conventional farms. Details of these analyses are outlined below.

All of the following analyses were calculated using bee or flower abundance per transect summed over all time points.

There were insufficient observations to analyse bee species separately but an examination of the data revealed no evidence for species-specific differences in relation to the explanatory variables examined.

6.3.3.4 Effects of habitat type and management practice on bumblebee and flower abundance within Rural Stewardship participant farms

In order to assess the effect of RSS Scheme prescriptions on bumblebee queen abundance, a Poisson loglinear analysis was carried out with farm, habitat type (hedge, field margin or grassland) and land management practice (RSS or conventional) as potential explanatory factors. Flower abundance, including only those species on which bumblebee queens had been observed to forage, was included as a covariate. This analysis used only data collected on RSS participant farms in order to exclude any effects of overall farm management. Separate analyses were carried out on nest site searching and foraging bumblebee queens. A test for two-way interaction effect between habitat type and land management practice was also included in the analysis relating to nest site searching bumblebee queens. Low numbers of foraging bumblebees were observed so an interaction effect could not be included in the analysis for foraging bumblebees. The final explanatory model was created by step-wise removal of non-significant factors.

A general linear model with normal errors was also carried out to assess the effect of RSS Scheme prescriptions on flower abundance (log transformed), with farm, habitat type (hedge, field margin or grassland) and land management practice (RSS or conventional) as explanatory variables. A two-way interaction effect between habitat type and land management practice was also included.

6.3.3.5 Comparison of conventionally managed land on Rural Stewardship participant vs. conventionally-managed farms

In order to identify effects of RSS participation on bumblebee abundance (nest sites searching and foraging), a Poisson loglinear analysis was carried out with locality (each farm pair being classed as one locality), habitat type and farm type as explanatory variables and flower abundance as a covariate. These analyses excluded data collected on RSS managed habitat types (so that equivalent habitats were being compared on each farm type). Again, a two-way interaction effect between habitat type and farm type was included in the nest site searching analysis, but not for foraging bumblebees as numbers observed were low. The final explanatory models were created by step-wise removal of non-significant factors.

The effect of farm type on flower abundance was investigated using an additional generalised linear model with locality, habitat type and farm type as explanatory variables. A two-way interaction effect between habitat type and farm management practice was also included. Flower abundance data were log transformed prior to analysis in order to normalise the data.

Subgenus *Psithyrus* species (kleptoparasitic bumblebees) were not included in any data analysis as they display different life history strategies to that of social *Bombus* species, and only small numbers were observed. It is likely that management benefiting social bumblebees will also profit these species as their numbers are likely to be directly influenced by the abundance of their host species.

6.4 Results

6.4.1 Bee species

During the course of the study, six species of true bumblebee were recorded, but the majority of observations (over 90%) belonged to just four. These were *Bombus terrestris* (29.4%), *B. pascuorum* (24.2%), *B. lucorum* (22.3%), *and B. hortorum* (16.7%). *Bombus pratorum* and *B. lapidarius* were also observed in small numbers (5.0% and 2.3% of observations respectively). Fewer than 0.5% of observations were of cuckoo bumblebees (those belonging to the subgenus *Psithyrus*).

6.4.2 Timing of queen activity

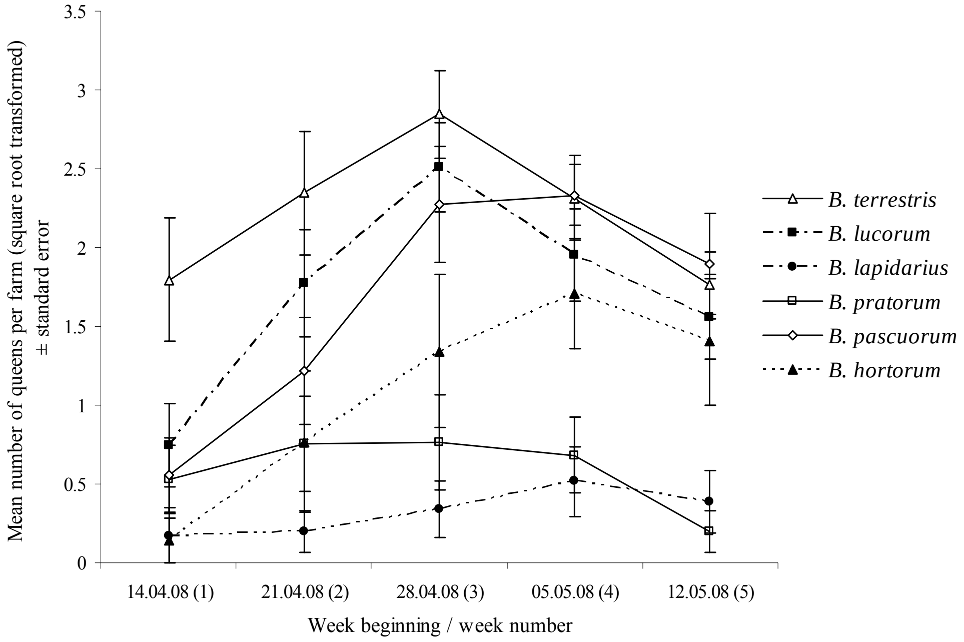
Abundance of bumblebee queens changed over the course of the study, with low numbers observed in mid April increasing towards the end of April, then declining ($F_{(1,54)} = 20.02$, $p < 0.001$). Species-specific differences were also found, with numbers of *B. terrestris* and *B. lucorum* peaking approximately a week earlier than *B. hortorum* and *B. pascuorum,* which reached their maximum in early May ($F_{(5,54)} = 5.15$, $p = 0.001$, figure 6.1). The abundance of queens of *B. pratorum* observed shows no clear peak, but declines towards the end of the recording period in mid May. Sightings of *B. lapidarius* were rare and no clear pattern is evident in the timings of observations of this species.

Small numbers of workers of each species except *B. lapidarius* were also observed during the final three weeks of observations.

6.4.3 Queen forage plant usage

Bumblebee queens were seen foraging on 24 different plant species spanning 13

Figure 6.1: Number of bumblebee queens of different species averaged across all farms at each time point.



different families. However, most of these plant species individually accounted for a very small percentage of observations. Over 60% of bumblebee flower visits were to white deadnettle (*L. album*), red deadnettle (*L. purpureum*), cherry (*Prunus* spp.) and comfrey (*S. officinale*) (table 6.1). Combined, these plant species made up only 21% of inflorescences of bumblebee forage plants observed.

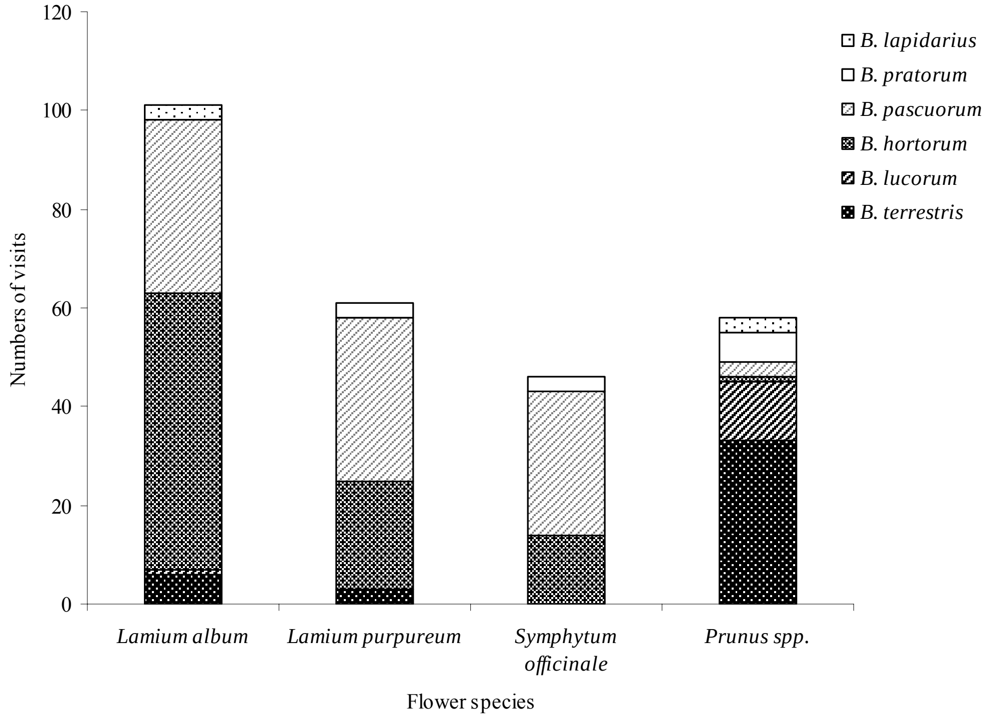
Clear species-specific differences were observed in queen forage use between these four plant species ($\chi^2_6 = 167.33$, $p < 0.001$, figure 6.2). *Bombus lucorum* and *B. terrestris* were most commonly observed foraging on *Prunus* blossoms, whilst the longer tongued *B. hortorum* and *B. pascuorum* were observed foraging most commonly on flowers with a long corolla such as *S. officinale*, *L. purpureum* and *L. album*. *Bombus hortorum* was observed particularly often on *L. album*. *Bombus lapidarius*, *B. pratorum* and *B. lucorum* were excluded from statistical analysis as the number of observations for these species was low.

Table 6.1: Numbers of foraging visits made by queens of the six species observed to different flower species from both 100m transect walks and additional 30 minute farm searches.

	Flower species											
	Asteraceae	Boraginaceae			Brassicaceae		Caryophyllaceae	Fabaceae			Grossulariaceae	
Bumblebee Species	*Taraxacum officinale*	*Pentaglottis viridis*	*Pulmonaria officinalis*	*Symphytum officinale*	*Aubretia* spp.	*Raphanus raphanistrum*	*Silene dioica*	*Cytisus scoparius*	*Viccia cracca*	*Ulex europaeus*	*Ribes sanguineum*	*Ribes uva-crispa*
B. terrestris	10	0	2	0	0	0	0	3	0	4	10	0
B. lucorum	0	0	1	0	1	0	0	3	0	12	6	1
B. pascuorum	11	1	8	29	1	0	0	4	2	1	1	0
B. pratorum	7	0	4	3	0	1	0	0	0	0	2	1
B. lapidarius	0	0	0	0	1	0	0	0	0	2	0	0
B. hortorum	1	0	9	14	0	0	1	1	0	7	0	0
Total	29	1	24	**46**	3	1	1	11	2	26	19	2

	Flower species												
	Lamiaceae				Ranunculaceae	Rosaceae			Salicaceae	Sapindaceae	Scrophulariaceae	Violaceae	
Bumblebee Species	*Glechoma hederacea*	*Lamiastrum galeobdolon*	*Lamium album*	*Lamium purpureum*	*Ranunculus ficaria*	*Crataegus monogyna*	*Malus* spp.	*Prunus* spp.	*Salix* spp.	*Aesculus hippocastanum*	*Cymbalaria muralis*	*Viola odorata*	Totals
B. terrestris	0	0	6	3	0	0	5	33	8	1	0	0	**85**
B. lucorum	0	0	1	0	0	0	2	12	7	0	0	0	**46**
B. pascuorum	0	2	35	33	1	0	0	3	0	1	1	1	**135**
B. pratorum	1	0	0	3	1	1	0	6	2	0	0	0	**32**
B. lapidarius	0	0	3	0	0	0	0	3	1	0	0	0	**10**
B. hortorum	0	0	56	22	0	0	0	1	0	0	0	0	**112**
Total	1	2	**101**	**61**	2	1	7	**58**	18	2	1	1	**420**

Figure 6.2: Numbers of visits by bumblebee queens of different species to the four most frequently visited forage plants.



6.4.4 Effects of habitat type and management practice on bumblebee and flower abundance within Rural Stewardship participant farms

Summary data on the vegetation characteristics of the different habitat types are presented in table 6.2.

Nest site searching bumblebee queens were observed more frequently in field margin habitats (FM1 and FM2) than in grassland habitats (G1 and G2), and more frequently in grassland habitats than in hedgerow habitats (H1 and H2) ($\chi^2_2 = 21.17$, $p < 0.001$, figure 6.3). Land managed according to RSS prescriptions (FM1, G1 and H1) also attracted greater numbers of nest site searching queens than conventionally managed land (FM2, G2 and H2) on the same farm ($\chi^2_1 = 8.93$, $p = 0.003$). The

Table 6.2: Average width and vegetation characteristics of different transect types on farmland. Standard errors in brackets.

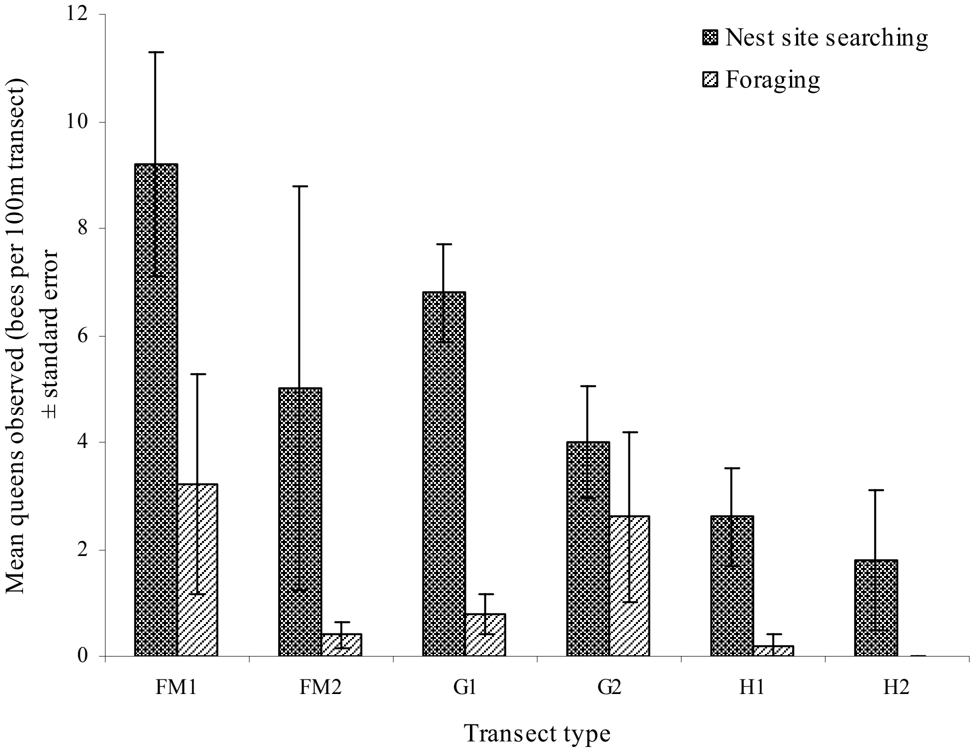
	Width of margin (m)	Height of vegetation (m)	Grass spp. (% cover)	Vegetation litter (% cover)	Exposed earth (% cover)	Broad-leaved spp. (% cover)	Moss (% cover)
RSS species-rich grassland	N/A	1.30 (0.21)	46.4 (17.33)	13.8 (12.35)	27.2 (9.43)	8.6 (2.80)	4.0 (2.53)
Conventional grassland	N/A	1.00 (0.14)	47.4 (5.92)	2.0 (1.36)	8.9 (3.62)	40.9 (5.57)	0.73 (0.67)
RSS hedgerows	2.40 (0.92)	0.96 (0.15)	48.6 (13.88)	8.6 (4.95)	38.4 (16.02)	4.2 (1.83)	0.2 (0.20)
Conventional hedgerow	1.78 (0.59)	0.53 (0.15)	56.5 (8.91)	5.0 (1.72)	19.7 (5.95)	15.1 (4.39)	3.6 (3.45)
RSS field margin	6.20 (1.06)	1.39 (0.07)	71.8 (8.32)	0.6 (0.40)	25.8 (7.10)	1.6 (1.60)	0.2 (0.20)
Conventional field margin	1.81 (0.54)	0.64 (0.10)	64.1 (5.30)	1.9 (1.35)	17.2 (4.31)	16.8 (4.35)	0.07 (0.67)

effect of land management (RSS versus conventional) on nest site searching bumblebee abundance was the same across all habitat types (interaction effect, χ^2_1 = 0.27, p = 0.607).

Habitat type did not explain the variation in the abundance of foraging bumblebee queens observed between transects (χ^2_2 = 2.33, p = 0.313), but the effect of land management practice was significant (χ^2_1 = 4.25, p = 0.039) with foraging bumblebees observed more frequently on RSS habitat than on conventional habitat. Interaction effects could not be examined as observations of foraging bumblebee queens were few, but these data suggest that greater abundances of foraging bumblebee queens were attracted to RSS field margins (FM1) than conventionally managed field margins (FM2), whilst conversely, non-prescription grassland (G2) appeared to be more attractive to foraging bumblebees than RSS species-rich grassland (G1) (figure 6.3). No difference was evident between RSS and conventionally-managed hedgerows (H1 and H2) (figure 6.3).

Habitat type was a strong predictor of the abundance of bumblebee forage flowers within RSS participant farms (χ^2_2 = 9.91, p = 0.007). Flower abundance was greatest in the field margin habitat type (FM1 and FM2) and lowest in the hedgerow habitat type (H1 and H2) (figure 6.4). There was a significant interaction between habitat type and land management practice (χ^2_2 = 10.20, p = 0.006), resulting from the low abundance of flowers observed in RSS species-rich grassland (G1) compared to non-prescription grassland (G2). Flower abundance did not differ between sites (χ^2_4 = 6.41, p = 0.171).

Figure 6.3: Mean number of bumblebee queens observed per transect for different transect type on Rural Stewardship participant farms. (Data summed over all time points, and pooled for bee species.) FM1 = Rural Stewardship arable field margin, FM2 = conventionally managed field margin, G1 = Rural Stewardship species-rich grassland, G2 = non-prescription grassland, H1 = Rural Stewardship hedgerow, H2 = conventionally managed hedgerow.

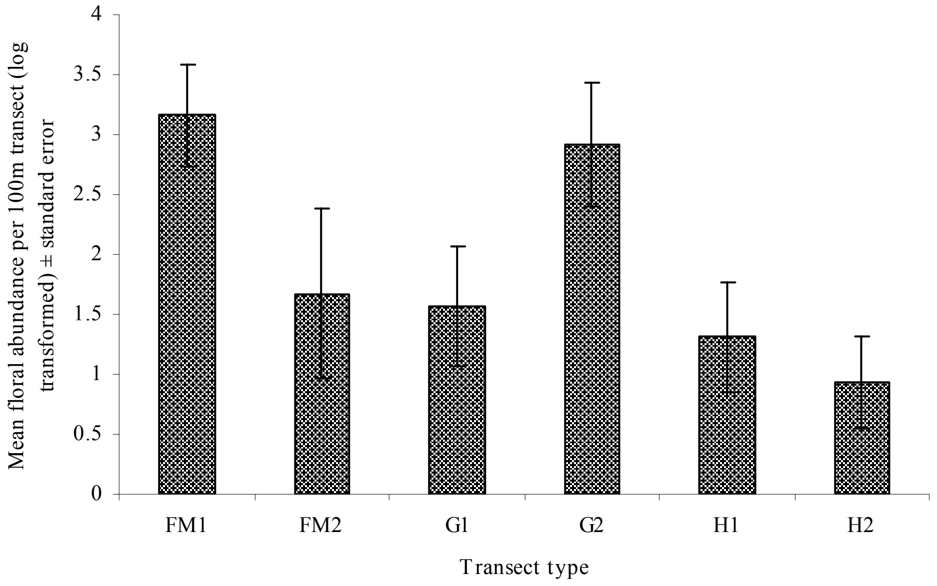


Transect types containing more flowers attracted significantly more foraging bumblebee queens ($\chi^2_1 = 17.8$, $p < 0.001$), but flower abundance had no effect on the abundance of nest site searching queens ($\chi^2_1 = 0.45$, $p = 0.503$, figures 6.3 and 6.5).

6.4.5 Comparison of conventionally managed land on Rural Stewardship participant vs. conventionally managed farms

Results for the effects of habitat type and farm type on bumblebee abundance

Figure 6.4: Mean number of inflorescences per transect (log transformed) for different transect type on Rural Stewardship participant farms. (Data summed over all time points.) Key to transect types as in figure 6.3.



between RSS participant and conventionally managed farms are presented in table 6.3. A significant interaction effect was found between farm type and habitat type, with nest site searching bumblebee queens being observed more frequently in field margins (FM2) on RSS participant farms than on conventional farms, but as frequently on non-prescription grassland (G2) and along hedgerows (H2) on both RSS participant farms and conventionally managed farms (figure 6.5a). Again, abundance of bumblebee forage plant inflorescences had no effect on numbers of nest site searching bumblebee queens observed ($\chi^2_1 < 0.001$, $p = 0.994$).

Habitat type was the best predictor of foraging bumblebee abundance (table 6.3). Foraging queens were observed most frequently in non-prescription grassland (G2) habitat type and were much less abundant in the field margin and hedgerow habitat types (FM2 and H2) (figure 6.5b). When considering only conventionally managed

Table 6.3: Table of results for the effects of habitat type and farm type (RSS participant vs. conventional) on bumblebee abundance using Poisson loglinear analyses with nest site searching and foraging bumblebees as response variables.

	Nest site searching bees			**Foraging bees**		
	Wald Chi-Square	Degrees of freedom	Significance	Wald Chi-Square	Degrees of freedom	Significance
(Intercept)	75.76	1	<0.01	15.51	1	<0.01
Habitat	5.76	2	0.06	35.46	2	<0.01
Management	2.56	1	0.11	5.51	1	0.02
Locality	36.28	4	<0.01	17.83	4	<0.01
Flowers	-	-	-	4.69	1	0.03
Habitat * Management	6.69	2	0.04	-	-	-

habitats, RSS participant farms attracted fewer foraging bumblebee queens than conventionally managed farms. Again, number of bumblebee forage plant inflorescences was a significant predictor of abundance of foraging bumblebee queens. However, the data were insufficient to provide a reliable assessment of any interaction effects between habitat type and farm type.

The locality of each farm pair was a significant predictor of the abundance of both nest site searching and foraging bumblebee queens (table 6.3).

Flower abundance differed between habitat types, again being highest in the grassland habitat type (G2) and lowest in the hedgerow habitat type (H2) ($\chi^2_2 = 13.81$, $p = 0.001$, figure 6.5c). No overall effect of farm type was observed nor was there an interaction between farm type and habitat ($\chi^2_1 = 0.42$, $p = 0.518$, $\chi^2_2 = 2.80$, $p = 0.247$ respectively). There was also no effect of locality on flower abundance ($\chi^2_4 = 5.17$, $p = 0.271$)

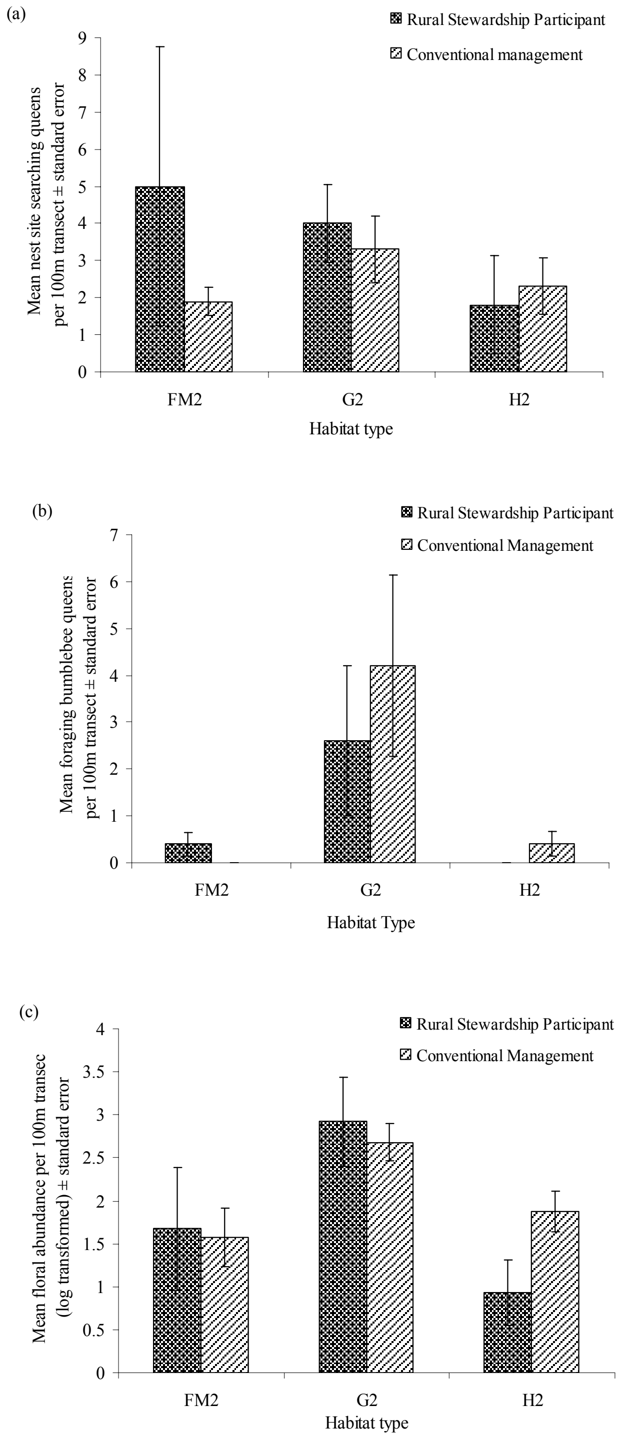


Figure 6.5: Mean number of (a) nest site searching queens (b) foraging queens and (c) inflorescences per transect on conventionally managed habitat types on Rural Stewardship participant vs. conventionally managed farms. (Data summed over all time points, only conventionally managed habitat included.) Key to transect types as in figure 6.3.

6.5 Discussion

6.5.1 Bee species

All social bumblebees observed belonged to the ‘big six’ British bumblebee species, so-called because they are common and widespread throughout most of the British Isles. The relative abundances of each species recorded in this study are largely consistent with those reported in previous studies on farmland in England. However, there was a notable scarcity of *B. lapidarius*, a species that usually accounts for a high proportion of bumblebee observations in this type of study (Kells et al., 2001; Carvell et al., 2004, 2006b; Pywell et al., 2005). This may be due in part to the fact that *B. lapidarius* is at the northern edge of its range in Scotland (Goulson et al., 2005) and is therefore likely to be less common here than in England where previous work has been carried out. However, in addition to this, *B. lapidarius* was found to be unusually rare in the north of the UK in 2008 (Bumblebee Conservation Trust ‘Beewatch’ Survey, unpublished data), possibly as a result of poor weather in the period of 2007-2008 which may have differentially affected this species at the edges of its range.

6.5.2 Timing of queen activity and species-specific patterns

It is well documented that bumblebee species differ in their choice of forage plant (Alford, 1975), and these differences were apparent in this study. As was expected, short-tongued species such as *B. terrestris* and *B. lucorum* were more frequently observed foraging on flowers with short corolla lengths, in this case largely *Prunus* spp. (excluding *P. spinosa*), whilst *B. hortorum* and *B. pascuorum* (the two longer tongued species represented in this study) were more frequently observed feeding on flowers with long corolla lengths, particularly *L. album*, *L. purpureum* and *S. officinale*.

Bumblebee activity varied between species with peak activity levels being reached first by *B. pratorum* between April 21st and 28th then by *B. terrestris* and *B. lucorum* and finally by *B. hortorum* and *B. pascuorum* in the week of May 5th. Similar abundances of each species of bumblebee were observed displaying foraging behaviour over the course of the study, but nest site searching behaviour was more commonly displayed by *B. terrestris* and *B. lucorum* than by other species (notably *B. hortorum* and *B. pascuorum*). These patterns reflect known phenological differences between these different species (Goulson et al., 2005). As the study was carried out early in the year, it would be expected that the lag time between queen emergence and commencement of nest site searching behaviour would result in earlier emerging species such as *B. terrestris* and *B. lucorum* being represented in higher abundances in the subset of queens searching for nest sites.

6.5.3 Effects of habitat type and management practice on bumblebee and flower abundance

A comparison of habitat types managed either conventionally or according to RSS prescriptions within the same farms allowed the local effects of each management prescription to be assessed excluding any influence of whole farm management, whilst comparing the same conventionally managed habitat types on RSS participant farms and conventionally managed farms allowed examination of effects of RSS participation at the farm scale.

Non-prescription grasslands (G2) tended to be relatively rich in broad-leaved plants including several spring-flowering forage plants such as *L. album* and *L. purpureum*, and as a result, this habitat type attracted the greatest abundance of foraging bumblebee queens. RSS species-rich grassland sites (G1) contained fewer spring forage flowers and this translated into a lower abundance of foraging bumblebee queens. This is in marked contrast to previous studies carried out in England, which have shown that arable field margins sown with a grass and wildflower mix (similar to that used in the RSS species-rich grassland prescription) were of greater value for providing bumblebee forage than those allowed to undergo natural regeneration (Carvell et al., 2004; Pywell et al., 2005). However, these studies focussed on foraging workers in summer, thus not addressing provision of spring forage to support queens early in the year. Unimproved grassland prescriptions usually aim to promote legumes such as *Trifolium pratense* and *Lotus corniculatus*, which flower in late spring and summer. These prescriptions provide little during the early stages of colony foundation and development.

Despite the low availability of spring forage, nest site searching bumblebee queens were observed more frequently on RSS species-rich grassland (G1) than on non-prescription grassland (G2). This is not unexpected as at this time of year, these areas appeared to be dominated by grasses, giving rise to a tall, dense and tussocky vegetation structure with few spring-flowering plants. Such habitat is probably ideal for providing suitable nest sites for bumblebees as it creates the sheltered sites at the base of grass plants favoured by surface-nesters and also attracts small mammals that will give rise to nest sites suited to colonisation by subterranean nesters.

Conventionally managed field margins (FM2) appeared to be of little benefit to foraging bumblebee queens, containing few spring flowering bumblebee forage plants and attracting low numbers of foraging bumblebees. However, management according to the RSS arable field margin prescription (FM1) resulted in a marked increase in the abundance of early forage flowers for bumblebees (notably *L. purpureum, S. officinale, Silene dioica* and *Ulex europaeus*) and an associated increase in abundance of foraging bumblebee queens observed, despite the lack of forbs included in the seed mix sown under this management prescription. Similarly, conventionally managed field margins (FM2) attracted fewer nest site searching bumblebee queens than RSS margins (FM1), which attracted the greatest number of nest site searching bumblebee queens of all habitat types studied. The grass mix sown on RSS managed field margins had become established over the three years since the scheme was implemented and the vegetation structure of these margins was similar to that of the RSS species-rich grassland. However, they appeared to receive more disturbance (e.g. as a result of the movement of farm machinery) than did the species-rich grassland, facilitating invasion by other plant species including those favoured by foraging bumblebee queens, notably *L. purpureum*, which is indicated as an important source of spring forage in this study. These findings suggest that RSS field margins are able both to provide suitable nesting habitat and to enhance spring forage availability for bumblebees which should promote colony foundation and early growth in these areas as a result.

Of the three broad habitat types examined, hedgerows appeared to be of least benefit to spring bumblebee queens. Although one of the aims of the RSS Scheme prescription for hedgerow management was to promote the development of a diverse hedge-bottom flora, abundance of spring bumblebee forage was found to be low in both conventionally managed and RSS hedgerows (H2 and H1 respectively) and this translated into low numbers of foraging queens in both management types. Despite the suggestion from previous studies that hedgerows are preferred nesting habitat for at least some of the bumblebees commonly recorded in this study (Kells and Goulson, 2003), nest site searching queens were found to be scarce in this habitat type.

Despite clear differences between the vegetation associated with RSS hedgerows and conventionally managed hedgerows, there was no evidence of a difference in attractiveness to nest site searching queens between the two hedgerow types. Although the vegetation associated with RSS hedgerows looked superficially like that of the RSS field margins and the species-rich grassland, RSS hedgerows seemed to be much less attractive to nest site searching queens. A possible explanation for this is that both the species-rich grassland and the field margin management prescriptions involve the sowing of a seed mix whilst the vegetation associated with RSS managed hedge-bottoms is a result of natural regeneration. More detailed analysis of the vegetation associated with these scheme types may help to explain the differences observed here.

When considering only habitats managed conventionally (i.e. FM2, G2, H2) there were some interesting interacting effects of habitat type and farm management on the abundance of nest site searching queens. It is sometimes argued that farmers choosing to adopt agri-environment schemes are likely to be more environmentally aware and may therefore manage their land differently to those farmers that choose not to take part in such schemes (even when managing features that are not specifically included in their agri-environment scheme agreement). The data presented here suggest that such differences probably do exist, for example nest site searching queens were more abundant in field margins on farms with RSS agreements than on equivalent margins on conventional farms, even when these were not part of management agreements. However, this could also be due to an effect of the management agreements on bumblebee abundance at the farm scale such that bumblebee numbers were generally higher on RSS managed farms than on conventionally managed farms.

It could be argued that numbers of nest site searching queens may not be a good indicator of subsequent nest density or even of habitat suitability (see methods). However, the data presented in this study correspond well with what would be expected given the body of evidence for bumblebee nest site choice already present in the literature (Sladen, 1912; Alford, 1975; Svensson et al, 2000; Kells and Goulson, 2003). This suggests that abundance of nest site searching bumblebees is a reasonable measure for assessing the relative quality of habitat for nesting bumblebees, although evidence for this would require both the density of nest-searching queens and then the density of subsequent nests.

6.6 Conclusions

The maintenance of a healthy and diverse assemblage of wild bees in the rural environment can ensure maximum yields from flowering crops with little or no input from expensive commercially reared or domesticated pollinators (Mohr and Kevan, 1987; Kremen et al., 2004). It is also of value for conservation, promoting the survival of wildflower species associated with rural environments (Osborne and Williams, 1996). Of all the wild bees native to the UK, bumblebees are almost certainly the most important wild pollinator taxa (Goulson, 2003a), but the maintenance of robust bumblebee populations requires the provision of suitable resources. Perhaps the most critical period for the establishment of strong bumblebee populations is spring, when a queen must locate a suitable nesting site and single-handedly feed and incubate her first brood of workers.

Rural Stewardship species-rich grassland and field margin prescriptions were found to provide benefits for spring bumblebee queens, and the field margin prescription creates habitat that is both attractive to nest site searching bumblebee queens and provides spring foraging resources, presumably promoting colony foundation and early growth in these areas. Notably, species-rich grassland prescriptions were favoured by nest-searching bumblebees and are likely to provide plentiful forage in summer, but they provided little early spring forage. In contrast, unsown grasslands created by natural regeneration were rich in spring flowers such as *Lamium* spp. and appeared to provide a valuable forage resource at this time. These findings demonstrate that it is possible to provide both spring forage and sites attractive to nest-searching bees by the implementation of a small number of simple management prescriptions, and that this may be an effective method of promoting bumblebee population density in agricultural environments.

6.7 Acknowledgements

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Chapter 7

Forage use and niche partitioning by non-native bumblebees in New Zealand

7.1 Abstract

British bumblebees were introduced into New Zealand at the turn of the last century and of these, four species became established and continue to persist. Two of these, *B. terrestris* and *B. hortorum*, are common in the UK whilst two, *B. ruderatus* and *B. subterraneus*, have experienced dramatic declines. The latter is now extinct in the UK. The presence of *B. ruderatus* and *B. subterraneus* in New Zealand present an opportunity to study their ecology in an environment that is presumably more favourable to their survival than that found in the UK. Forage visits made by bumblebees in New Zealand were recorded across a season. Ninety six percent of visits were to six non-native forage plants (*Cirsium vulgare*, *Echium vulgare*, *Hypericum perforatum*, *Lotus corniculatus*, *Lupinus polyphyllus* and *Trifolium pratense*) suggesting a heavy reliance on these species. Several of these plants have decreased in abundance in the UK, providing a potential explanation for the observed declines of *B. ruderatus* and *B. subterraneus* in Britain. In contrast to studies conducted within their native range, *B. ruderatus*, *B. terrestris* and *B. hortorum* did not differ in diet breadth, and overlap in forage use between the three species was high, probably as a result of the reduced diversity of bumblebee forage plants present in New Zealand. Diel partitioning of forage use between the species was observed, with foraging activity of *B. hortorum* greatest in the morning and the evening, *B. ruderatus* greatest in the middle of the day and *B. terrestris* intermediate between the two. These patterns correspond well with the climatic preferences of each species as evidenced by their geographic range. The relevance of these findings for bumblebee conservation in the UK is discussed.

7.2 Introduction

British bumblebees were introduced into South Island, New Zealand at the turn of the last century for the pollination of red clover, which was widely cultivated as a fodder crop (Hopkins, 1914). Four species (*Bombus terrestris*, *B. hortorum*, *B. ruderatus* and *B. subterraneus*) became established and spread rapidly (MacFarlane and Gurr, 1995). *Bombus terrestris* is now ubiquitous throughout the North and South Islands, *B. ruderatus* and *B. hortorum* are widely distributed and at least locally common and *B. subterraneus* persists in central South Island.

In the British Isles, *B. terrestris* and *B. hortorum* are common and widespread, but *B. ruderatus* has suffered severe declines in recent decades and *B. subterraneus* was declared extinct in the United Kingdom in 2000 (Edwards and Jenner, 2005). The decline of these two species and of several others in the UK have been attributed to habitat degradation as a result of agricultural intensification (Williams, 1986; Goulson et al., 2008a) and particularly to associated declines in the availability of the wildflowers on which these species feed (Carvell et al., 2006a).

Many factors may have facilitated the successful invasion of British bumblebees into New Zealand, including the similar climate and freedom from natural enemies (Donovan and Weir, 1978). However, the most important factor was probably the presence of an abundance of non-native plant species that had evolved alongside bumblebees in Europe and elsewhere. Bumblebees in New Zealand are rarely observed visiting native plant species (MacFarlane, 1976; Donovan, 1980; Goulson and Hanley, 2004).

Understanding why *B. ruderatus* and *B. subterraneus* persist in New Zealand when they have done so poorly in the UK could provide important insights for future conservation efforts for these species. This is of particular relevance since a project is currently underway to reintroduce *B. subterraneus* into the United Kingdom from New Zealand (Howlett et al., 2009). A major component of this project involves management of land for bumblebees adjacent to the proposed reintroduction sites, which currently support several rare British bumblebee species including *B. ruderatus*. In order for this to be successful, the forage requirements of these species throughout the season must be understood.

The exact details of the introduction of bumblebees to New Zealand are unknown but it is reported that at least six British bumblebee species were released in New Zealand (Hopkins, 1914). Although *B. ruderatus* and *B. subterraneus* would have been more common in the UK than today, it seems likely that random selection of British bumblebees would have resulted in equal if not greater representation of other common species such as *B. lucorum*. It is not immediately obvious why these four species should have survived whilst others did not.

Bombus terrestris is a generalist, short-tongued bumblebee species that is able to make use of a wide range of different plant species for forage (Goulson and Darvill, 2004; Goulson et al., 2005) and has shown high invasiveness, having become established in Tasmania, Japan and Israel (Semmens et al., 1993; Goulson, 2003b; Matsumara et al., 2004). However, *B. ruderatus*, *B. hortorum* and *B. subterraneus* are all long-tongued Fabaceae specialists (Goulson et al., 2005). All three have a known preference for red clover (*Trifolium pratense*) and studies have reported strong overlaps in forage use between these species (Goulson et al., 2005; Goulson et al., 2008b). Therefore it might be predicted that competition between these three species should be high, particularly when introduced into a novel environment which is likely to provide a limited breadth of resources in comparison to those available within their native range.

Data collected by Goulson and Hanley (2004) indicate that the diet breadth of New Zealand bumblebee populations are indeed reduced compared with figures calculated within their native ranges and that forage visits by all four species are largely restricted to a handful of non-native forage plants. Overlap in forage use was evident between the species, and as might be expected, this was particularly true for the three long-tongued species.

These findings were based on records collected over a three week recording period beginning towards the end of early colony foundation, providing only a snapshot view of the forage requirements of these species. In order to thrive, bumblebees require a continuous supply of forage throughout the spring and summer. Changes in forage use across the season are currently unknown for New Zealand bumblebees.

In this study, forage visits are described across a whole season in order to provide a more complete picture of forage use by British bumblebees in New Zealand. These data could help to inform management practices for the conservation of rare UK bumblebees and may be of particular relevance to the development of suitable strategies for the reintroduction of *B. subterraneus.* Aspects of niche partitioning between the three most abundant bumblebee species in New Zealand are also investigated in order to assess how competitive interactions might be reduced by differences in forage use and/or timing of foraging.

7.3 Methods

7.3.1 Field work

Field work was carried out in the MacKenzie District and Central Otago regions of South Island, New Zealand between 11th December and 15th February 2008-2009 as this is the only area of New Zealand in which the four bumblebee species coexist (Goulson and Hanley, 2004).

Searches of one man hour were conducted at 121 sites across the study area, following an established technique which has been used for a number of previous studies of forage use by bumblebees, facilitating comparisons across studies (Goulson and Darvill, 2004; Goulson and Hanley 2004; Goulson et al., 2005; Goulson et al., 2008b). All sites were at least 1km away from neighbouring sites and the locations of the sites were chosen at random so that all areas were represented across the full temporal range of the study. The sites searched were approximately 100m in radius and were selected based on habitat type and the presence of at least some known bumblebee forage plants. Sites were either lake or river margins or areas of rough pasture or scrub, since these habitat types were found by Goulson and Hanley (2004) to attract all four bumblebee species present in New Zealand. Searches were conducted between 9am and 7pm, during warm, dry weather and the exact location, date and time of day was recorded for each search. All bumblebees observed were identified to species and caste and their behaviour was recorded as either pollen collecting (if active brushing of pollen into the corbicula was observed) or nectar collecting. The flower on which the bee was foraging was also recorded. At each site, the number of open flowers or inflorescences of each plant species present within the study site was estimated. The recording period was chosen such that it would span the full range of bumblebee activity in the region: from emergence of spring queens through colony development and growth to the production of new queens and males at the end of the season. As a result, all castes were represented and the requirements of each species across a whole season could be identified.

Dawn until dusk studies were also carried out to look for differences in activity patterns throughout the day between the four bumblebee species. Ten surveys were conducted between 2nd and 19th February 2009. These were conducted at distant sites spread across the study area and only in dry weather. At each survey site transects of 110m in length were marked out through high quality patches of forage, chosen to include plants known to be attractive to all four of the species present. The transect was walked at a constant speed at sixteen regular intervals between first light (approximately 6am) and sundown (usually approximately 9.30pm) and any foraging worker bumblebees seen within a distance of three metres on either side of the transect were recorded to species level. Prior to each transect walk, the temperature and relative humidity were recorded.

7.3.2 Analysis

B. subterraneus was excluded from all analyses due to low numbers of observations of this species. Statistical analyses were conducted using SPSS 16.0.

A chi-square test of independence was used to examine differences in forage use across the whole recording period between species. Only the six most commonly visited plant species were included in this analysis since number of visits to other species were low. Use of the six most commonly visited plant species as sources of pollen and nectar was also investigated by comparing the proportion of total visits to all plant species made to each plant species by nectar-collecting and pollen-collecting bumblebees (all species combined).

Simpson's index of diversity (Simpson, 1949) was calculated for the forage plants visited by each species at each site in order to provide a measure of diet breadth. Data was summed across caste and foraging behaviour and only those sites in which five or more individuals of that species were recorded were included. All plant species were included in this analysis. A Kruskall-Wallis test was used to compare diet breadths among species.

Niche overlap was calculated (following Colwell and Futuyma, 1971) between each species pair at each site in which both species in the pair were represented by five or more individuals. Again, all plant species were included in this analysis.

In order to assess changes in forage use over time, the study period was divided into four recording periods (11th-31stDecember, 1st-15th January, 16th-31st January, 1st-18th February). The proportion of available forage plants visited by each species was calculated for each study period. A plant species was classified as a forage plant if five or more observations of visitation were made during the course of the study. The aim of this was to remove plants such as those belonging to *Heracleum* spp. which were abundant but despite occasional visitation, obviously were not commonly used for forage. The proportion of bumblebee visits (all species combined) attributable to each plant species was also calculated.

Bee visits recorded during dawn until dusk sampling were summed over each two consecutive transect walks to divide the day into eight regular recording periods and then expressed as proportions of the total visits observed across the recording period for each species. Data were normalised using an arcsine transformation and a repeated measures analysis of variance was used with species as a factor in order to compare temporal influences on the daily activity patterns of each species.

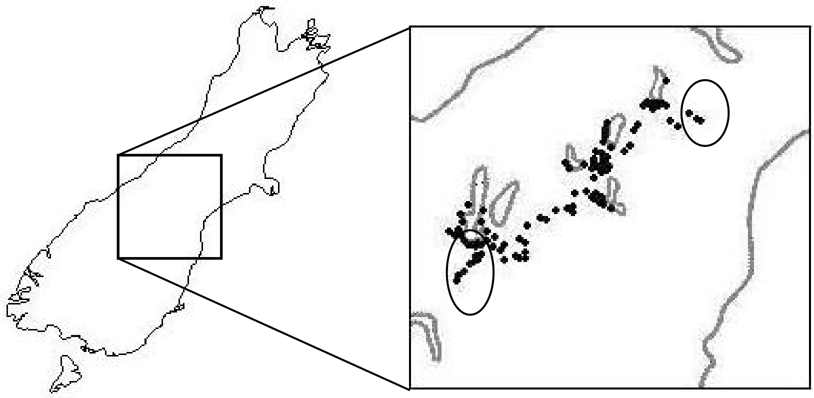
7.4 Results

In total 7,612 foraging bees were recorded including queens, workers and males of all four bumblebee species present in New Zealand (table 7.1). *Bombus ruderatus* and *B. terrestris* were by far the commonest bumblebee species, constituting 95% of all observations and being found throughout the study area. *Bombus subterraneus* was also found throughout the study area, but in very low numbers (constituting <1% observations), whilst observations of *B. hortorum* were largely restricted to the region around Wanaka in the south-west of the study area and the region around Fairley in the north-east of the study area (figure 7.1).

Table 7.1: Numbers of bumblebee forage visits observed divided by species, caste and pollen or nectar collection

	Queen		Worker		Male	
	Nectar	Pollen	Nectar	Pollen	Nectar	**Total**
B. hortorum	4	0	228	23	73	**328**
B. ruderatus	120	9	1628	222	534	**2513**
B. subterraneus	2	0	16	1	14	**33**
B. terrestris	164	37	2431	941	1165	**4738**
Total	**290**	**46**	**4303**	**1187**	**1786**	**7612**

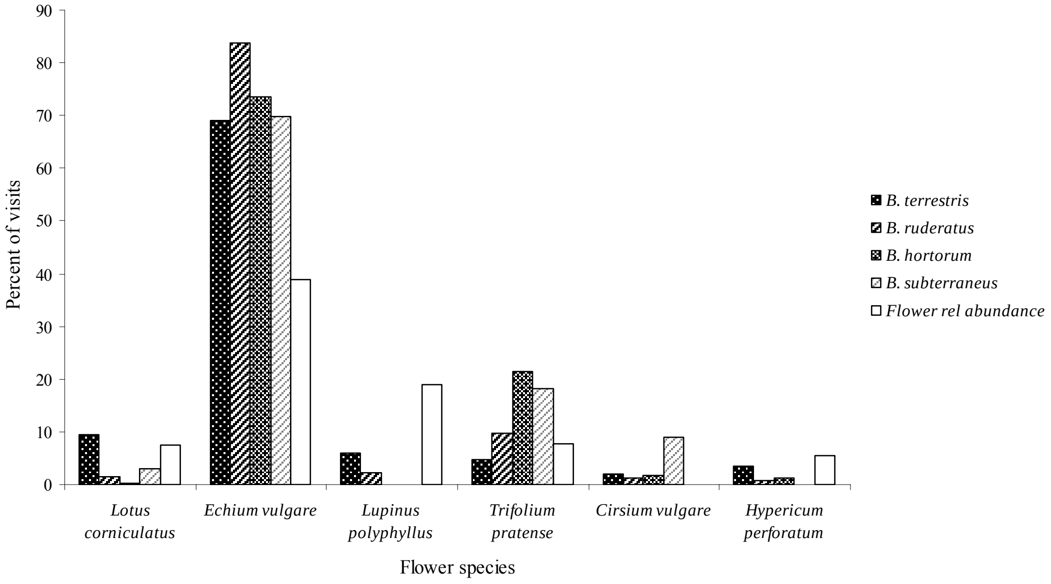
Figure 7.1: Sites within South Island, New Zealand at which hour bumblebee searches were conducted. Circled areas indicate areas where *B. hortorum* were commonly observed.



7.4.1 Forage use between species

Bumblebees were recorded visiting 28 different introduced and one native plant species (appendix 7.1) but 96% of all forage visits were to just six of the introduced plant species (*Cirsium vulgare*, *Echium vulgare*, *Hypericum perforatum*, *Lotus corniculatus*, *Lupinus polyphyllus* and *T. pratense*). Visits to *E. vulgare* made up the majority of observations (74%). *Bombus terrestris* was the only species to be observed foraging on a native plant species (*Acaena saccaticupula*) and these visits accounted for just 0.2% of total visits by this species. When forage visits were combined across sites and between castes, no species-specific differences were observed in visitation rates to the six most commonly used forage plants ($\chi^2_{10} = 0.65$, $p \approx 1$; figure 7.2).

Figure 7.2: Percentages of forage visits made by four British bumblebee species in New Zealand to the six most commonly visited wild flower species



Diet breadths calculated per site provided no evidence for differences in diet breadth between *B. terrestris, B. ruderatus* and *B. hortorum* ($\chi^2_2 = 1.30$, p = 0.523; table 7.2). Diet breadth is low for all three species compared to values calculated for the same species in previous studies (table 7.2). Niche overlaps for forage use were high for all pairs of species (table 7.2) suggesting that all three species are utilising very similar resources.

Table 7.2: Indices of diet breadth and niche overlap calculated for the three bumblebee species present in New Zealand in different studies. (Indices calculated with data collected in this study include standard error in brackets.)

Reference	Country	*B. ruderatus*	*B. hortorum*	*B. terrestris*
Goulson and Hanley, 2004	New Zealand	2.07*	2.05*	4.43*
Goulson and Darvill, 2004	United Kingdom	NA	2.57*	7.27*
Goulson et al., 2008b	Poland	3.5	3.02*	8.63*
Current study	New Zealand	1.56 (±0.101)	1.36 (±0.096)	1.67 (±0.097)

Reference	Country	*B. rud/B. hort*	*B. rud/B. terr*	*B. terr/B. hort*
Goulson and Darvill, 2004	United Kingdom	NA	NA	0.19*
Goulson et al., 2008b	Poland	0.78	0	0.02
Current study	New Zealand	0.83 (±0.047)	0.7 (±0.036)	0.67 (±0.085)

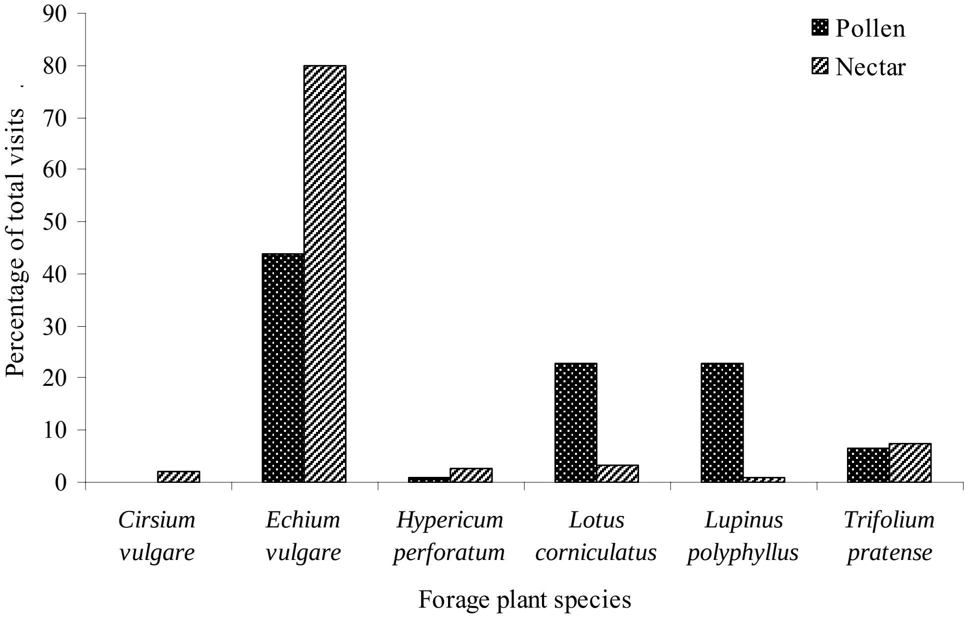
* where values were calculated separately for caste or foraging behaviour, the average value is presented

Nectar collecting bumblebees demonstrated different patterns of forage use from pollen collecting bumblebees (figure 7.3). In this study, *E. vulgare* accounted for 80% of nectar collecting visits but only 44% of pollen collecting visits. Conversely, *L. corniculatus* and *L. polyphyllus* (both belonging to the Fabaceae family) were rarely visited by nectar collectors but attracted many more pollen collecting bumblebees. *C. vulgare* was only ever visited for nectar whilst *T. pratense* accounted for approximately 7% of visits by both pollen and nectar collecting bees.

7.4.2 Forage use over time

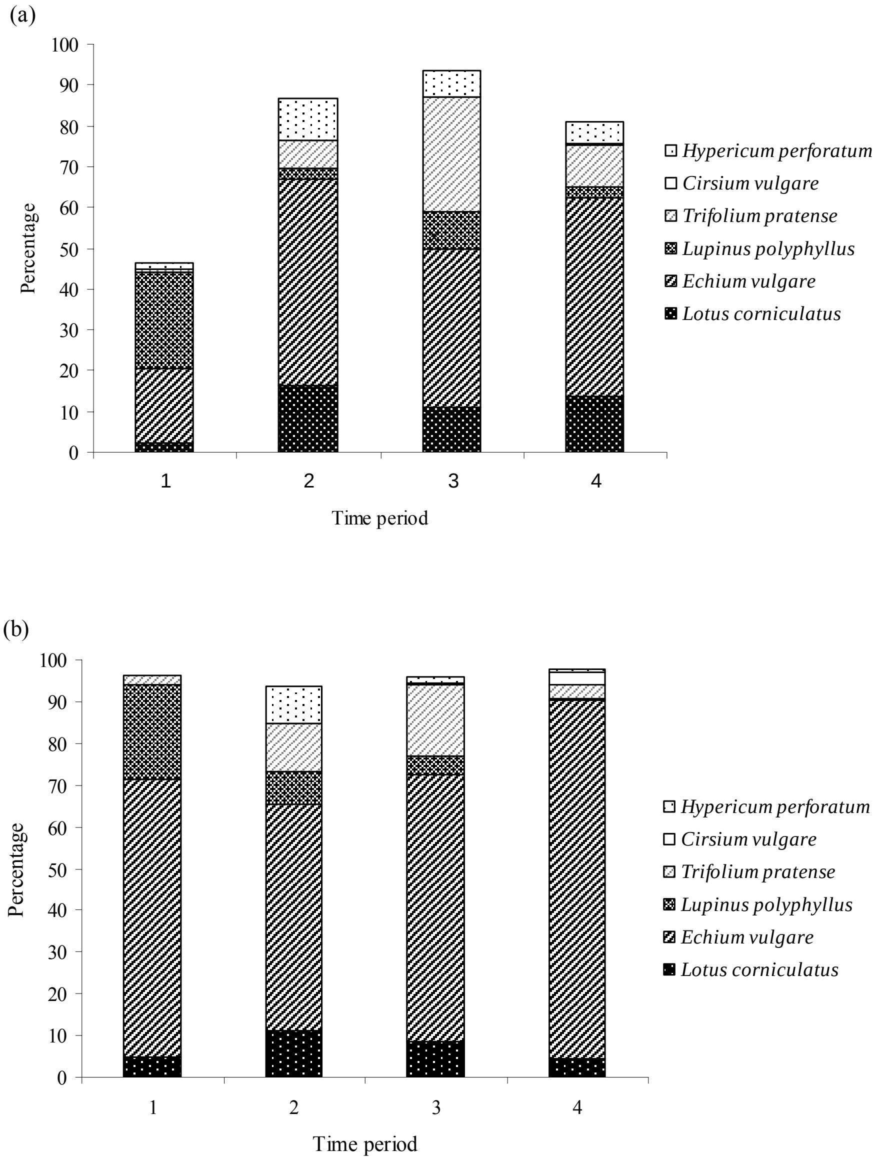
In the first recording period, the six preferred forage plants made up just 46% of total forage plant availability (figure 7.4a), but accounted for 96% of foraging visits (figure 7.4b). Visitation to *E. vulgare* was always high (accounting for between 54% and 86% of total visits) regardless of the abundance of this species in relation

Figure 7.3: Percentages of forage visits made by British bumblebees in New Zealand to the six most commonly visited wild flower species split by pollen and nectar collecting visits



to that of other forage plants. The proportion of visits received by *L. corniculatus* and *T. pratense* generally reflected the relative abundance of these species, whilst use of *L. polyphyllus* reflected the relative abundance of this plant early in the season, but decreased as other plant species increased in relative abundance over the season. Use of *H. perforatum* showed a peak in the second recording period, when the relative contribution of this species to overall forage was at its highest, but visits to this species decreased over subsequent time periods. *C. vulgare* was always relatively uncommon, and being late flowering, contributed greater than 1% to overall forage abundance only in the final recording period. Visits to this species in this period were disproportionately high, mainly as a result of the preference of males for feeding on this species (appendix 7.1). It was not uncommon to see multiple individuals on a single inflorescence of this species.

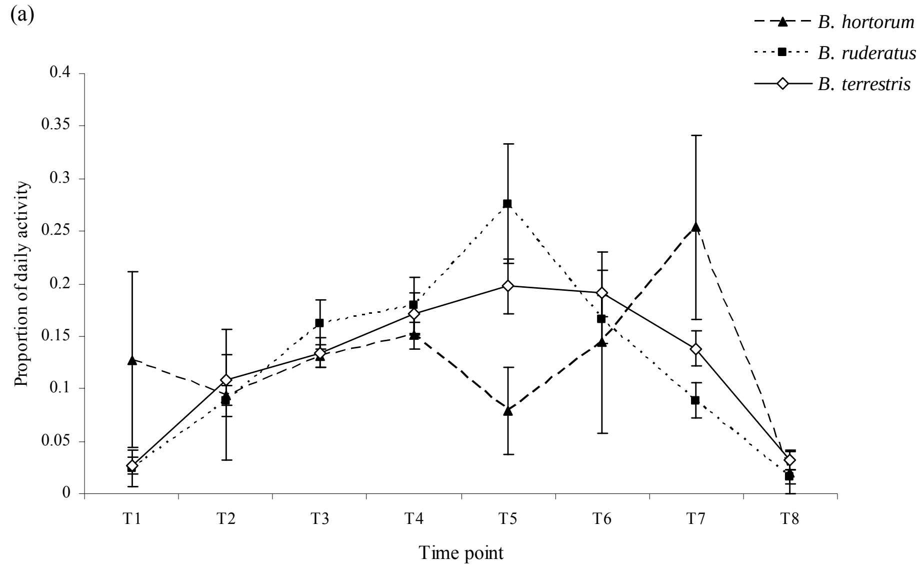
Figure 7.4: Percentages of (a) available forage attributable to the six most commonly visited forage plant species and (b) foraging visits made to the six most commonly visited forage plant species split by recording period. (Recording period 1 = 11th-31st December, 2 = 1st-15th January, 3 = 16th-31st January, 4 = 1st-18th February)

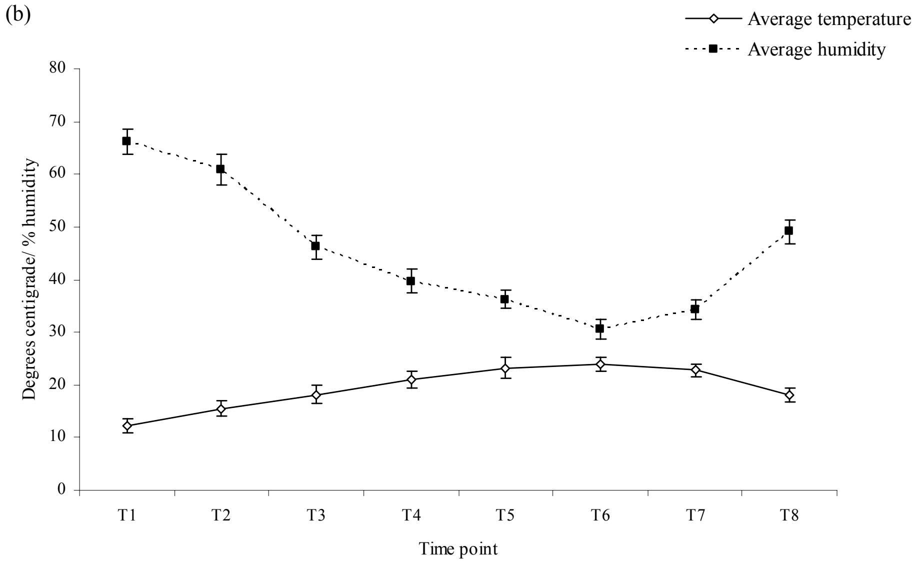


7.4.3 Differences in daily activity patterns between species

Activity of all bumblebee species was affected by time of day ($F_{7,140} = 8.09$, $p < 0.001$) but there were also species-specific differences in activity patterns across the

Figure 7.5: Average (a) proportion of daily foraging activity of three bumblebee species and (b) daily temperature and humidity at eight evenly spaced time points over a day (± standard error)





day (interaction effect, $F_{14,140} = 2.12$, $p = 0.014$). Both *B. terrestris* and *B. ruderatus* demonstrated low levels of activity in the early morning and late evening, but showed a peak of activity in the early afternoon (figure 7.5a). This pattern was more pronounced for *B. ruderatus* which showed a very steep activity curve with a high peak activity rate. Activity of *B. terrestris* was more evenly distributed with activity remaining high across time points 4-6. *Bombus hortorum* showed very different patterns of activity compared to the other two species, being most active early in the morning and at time points 4 and 7, either side of the peak of activity for *B. ruderatus*.

Temperature increased throughout the day until time point 6 and then began to drop off towards the end of the recording period (figure 7.5b). Relative humidity was negatively correlated with temperature.

7.5 Discussion

As in previous studies, bumblebee populations in New Zealand were found to rely almost entirely on non-native plant species for forage and of these, a very small number made up the majority of forage visits across all four species in the study area. Despite the long duration of this study to encompass the early nest founding and late reproductive production stages of colony growth, patterns of forage use were fairly consistent across the study period, although the importance of *C. vulgare* increased dramatically in the final recording period and this species seemed to provide an important forage source for males. That males of a species may differ in forage requirements to workers and queens has previously been shown in the UK (Carvell et al., 2006b) and may be an important consideration for the development of management strategies for bumblebees. In this study, *E. vulgare* was a particularly important forage plant, accounting for the majority of visits observed. The phenology of this species is such that it continued to flower throughout the recording period and the continued availability of this favoured forage plant throughout the period during which bumblebees are active may be at least partially responsible for the success of bumblebees in New Zealand.

Whilst *E. vulgare* made up the majority of nectar collecting visits, *L. polyphyllus*, *L. corniculatus* and to a lesser extent, *T. pratense* were also commonly visited for pollen. *E. vulgare*, *L. corniculatus* and *T. pratense* have all been found to produce high quality pollen in terms of protein content and provision of essential amino acids (Hanley et al., 2008). *L. corniculatus*, *T. pratense* and *L. polyphyllus* all belong to the Fabaceae family, with which *B. ruderatus* and *B. hortorum* are strongly associated within their native ranges (Goulson et al., 2005), and which generally produce higher quality pollen than that of other species (Hanley et al., 2008). *L. polyphyllus* flowered early in the season and in the first recording period foraging visits to this species were high. *L. corniculatus* and *T. pratense* flowered later in the season, but when these species became more abundant, *L. polyphyllus* was visited proportionately less. *L. corniculatus* and *T. pratense* are important sources of forage for bumblebees in the UK (Goulson and Darvill, 2004; Carvell et al., 2007; Carvell et al., 2004) whilst *L. polyphyllus* originates from North America (Hanley and Goulson, 2003) and although it has evolved alongside bumblebees, its native range does not overlap with that of the bumblebees present in New Zealand. However, this study suggests that *L. polyphyllus* may provide an important source of high quality pollen early in the season.

It is notable that three of the six most commonly visited species (*E. vulgare*, *H. perforatum* and *C. vulgare*) were listed as pest plants under the New Zealand Noxious Weed Act in 1950 (http://www.maf.govt.nz/mafnet/rural-nz/sustainable-resource-use/land-management/emerging-weeds/appendices/appendix-a.htm). Several studies demonstrate that the spread of weeds is often facilitated by the presence of non-native pollinator species (Barthell et al., 2001; Stout et al., 2002; Goulson and Derwent, 2004) and the high rate of visitation to these plants by bumblebees indicates that they may play an important role in the pollination and/or out-crossing of these weed species, potentially facilitating their spread throughout New Zealand.

A comparison of forage use reported by Goulson and Hanley (2004) and data presented here reveals some differences. In this study, *E. vulgare* accounted for the majority of forage visits observed, whilst Goulson and Hanley found that *T. pratense* was more commonly visited. This is partially accounted for by the fact that *T. pratense* was not in flower for the full duration of this study. However, visits to *E. vulgare* remained dominant even when *T. pratense* was in flower. It is also notable that diet breadth indices calculated by Goulson and Hanley were larger than those calculated in this study. Both of these differences can be accounted for by the fact that Goulson and Hanley sampled a wider range of habitat types and covered a wider area of New Zealand. Floral availability was not reported by Goulson and Hanley, but it is likely that the differences in foraging patterns observed between the two studies are largely a reflection of differences in the availability of different species as forage plants.

Both studies demonstrate a heavy reliance of New Zealand bumblebees on a small number of plant species. Of these, several (including *E. vulgare*, *L. corniculatus* and *T. pratense*) have shown marked declines in the UK (Grime et al., 1988; Rich and

Woodruff, 1996; Carvell et al., 2006a) and this may explain rarity of *B. ruderatus* and extinction of *B. subterraneus*. The promotion or supplementation of populations of these plant species in the proposed area of release of *B. subterraneus* in the UK may be beneficial for the survival of reintroduced individuals and is also likely to confer benefits to those bumblebee species currently persisting within these areas.

In this study and in that of Goulson and Hanley (2004), diet breadth indices were low for all species compared to those reported elsewhere. This is particularly notable for *B. terrestris* which is a very generalist species and is typically observed foraging on a wide range of different plant species including many that are not native within its natural range (Hingston and McQuillan, 1998; MacFarlane, 1976). The polylectic nature of this species generally results in high diet breadth indices where they are calculated (Goulson and Darvill, 2004; Goulson et al., 2008b). *Bombus hortorum* and *B. ruderatus* generally demonstrate greater levels of dietary specificity resulting in lower diet breadth indices (Goulson and Darvill, 2004; Goulson et al., 2008b) but even in these species, a reduction in diet breadth is seen between data collected within their native range and that collected in this study. Since New Zealand native bees are generally much smaller than bumblebees (Donovan, 1980), native plant species are unlikely to be suitable for exploitation by bumblebees, rendering them almost entirely dependent on introduced plant species. The limited presence or abundance of suitable forage plant species in New Zealand therefore presumably explains the reduction of dietary breadth of these species.

A strong overlap in forage use between *B. ruderatus* and *B. hortorum* is consistent with the findings of Goulson et al. (2008b), but high levels of overlap between *B.*

terrestris and the two long-tongued species are in contrast to values calculated elsewhere. These findings suggest that the limited diversity of suitable bumblebee forage plants present in the study area forces long-tongued and short-tongued species to share the same floral resources. Within their native range, overlap in forage use between long- and short-tongued species is often low since bumblebees tend to visit flowers with corolla-lengths that correspond to the length of their tongue (Ranta and Lundberg, 1980; Harder, 1985). This is believed to be a mechanism of niche partitioning, preventing competitive exclusion and allowing several species of bumblebee to coexist (Inouye, 1978; Pyke, 1982; Goulson et al., 2008b). The narrow range of suitable forage plant species for bumblebees in New Zealand may provide a partial explanation for the disappearance of some of the species introduced.

Differences in tongue-length are not sufficient to explain coexistence in bumblebee assemblages since many stable bumblebee communities consist of several species of overlapping tongue-length and forage use (Goulson et al., 2005; Goulson et al., 2008b). In order for coexistence to occur, species must differ in some ecological parameter in order to avoid competitive exclusion. However, partitioning by resource usage is not the only way in which this can be achieved. For example, avoidance of competition can also be achieved by the partitioning of resource use over time either as a result of behavioural responses by the species involved or as a result of stochastic influences such as changing patterns of resource availability. The findings of this study suggest that bumblebees in New Zealand may partition forage use throughout the day such that different species demonstrate different rates of foraging activity at different times of day. In particular, *B. ruderatus* was found to forage predominantly in the middle of the day, whilst *B. hortorum* foraged early in the morning and either side of the peak activity time for *B. ruderatus*. *Bombus ruderatus* has a more southerly distribution than *B. hortorum* in Europe (MacFarlane and Gurr, 1995) and *B. ruderatus* also has shorter hair than that of *B. hortorum* (Sladen, 1912), suggesting that *B. ruderatus* may be adapted to warmer and drier environmental conditions than *B. hortorum*. This could explain the observed differences in activity between these two species, since *B. ruderatus* is active during the hottest and driest part of the day whilst *B. hortorum* is active when it is cooler and humidity levels are higher. *Bombus ruderatus* and *B. hortorum* have always been known to have very similar ecological niches, exhibiting almost identical tongue-lengths (Goulson et al., 2005) and very similar morphology (Alford, 1975) so it is possible that they exhibit similar temporal niche partitioning elsewhere. Similarly, most other coexisting bumblebee species do not share identical geographic ranges (Williams, 2005), again indicating differences in environmental tolerances. Therefore, situations such as this one may be widespread and could provide another explanation for coexistence. Indeed, similar patterns were reported from Sweden by Hasselrot, (1960) who found that nest traffic commenced earlier and continued until later in *B. hypnorum* than *B. terrestris* and *B. lapidarius* (as found here for *B. hortorum*) and that the *B. lapidarius* demonstrated pattern of activity very similar to that observed here for *B. ruderatus*.

However, these descriptions of niche partitioning as a mechanism of avoidance of competition all assume that forage availability limits bumblebee populations. Although this has sometimes been shown to be the case (Pelletier and McNeil, 2003) high levels of niche overlap may simply reflect relaxed selection for partitioning of dietary niche space, allowing the highest quality resources available to be used by all (Pianka, 1974). Other ecological parameters may limit bumblebee populations in New Zealand. For example, since bumblebees often build their nests in the abandoned homes of small mammals (Sladen, 1912; Svensson and Lundberg, 1977; Donovan and Weir, 1978), and New Zealand lacks a diverse small mammal fauna (King, 1990) availability of nest sites may be a limiting factor for bumblebee populations in New Zealand.

7.6 Conclusions

British bumblebees in New Zealand rely on a small number of non-native plant species on which to forage. Several of these species have declined in the United Kingdom, perhaps providing an explanation for the declines of two out of the four New Zealand bumblebee species in Britain. The provision of these plant species should be considered in management targeted towards the conservation of these species and could form a basis for habitat management strategies associated with the reintroduction of *B. subterraneus* into the UK.

An understanding of mechanisms of coexistence is also vital for the effective conservation of communities of related species. *Bombus ruderatus* and *B. hortorum* may exhibit temporal partitioning of resources throughout the day and this can be explained as a result of differences in environmental tolerances of these two, otherwise very similar, species. This suggests that the balance between these species may be maintained by environmental conditions and that alteration in climatic conditions could shift the balance such that one species is favoured and may exclude the other. This phenomenon may be widespread and further investigation is required.

7.7 Acknowledgements

I would like to thank Roddy Hale and all at the University of Lincoln, New Zealand for the use of facilities and help with the project. Special thanks to Jenny Kaden for field assistance and to Mairi Hale for support. I would also like to thank the University of Stirling, and the Natural Environment Research Council, the Game and Wildlife Conservation Trust, The British Entomological and Natural History Society (Maitland Emmett travel grant), the Society for Experimental Biology (Company of Biologists travel grant) and the Percy Sladen Memorial Fund for funding.

Appendix 7.1: All forage visits by bumblebees to different flower species split by species, caste and pollen (P) or nectar (N) collection

	B. hortorum					*B. ruderatus*					*B. subterraneus*					*B. terrestris*					
	Queen		Worker		Male	Queen		Worker		Male	Queen		Worker		Male	Queen		Worker		Male	
	N	P	N	P	N	N	P	N	P	N	N	P	N	P	N	N	P	N	P	N	**Total**
Acaena saccaticupula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	**8**
Buddleja davidii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	**4**
Calystegia sepium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	**1**
Cirsium palustre	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	8	31	**56**
Cirsium vulgare	0	0	1	0	5	0	0	10	0	20	0	0	0	0	3	0	0	12	0	84	**135**
Digitalis purpurea	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	1	2	0	0	**6**
Echium vulgare	2	0	173	9	57	103	5	1399	124	476	1	0	11	0	11	150	21	1755	381	960	**5638**
Eschscholzia californica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**
Heracleum spp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	**3**
Hypericum perforatum	0	0	4	0	0	0	0	15	1	0	0	0	0	0	0	1	0	149	11	1	**182**
Leontodon spp.	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	20	0	17	**41**
Linarea purpurea	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	4	**7**
Lotus corniculatus	0	0	0	1	0	1	0	17	16	2	0	0	0	1	0	0	0	151	264	34	**487**
Lupinus arboreus	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	2	0	**6**
Lupinus polyphyllus	0	0	0	0	0	8	2	19	28	1	0	0	0	0	0	3	13	25	239	1	**339**
Medicago sativa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38	9	0	**47**
Mentha × piperita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**
Origanum vulgare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	**1**
Papaver rhoeas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**
Prunella vulgaris	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	**1**
Reseda luteola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	1	**9**
Rosa rubiginosa	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	2	1	**6**
Rubus fruticosus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	**4**
Silene vulgaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	**1**
Trifolium pratense	2	0	49	12	7	2	0	160	49	33	1	0	5	0	0	10	0	183	20	17	**550**
Trifolium repens	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	32	4	5	**43**
Trifolium vesiculosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	**2**
Verbascum thapsus	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	15	1	2	**20**
Verbascum virgatum	0	0	0	0	0	2	0	4	1	0	0	0	0	0	0	0	1	4	0	0	**12**
Total	**4**	**0**	**228**	**23**	**73**	**120**	**9**	**1628**	**222**	**534**	**2**	**0**	**16**	**1**	**14**	**164**	**37**	**2431**	**941**	**1165**	**7612**

Chapter 8

Genetic divergence and diversity loss of British bumblebees in New Zealand: Is the New Zealand population of *Bombus subterraneus* a good candidate for reintroduction into the UK?

8.1 Abstract

Four British bumblebee species (*Bombus terrestris*, *Bombus hortorum*, *Bombus ruderatus* and *Bombus subterraneus*) became established in New Zealand following their introduction at the turn of the last century. Of these, two have remained common in the UK (*B. terrestris* and *B. hortorum*), whilst two (*B. ruderatus* and *B. subterraneus*) have shown marked declines, the latter being declared extinct in 2000. A reintroduction attempt is currently underway in which it is hoped that the New Zealand population of *B. subterraneus* can be used to re-stock the UK. However, the validity and success of this attempt relies on the genetic health of the New Zealand population of *B. subterraneus* and also upon its similarity to the original UK population. New Zealand bumblebees are likely to have undergone a major population bottleneck during their introduction. Therefore, it might be predicted that the genetic diversity of these populations will be lower than that of the original UK populations and that genetic composition will differ between the two localities, giving rise to low suitability of New Zealand *B. subterraneus* as a source population for reintroduction into the UK. Here, microsatellite markers are used to compare modern populations of *B. terrestris*, *B. hortorum* and *B. ruderatus* in the UK and New Zealand and also to compare museum specimens of *B. subterraneus* from the original British population with the current New Zealand population. Species-specific patterns found were consistent with predictions based on the presumed history of these populations. Importantly, the New Zealand population of *B. subterraneus* exhibited low genetic diversity compared to the original UK population and differentiation from the original UK population was

high, suggesting that the New Zealand population may not be a good candidate for reintroduction into the UK.

8.2 Introduction

British bumblebees were introduced into South Island, New Zealand at the turn of the last century for the pollination of the fodder crop, *Trifolium pratense* (Hopkins, 1914). Four species became established (*Bombus terrestris*, *B. hortorum*, *B. ruderatus* and *B. subterraneus*) and these still persist in New Zealand today. Following their introduction, these four species spread rapidly across the South Island and by 1965 all but *B. subterraneus* were also present in the North Island (MacFarlane and Gurr, 1995). This success was probably facilitated by release from natural enemies and an abundance of introduced bumblebee forage plant species such as *Trifolium pratense*, *Echium vulgare* and *Lotus corniculatus* (Donovan and Weir, 1978; Goulson and Hanley, 2004; chapter 7).

At the time of their introduction into New Zealand, *B. terrestris*, *B. hortorum* and *B. ruderatus* were all common in England and *B. subterraneus* was also described as abundant or common in many localities in the south (Sladen, 1912). Today, *B. terrestris* and *B. hortorum* remain common and ubiquitous throughout the UK, but *B. ruderatus* and *B. subterraneus* have both suffered severe declines, believed to be due to habitat loss as a result of land use changes associated with agricultural intensification (Williams and Osborne, 2009). *Bombus ruderatus* now exists in scattered populations across the south of England (Goulson, 2003a) and *B. subterraneus* was declared extinct in the UK in 2000 (Edwards and Jenner, 2005).

Recent evidence suggests that these patterns of decline are now mirrored in New Zealand, probably due to similar factors and perhaps especially as a result of the withdrawal of government subsidies for farmers to sow leguminous crops such as *Trifolium pratense* and *Lotus corniculatus*, which provide important forage sources for bumblebees in New Zealand (Goulson and Hanley, 2004; chapter 7). A comparison of surveys of the distributions of New Zealand bumblebee populations published in 1995 (MacFarlane and Gurr) and in 2004 (Goulson and Hanley), suggests that both *B. subterraneus* and *B. ruderatus* have become more restricted in their range.

It is something of a surprise that bumblebees have survived so successfully in New Zealand until the present day, since the numbers of individuals of each species released are likely to have been very small. Two successful introduction attempts were made, and these consisted of 93 bumblebee queens in 1885 and a further 143 bumblebee queens in 1906. It is believed that at least six species of bumblebee were included in the 236 bumblebee queens brought to New Zealand, suggesting that the founder populations of each species must have been very small. In addition, adverse conditions during transit and differences in environmental conditions between the UK and New Zealand are likely to have resulted in high initial rates of mortality, further reducing the number of individuals contributing to the populations found in New Zealand today.

Severe population bottlenecks such as those presumably experienced by New Zealand bumblebee populations can lead to a number of deleterious genetic effects. A bottleneck event inevitably results in loss of genetic diversity and this initial loss of genetic variation is likely to result in a reduced ability of the population to adapt to environmental change. Small populations are also more susceptible to genetic drift (chance changes in allele frequency between generations) which can cause chance fixation of deleterious, or loss of beneficial alleles from the population. Inbreeding may also lead to negative fitness consequences in populations that have undergone such processes through expression of deleterious recessive alleles (Frankham et al., 2004).

Inbreeding depression is any negative effect arising as a result of reproduction between individuals of similar genetic make-up. This process has variously been found to lower survival, growth rate and fecundity as well as causing greater susceptibility to disease, predation and environmental stress in a wide range of animal species (reviewed in Keller and Waller, 2002). Haplodiploid species may suffer reduced effects of inbreeding depression as a result of the exposure of deleterious alleles to selection in the haploid male phase (Werren, 1993; Antolin, 1999). However, there are likely to be many female-specific traits to which this does not apply (Darvill et al., 2006). Additionally, the method of sex determination in bumblebees is such that inbred populations produce ‘diploid males’. These individuals arise as a result of homozygosity at sex determination loci and replace half the worker force in affected colonies (Duchateau et al., 1994). Diploid males are reared to adulthood within the nest, using up valuable resources, but since they are unable to contribute to future generations or carry out the duties of workers (Duchateau et al., 1994; Cook and Crozier, 1995), they confer considerable cost to the colony (as demonstrated by Plowright and Pallett (1979) and Whitehorn et al. (2009)). This therefore represents a further cost of reduced genetic diversity to bumblebee populations.

The genetic effects of the bumblebee introductions to New Zealand are of particular relevance in light of a current collaborative project led by British conservation organisations seeking to reintroduce *B. subterraneus* from New Zealand into the UK. The funding for the project was secured on condition that New Zealand bumblebees be used as the source population since this population is of British origin (D. Shepherd, pers. comm.). However, whether New Zealand's population of *B. subterraneus* is representative of the original British population is dependent on the New Zealand population having remained genetically similar to the original UK population. Additionally, the ability of the New Zealand population to re-adapt to the conditions in the UK is crucial for the success of the reintroduction project since the population is likely to have become adapted to different environmental conditions and will have experienced relaxed selection for defences against natural enemies in New Zealand. (Just three of the many bumblebee parasites and pathogens present in the UK are known to exist in New Zealand (Donovan and Weir, 1978) and bumblebee nest predators such as badgers and shrews are also absent). However, the genetic processes associated with an initial bottleneck event and relatively small population size may have greatly diminished the adaptive potential of this population.

Although it is certain that New Zealand bumblebee populations experienced an initial population bottleneck, the magnitude of this effect and its impacts on the genetic structure and diversity of these populations are largely unknown. Recent data presented by Schmid-Hempel et al. (2007) suggests that New Zealand populations of *B. terrestris* exhibit similar levels of genetic diversity to populations in the UK, but also demonstrate significant differentiation from the UK population.

It is slightly surprising that this species should demonstrate such a high level of genetic diversity in New Zealand. However, *B. terrestris* has always been extremely abundant in England and demonstrates great adaptability to environmental change as evidenced by its high invasive potential (Goulson 2003b), so it is likely that this species may have represented a large proportion of the surviving queens introduced into New Zealand. Other species are unlikely to have fared so well.

In the following study, molecular markers were used to compare the genetic diversity and structure of current British and New Zealand populations of *B. terrestris*, *B. hortorum* and *B. ruderatus* in order to study the genetic effects of a population bottleneck followed by approximately 110 generations of isolation. The current New Zealand population of *B. subterraneus* was also compared to museum specimens of the original British population of *B. subterraneus* in an attempt to assess the divergence of the genetic structure of this population from the original source population and to assess the potential of New Zealand *B. subterraneus* as a viable source population for introduction into the UK. *Bombus subterraneus* of Swedish origin were also genotyped to provide a comparison with a current European population.

8.3 Methods

8.3.1 Sample collection

Non-lethal tarsal clips (Holehouse et al., 2003) were collected from live workers or queens of *B. terrestris*, *B. hortorum*, *B. ruderatus* and *B. subterraneus* in the

MacKenzie District of New Zealand and from *B. terrestris*, *B. hortorum* and *B. ruderatus* in the south of England in the summers of 2003. Sample sizes of *B. hortorum* and *B. ruderatus* in England and New Zealand, and of *B. subterraneus* in New Zealand were supplemented by additional collections made in the summer of 2007. Tarsal clips from individuals of the original British population of *B. subterraneus* were taken from dried workers or queens held at the Museum of Natural History in Oxford. All specimens sampled originated from the south of England but due to low availability, dates of collection associated with individuals sampled ranged from 1940-1965. An additional sample consisting of workers and queens of *B. subterraneus* collected from the Uppland province of Sweden in the summers of 2007 and 2008 was also analysed. All samples were preserved in 100% ethanol. Sample sizes are presented in table 8.1.

Table 8.1: Raw sample sizes, colonies represented in each sample (as detected by analysis of data using Colony (Wang, 2004)) and final sample sizes of bumblebees of English, New Zealand and Swedish origin for genetic analysis.

Species	Location	Year	Sample size	Colonies represented	Final sample size
B. terrestris	England	2003	209	141	141
	New Zealand	2003	66	56	56
B. hortorum	England	2003	19	18	46
	England	2007	31	28	
	New Zealand	2003	30	28	37
	New Zealand	2007	9	9	
B. ruderatus	England	2003	33	24	28
	England	2007	4	4	
	New Zealand	2003	16	14	54
	New Zealand	2007	81	40	
B. subterraneus	England	1940-1965	58	41	41
	New Zealand	2003	44	24	38
	New Zealand	2007	25	14	
	Sweden	2007	17	13	46
	Sweden	2008	35	33	

8.3.2 Molecular techniques

DNA was extracted from fresh bees using the HotShot protocol (Truett et al, 2000). However, this protocol was inadequate for extraction of DNA from museum specimens, so the QIAGEN QIAamp DNA Micro Kit (generally used for forensic analysis) was employed for DNA extraction from these individuals.

All bees were genotyped at 8 microsatellite loci (B100, B132, B11, B10, B96, B126, B124 and B121) using primers developed by Estoup et al. (1995, 1996). Amplification at these loci was achieved by means of the polymerase chain reaction using the QIAGEN Multiplex PCR kit. PCR reactions were 10µL in volume and consisted of approximately 1µL Q-solution, 5µL PCR Master Mix, 1µL primer solution (3 x 0.2µM of each primer, forward primers labelled with NED, HEX or FAM dyes, Applied Biosystems), 1µL template DNA (of variable concentration dependent on the extraction technique used) and 2 µL HPLC H_2O. Samples were denatured at 95°C for 15 minutes, and this was followed by thirty-four 210 second cycles consisting of a denaturing step at 94°C for 30 seconds, an annealing step at 49°C for 90 seconds and an extension step at 72°C for a further 90 seconds. This was then followed by a final extension step at 72°C for 10 minutes. An ABI PRISM 377 semi-automated slab gel sequencer was used to visualise PCR products and fragment size was determined using an internal size standard (GeneScan ROX 350, Applied Biosystems). Fragments were scored using Genotyper (Applied Biosystems). Samples for which amplification was not successful, or scoring was uncertain, were re-run and re-extraction of DNA was carried out if necessary. For all museum specimens, the amplification procedure was repeated twice and data were compared between amplifications to test for consistency of scoring. If genotypes were not scored consistently, the individual was discarded. Individuals were also removed from the dataset if amplification failed at more than three loci, since level of genetic degradation within these individuals was likely to be high (Lozier and Cameron, 2009).

8.3.3 Data Analysis

Datasets were checked for unexpected mutation steps, large gaps in fragment lengths and unusually sized fragments using MSA version 4.05 (Dieringer and Schlotterer, 2003). Colony version 2.0.0.1 (Wang, 2004) was then used to identify sister pairings within each time period, species and population. Corrections were made for genotyping errors of 0.5% at each locus. For each sisterhood identified, all but one individual was removed from the dataset prior to further analysis. Since allele frequencies within a population vary among generations, genetic differentiation between samples collected in different years at the same locations was assessed for each species by calculation of Weir and Cockerham's estimator of Fst (θ). Significance was determined following 10,000 allele permutations implemented in MSA. Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium between loci were tested for using GenepopV4 (Raymond and Rousset, 1995). In order to minimise type I errors, strict sequential Bonferroni corrections were applied.

Genetic diversity within populations was assessed by means of allelic richness and Nei's unbiased measure of gene diversity, calculated for each species and population at each locus using Fstat version 2.9.3 (Goudet, 2001). A Wilcoxon signed-rank test was used to assess differences in allelic richness and gene diversity for each species, with the exception of *B. subterraneus* for which a Friedman test was employed. These analyses were carried out using SPSS version 16.0. Wright's measure of population differentiation, Fst, was used to assess genetic differentiation between New Zealand and British populations for each species (Wright, 1951). These were calculated in Fstat according to the Weir and Cockerham (1984) estimator (θ). Global θ values were calculated for all species, and means and standard deviations were calculated by jack-knifing over loci. Pairwise θ values were also calculated for all combinations of the three populations of *B. subterraneus* sampled. A permutation procedure (10,000 allele permutations) was employed to test for departure of global and pairwise θ values from 0 using MSA. Since Fst estimates are dependent on levels of genetic variation displayed at the markers used, these values cannot be used to make comparisons between species. Global values for the standardised measure G'st were therefore also calculated (following Hedrick, 2005). The Swedish population of *B. subterraneus* was not included in this analysis so that differentiation among British and New Zealand populations of each species could be compared directly.

8.4 Results

8.4.1 Bombus terrestris

Clusters of sisterhoods were identified within both the New Zealand and UK samples of *B. terrestris* (table 8.1). Upon removal of all but one individual from each cluster, no significant deviation from Hardy-Weinberg equilibrium was found at any locus and there was no evidence for linkage disequilibrium among any locus pairs.

Allelic richness was significantly lower in New Zealand than the UK (Z = -2.1, p = 0.036, figure 8.1a) although no difference was observed in gene diversity between the two populations (Z = -0.7, p = 0.484, figure 8.1b). Population differentiation between UK and New Zealand populations was low although this difference was highly significant (θ = 0.019 ± 0.004, p < 0.001). Global G'st was lower for this species than all other species investigated, confirming that this species demonstrates the lowest differentiation among populations of the four species included in the study (table 8.2).

Table 8.2: Values of and figures used to calculate the standardised measure of genetic differentiation G'st for New Zealand and UK populations of *B. terrestris, B. hortorum, B. ruderatus* and *B. subterraneus* (follows Hedrick, 2005)

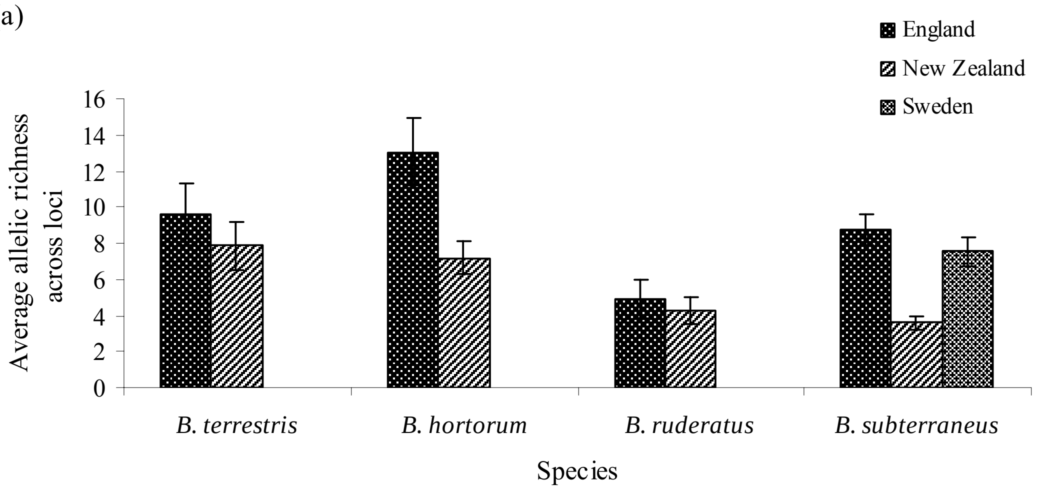
Species	H_T	H_S	G_{ST}	k	$G_{ST(max)}$	G'_{ST}
B. terrestris	0.75	0.74	0.01	2	0.15	0.06
B. hortorum	0.84	0.81	0.04	2	0.11	0.35
B. ruderatus	0.53	0.51	0.05	2	0.33	0.14
B. subterraneus	0.79	0.68	0.14	2	0.19	0.75

8.4.2 Bombus hortorum

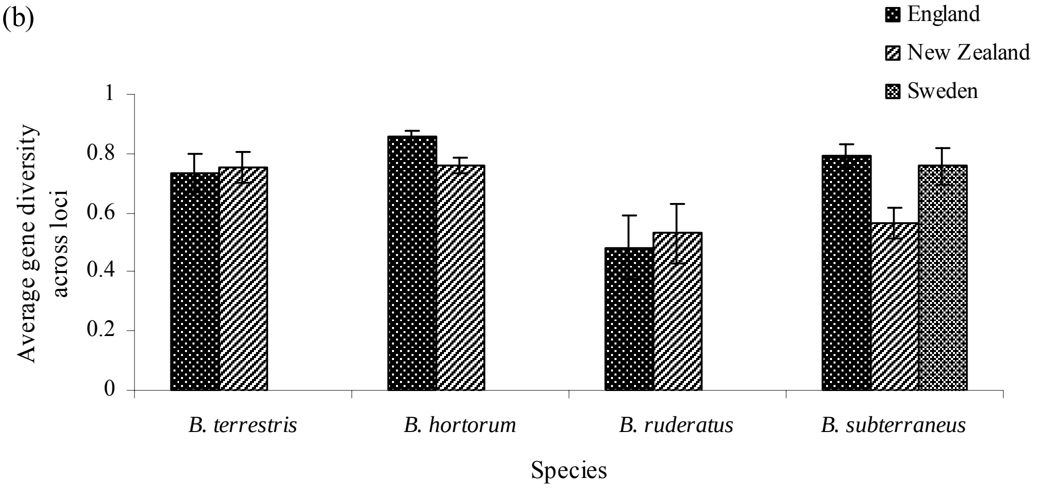
Clusters of sisterhoods were identified in all samples of *B. hortorum* with the exception of that collected from New Zealand in 2007 (table 8.1). When sisterhoods

Figure 8.1: Average allelic richness* (a) and gene diversity (b) across eight microsatellite loci in New Zealand and UK populations of *B. terrestris, B. hortorum* and *B. ruderatus* and in New Zealand, UK and Swedish populations of *B. subterraneus* (±standard error). *Calculated based on a minimum sample of 55, 34, 26 and 19 individuals respectively.

(a)



(b)



were eliminated from the dataset, no significant deviation from Hardy-Weinberg equilibrium was found at any locus except B100. On further examination, this deviation was only apparent within the New Zealand population and was attributable to heterozygote deficit. There was no evidence for linkage disequilibrium among any locus pairs. All further analyses were conducted with and without data for B100, but since differences between corresponding analyses were negligible, results presented here refer to the full dataset (B100 included).

Allelic richness and gene diversity were significantly lower within the New Zealand population of *B. hortorum* than within the UK (Z = -2.521, p = 0.012 and Z = -2.521, p = 0.012 respectively, figure 8.1). No genetic differentiation was found between samples collected in the same localities at different sampling periods (UK: $\theta = 0.005$, p = 0.14, NZ: $\theta = 0.011$, p = 0.15). However, significant differentiation was found between the New Zealand and UK populations of *B. hortorum* ($\theta = 0.07 \pm 0.01$, $p<0.001$). This Fst value suggests moderate differentiation (Wright, 1978). Global G'st was higher than that of *B. terrestris* and *B. ruderatus* so that of the four species, the British and New Zealand populations of *B. hortorum* show the second highest differentiation from one another (table 8.2).

8.4.3 Bombus ruderatus

Sisterhoods were detected within all samples, with the exception of the English sample collected in 2007 (table 8.1). Upon removal of all but one individual from each sisterhood, genotypes did not deviate from Hardy-Weinberg equilibrium at any locus and there was no evidence for linkage disequilibrium amongst any two loci.

No significant difference was found for allelic richness (Z = -1.12, p = 0.263, figure 8.1a) or gene diversity (Z = -0.7, p = 0.484, figure 8.1b) between the New Zealand and UK populations of *B. ruderatus*. There was also no evidence for significant genetic structuring between samples collected at the same locations in different years (UK: $\theta = 0.029$, $p = 0.17$, NZ: $\theta = 0.019$, $p = 0.05$). However, moderate genetic structuring was found between the New Zealand and UK populations of *B. ruderatus* and these differences were highly significant ($\theta = 0.083 \pm 0.025$, $p<0.001$). Global G'st was comparatively low, with this species showing the second lowest level of differentiation of the four (table 8.2).

8.4.4 Bombus subterraneus

Sisterhoods were detected in all sample sets of *B. subterraneus* (table 8.1). (That sisterhoods were detected within the museum samples was consistent with the fact that some individuals sampled were collected from the same locality in the same year). When all but one individual from each sisterhood was removed from each dataset, no deviation from Hardy-Weinberg was found at any locus for the New Zealand sample of this species. Five out of the eight microsatellite loci were out of Hardy-Weinberg equilibrium for the British sample of *B. subterraneus* due to heterozygote deficit. Swedish individuals also demonstrated significant deviation from Hardy-Weinberg equilibrium at B96 and B121, again as a result of heterozygote deficit at these loci. Significant linkage disequilibrium was detected between B100 and B11 in the British sample of *B. subterraneus*. Linkage was also identified between B132 and B11 in the New Zealand population of *B. subterraneus*. All further analyses were conducted with and without problematic loci (by removal of B96, B121 and B11 for the latter) but since differences between corresponding analyses were negligible, statistics presented here are those calculated across all loci.

Significant differences in allelic richness ($\chi^2_2 = 13$, p = 0.002) and gene diversity ($\chi^2_2 = 10.75$, p = 0.005) were observed between the three populations of *B. subterraneus* with the New Zealand population demonstrating lower allelic richness and gene diversity than both the English and Swedish population (figure 8.1).

No significant genetic structuring was found between samples collected at the same locations in consecutive years (NZ: $\theta = -0.013$, p = 0.91, Sweden: $\theta < 0.001$, p = 0.45). However, global θ among populations was high and significant ($\theta = 0.197 \pm 0.031$, $p<0.001$) suggesting high genetic differentiation between the three populations. Pairwise comparisons revealed that differentiation between New Zealand and Britain is greatest ($\theta = 0.256$, $p<0.001$), differentiation between Sweden and New Zealand is also high ($\theta = 0.225$, $p<0.001$) and differentiation between Sweden and the UK is moderate ($\theta = 0.113$, $p<0.001$). Global G'st for this species was extremely high with this species demonstrating by far the highest level of differentiation of all the species studied between the British and New Zealand populations (table 8.2).

8.5 Discussion

8.5.1 Linkage disequilibrium and deviation from Hardy-Weinberg equilibrium

Heterozygote deficit can result in deviation from Hardy-Weinberg equilibrium at a given locus if: (1) selection is acting at that locus; (2) there is strong inbreeding (in the sense of assortative mating); (3) the population includes 'null alleles' at that locus (alleles that fail to amplify under the PCR conditions applied); (4) if more than one discrete interbreeding deme are included within a sample (Selkoe and Toonen, 2006). In the case of *B. hortorum* and *B. subterraneus* from Sweden, deviation from Hardy-Weinberg equilibrium at just one locus suggests that explanations 2 and 4 are unlikely, so confirmation of results by repeating analyses with the exclusion of these loci should eliminate errors associated with this phenomenon. The high level of deviation from Hardy-Weinberg equilibrium detected within the British sample is unsurprising since the sampling method used would have resulted in the inclusion of individuals from temporally segregated breeding populations (explanation 4).

Linkage disequilibrium occurs when transmission of one locus from parent to offspring becomes more likely as a result of transmission of another. This may occur if the loci are close to one another on a chromosome, if the loci are functionally linked or if selection pressure produces a bias towards transmission of the loci as a pair (Selkoe and Toonen, 2006). However, detection of linkage disequilibrium can also arise as a result of a recent immigration from a genetically differentiated population or due to a recent population bottleneck (Darvill et al., 2006). Linkage disequilibrium can result in increased type I error in microsatellite studies since it violates the assumption that loci are independent of one another. This effect is eliminated by removal of data from one locus of the pair.

8.5.2 Patterns of genetic divergence and diversity

Patterns of genetic diversity and differentiation between the populations of bumblebees examined here are consistent with the known and presumed histories of these populations in the UK and New Zealand. *Bombus terrestris* is common and ubiquitous in both the UK and in New Zealand. Since this species has always been one of the most common bumblebee species in England, it seems likely that it should have been well-represented in a sample of British bumblebees taken for introduction into New Zealand. The greater the number of founding queens released in New Zealand, the lower the likelihood of dramatic losses in genetic diversity or differentiation from the original population as a result of genetic drift. The similarity of the genetic structure between New Zealand and England found in this study suggests that the founder effect associated with the introduction of this species into New Zealand was small. That genetic diversity remains high within the New Zealand population is probably reflective of the buffering capacity of the large population size found there. However, consistent with data presented by Schmid-Hempel et al. (2007), *B. terrestris* populations in New Zealand do exhibit slightly reduced genetic diversity in comparison to UK populations and though there is little differentiation between the two populations, this difference is highly significant, demonstrating that there have been genetic consequences of the initial introduction of this species into New Zealand and/or the subsequent isolation of the British and New Zealand populations.

Bombus hortorum is also common and ubiquitous in England but it is not as common as *B. terrestris* and might be predicted to have been less well represented in introductions into New Zealand. Additionally, whilst the current range of this species in New Zealand is unreported, historically it demonstrated a restricted range, having been largely confined to the south-east of the South Island (MacFarlane and Gurr, 1995). This species demonstrates lower genetic diversity in New Zealand than in the UK and also exhibits a higher level of differentiation from the UK population than does both *B. terrestris* and *B. ruderatus*. This might be expected if the initial founder population was lower than those of *B. terrestris* and *B. ruderatus*, but may also reflect lower success of this species in colonising New Zealand, leading to fluctuations in population sizes and resultant genetic drift.

Bombus ruderatus was probably similarly common to *B. hortorum* at the time of introduction into New Zealand (Sladen, 1912), and since these species share comparable ecological requirements, it is likely that these species were relatively equally represented. Following its introduction into New Zealand, this species spread rapidly throughout South Island and also the North Island (MacFarlane and Gurr, 1995). However, the same species has shown rapid declines in the UK since the time of its introduction into New Zealand and has become exceedingly scarce, being restricted to a handful of scattered sites in the south of England (Goulson, 2003a). This is likely to have resulted in loss of genetic diversity and genetic drift within the UK population. Data presented here support this hypothesis. Although extreme caution must be taken when comparing genetic diversity based on microsatellite data among species, it is notable that values of allelic richness and gene diversity for *B. ruderatus* in the UK are very low compared to those of other species in the UK. Whilst this could simply be explained by the fact that this species might generally show lower diversity at the loci used than other British species, it is perhaps more likely that this lower diversity genuinely reflects a reduction in overall genetic diversity as a result of its declines and subsequent existence in small, isolated populations. Genetic diversity in both the UK and New Zealand are similar, but these populations demonstrate highly significant differentiation and again, this differentiation is greater than that observed between populations of *B. terrestris*. It is likely that the genetic diversity present in the New Zealand population of *B. ruderatus* represents a fraction of that of the original UK population (as in *B. hortorum*), but that declines experienced by *B. ruderatus* in the UK have resulted in losses of genetic diversity such that the populations now exhibit similar diversity. The relatively high G’st value compared to that of *B. hortorum* may suggest that the founder effect for this species was reduced compared to that of *B. hortorum*, though this difference could also be explained by the lower success of *B. hortorum* in New Zealand subsequent to its introduction as compared to that of *B. ruderatus*.

B. subterraneus has probably always been less abundant than *B. terrestris*, *B. hortorum* and *B. ruderatus* in the UK, so it is likely that the size of the founder population of this species was the smallest of all four species. Additionally, this species persists within an extremely restricted range in New Zealand (MacFarlane and Gurr, 1995) and is far less common than the other three species (Goulson and Hanley, 2004; chapter 7). Given the strong bottleneck effect likely to have been associated with the introduction of *B. subterraneus* into New Zealand and the subsequent existence of this species within relatively small populations, it might be predicted that genetic diversity would be low and that similarity to the original British population is likely to be limited, and this is indeed the case. The New Zealand population of *B. subterraneus* exhibits extremely low genetic diversity in comparison to both the Swedish and original UK population of the same species and the New Zealand population of *B. subterraneus* is also significantly and highly genetically differentiated from both European populations.

8.5.4 Implications for the use of B. subterraneus from New Zealand as a source population for reintroduction into the UK

The consideration of genetic factors is key in the planning of successful reintroduction attempts since high levels of genetic diversity are likely to be important in determining the adaptive potential of the population and thus its ability to thrive despite novel environmental conditions associated with the introduction site. Introduction of a population with low genetic diversity will also increase the susceptibility of the newly established population to inbreeding depression. Since the New Zealand population of *B. subterraneus* exhibits extremely low genetic diversity, these effects are likely to be pronounced, dramatically reducing the likelihood of successful establishment of this population in the UK.

An additional genetic effect associated with reintroductions from captive-bred individuals is that of adaptation to captivity. For example, Araki et al. (2007) demonstrated that captive bred populations of salmonid fish suffered reductions in reproductive capabilities in the wild at a rate of roughly 40% per year spent in captivity and attribute this drop in fitness to relaxation of natural selection and adaptation to artificially modified rearing environments. Although New Zealand bumblebee populations still exist within a wild situation, they have experienced relaxed selection pressure for the natural enemies present in the UK and they also exist under different environmental conditions from those of the UK. The work of Araki et al. demonstrates that selective processes and/or relaxed selection pressure can act incredibly quickly to reduce the fitness of a population when reintroduced back into its native habitat and this raises considerable concerns regarding the likely fitness of New Zealand *B. subterraneus* in the UK.

The dissimilarity of the New Zealand population from the original UK population is also a concern from the point of view of the reintroduction attempt. The philosophy behind using New Zealand as a source population was that these individuals are representative of the original UK population, but the findings of this study suggests that current Swedish populations of this species are actually genetically more similar to the original population than current New Zealand populations.

The Swedish population of *B. subterraneus* exhibits greater genetic diversity than the New Zealand population and is also likely to be exposed and therefore adapted to similar environmental conditions and biotic interactions to those that will be experienced by reintroduced individuals in the UK. This is likely to dramatically increase the likelihood of successful establishment of this population. Swedish *B. subterraneus* also demonstrates greater genetic similarity to the original UK population and could therefore also be said to be a more representative population for use in a reintroduction attempt. For all of these reasons, the Swedish population could be considered to be a more realistic prospect as a source population for reintroduction into the UK. Whilst the thinking behind the use of New Zealand populations of *B. subterraneus* is clear, the findings of this study suggest that for this reintroduction attempt, consideration of other populations either to supplement the New Zealand population (since using both would lead to much greater genetic diversity) or instead of the New Zealand population may be of value.

8.5.5 Use of DNA extracted from museum specimens in conservation

The use of museum specimens for population genetic studies has been increasing in recent years and the potential of this methodology to examine genetic impacts on species of conservation concern is becoming apparent. DNA extracted from museum specimens provides baseline data which can act as a point of comparison for assessment of a range of genetic processes. These include loss of genetic diversity as a result of reduced population size, increased genetic structuring as a result of reduction in connectivity among sub-populations and introgression of introduced individuals with native species or sub-species (reviewed in Wandeler et al., 2007).

As with many other studies, little difficulty was presented in isolated and typing genomic DNA from museum specimens of 60 or more years in age, despite the crude preservation methods employed for conservation of these individuals. The use of this methodology has provided an interesting insight into the genetic processes acting on British bumblebees in the UK and New Zealand and has also allowed valuable assessment of the suitability of an invasive species for reintroduction back into its native range.

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Chapter 9

General Discussion

Bumblebees are suffering gradual declines across their range as a result of reductions in habitat quality and availability associated with human activity (Kosier et al., 2007; Colla and Packer, 2008; Grixti et al., 2009; Williams et al., 2009). In recent years, more precipitous declines have also been reported in North America and Japan due to other factors (Goka et al., 2001; Matsumara et al., 2004; Colla et al., 2006; Winter et al., 2006; Inoue et al., 2007; Nagamitsu et al., 2009). The need to preserve these vital pollinator species is well understood (Colla and Packer, 2008; Goulson et al., 2008a; Grixti et al., 2009) and much is being done in an attempt to halt their declines, including development of management strategies to boost forage availability (Carvell, 2002; Pywell et al., 2005, 2006; Carvell et al., 2007), initiatives designed to increase public awareness of bumblebee losses (Williams and Osborne, 2009) and compilation of reports highlighting potential causes of bumblebee declines (Winter et al., 2006; Goulson et al., 2008a; Colla and Packer, 2008; Williams and Osborne, 2009). However, despite these important advances, there is still much to learn about bumblebee ecology and the specific factors influencing their declines (Goulson et al., 2008a).

9.1 Provision of spring resources for bumblebees

Conservation management for bumblebees generally focuses on the provision of summer forage for bumblebees (e.g. Carvell et al., 2006b; Pywell et al., 2005, 2006) whilst other resources required for bumblebee success are often overlooked. Spring is a particularly sensitive time in the bumblebee life history, representing the stages of colony foundation and early colony growth (Goulson, 2003a). Many studies assess the efficacy of land management practices for providing summer forage for bumblebees (Carvell et al., 2004, 2006b, 2007; Pywell et al., 2005, 2006) but few consider the value of these for providing nest sites and spring forage. Whilst the availability of summer forage in the rural environment is required to maintain bumblebee populations in this environment, insufficient resource availability in spring may still restrict bumblebee population sizes.

The provision of suitable nesting habitat and spring forage might provide a means of attracting spring queens into the agricultural landscape thus providing a more reliable method of boosting bumblebee population sizes in the rural environment. Data presented here demonstrate that Scottish Rural Stewardship agri-environment prescriptions designed to promote floral abundance and diversity do not promote early flowering species and are unable to provide suitable forage resources for bumblebee queens in spring (chapter 6). This is also likely to be the case for many other similar schemes across Europe. However, prescriptions involving the sowing of tussock-forming grasses can provide attractive habitat for nest site searching queens of common bumblebee species, and infrequent disturbance to such habitat also allows the invasion of early flowering 'weed' species such as red and white deadnettle (*Lamium purpureum* and *L. album*) which can provide a source of spring forage for bumblebees. These conditions were found to be fulfilled by the Rural Stewardship field margins studied. Though disturbance of margins was prohibited by the management prescription under investigation, in the real farm situation, occasional disturbance by movement of farm machinery over the margins appeared to be inevitable. This appeared to increase the value of this prescription for bumblebees.

Research presented in this thesis suggests that simple field margin prescriptions can provide both nest sites and forage for spring bumblebee queens, perhaps thereby encouraging increased rates of colony foundation within the agricultural environment. However, field margin prescriptions already have an extremely high uptake rate by farmers in the UK compared with more targeted prescriptions such as those designed to provide forage resources for bumblebees (C. Carvell, pers. comm.). Since this work suggests that general prescriptions designed to promote floral abundance may do little to provide spring forage for bumblebees, continued investment in the development of flower mixes with a particular focus on ensuring forage availability across the whole season may be the most effective use of resources for supporting bumblebee populations in agricultural land.

Many studies examining the effectiveness of different agri-environment schemes for promoting biodiversity are carried out under experimental conditions in which prescriptions are followed by the investigator or institution undertaking the research. The study presented in this thesis (chapter 6) examines management prescriptions as they translate to genuine farm situations, as implemented and maintained by farmers. It therefore provides a much more realistic representation of the effectiveness of these schemes. However, perhaps as a result of this, there is considerable variation between replicates of each habitat and management type, for example the vegetation characteristics of the different treatments varied greatly among sites (see table 6.2). Though this study revealed interesting patterns regarding the overall efficacy of each treatment type for providing spring habitat for bumblebees, more detailed characterisation of vegetation structure and independent analysis of these factors against bumblebee abundance would have allowed a greater depth of information to be gathered on the exact habitat requirements of spring bumblebee queens. This approach would be particularly valuable if applied to nest site searching queens as little is known about the nesting habitat required by bumblebees yet this information is vital for ensuring that the conditions for a healthy bumblebee community are fulfilled.

Additionally, since nest site searching bumblebee queens may not be a good indicator of actual nest founding (see discussion in chapter 6), the development of a more reliable measure of bumblebee nest density would be of value, though this has been attempted many times with little success. Perhaps more realistically, it may be valuable to assess the effects of treatment type and/or vegetation structure on other factors that might be indirectly related to nest site availability. For example, those prescriptions that promote small mammals are likely to be beneficial since bumblebees often found nests in the abandoned homes of small mammals thus abundance of these mammal species may provide another indirect measure of nest site availability for bumblebees.

Whilst this research addresses the provision of forage and nest sites early in the season, the survival of local bumblebee population relies on the presence of many other resources not addressed here, including summer forage, male patrolling sites and hibernation sites. It would be useful to combine the methodology used here with that used in studies assessing seasonal forage availability and if possible, measures of nest survival and reproduction to get a better idea of the overall value of different land management practices for bumblebees. Whilst the latter is difficult due to the difficulty in locating nests, a current study addresses this question using genetic techniques to identify nest mates across time (Goulson et al. in prep) and methodology such as this may provide a valuable alternative to traditional observational work.

9.2 The nesting ecology of bumblebees

One difficulty in ensuring the successful provision of nest sites for bumblebees is that very little known about their species-specific nest site requirements. Determinants of colony survival are also little understood, yet knowledge of the effects of environmental change at the colony level are vital if conservation efforts for bumblebees are to be successful (Williams and Osborne, 2009). One method for increasing nest site availability and facilitating study of factors affecting colony success would be to use artificial domiciles within which bumblebee queens will found colonies that can then be observed and/or manipulated. This has been attempted with varying success in previous studies (chapter 2).

Variation in success of artificial domicile studies may be attributable to a range of factors including geographic or temporal variation in factors such as nest site availability for bumblebees or propensity of the bumblebee species present to utilise artificial domiciles. It also seems likely that published studies represent a subset of studies that is biased toward positive results due to a common tendency towards underreporting of negative results. It is notable that most recent studies attempting to attract bumblebee queens to nest in artificial domiciles report extremely low success rates (chapter 2).

In a study presented here, six different domicile styles trialled in the UK attained average occupancy rates of just 2% demonstrating that, in Britain at least, artificial domiciles may not be effective in providing nest sites for bumblebees or as a means of procuring colonies for study (chapter 3). However, even within this study, uptake rates were extremely variable with one domicile style achieving an uptake rate of 45% at one site, suggesting that a greater understanding of the factors affecting uptake rates might enhance the efficacy of this methodology. There are many alternative explanations for the observed variation in domicile uptake rates observed within this study. For example, due to the un-standardised nature of the design of the experiment presented in chapter 3, there is no way to distinguish between the efficacy of different domicile designs and the potential effects of geographic location and local habitat variables. Similarly, the appearance of the entrance to the domicile (in this case a simple tunnel versus the more natural-looking ‘tile overhang’ entrance style) may be very important in determining which domiciles are investigated by bumblebee queens. However, since domiciles of each entrance type were not trialled alongside one another, it is impossible to ascertain whether or not this was an important factor determining success rates. During the course of this PhD project, further trials attempting to test this were conducted in the woodland habitat type, including blocks consisting of every domicile type trialled during the course of the study, each present both with and without tile overhang entrances. However, due to extremely low occupancy rates (<1% overall), results were not reported in this thesis.

It seems likely that low occupancy rates of artificial domiciles for bumblebees often occur as a result of poor placing. Both from the study presented here (chapter 3) and from those summarised in the domicile review (chapter 2), it appears that success is generally higher when domiciles are placed in sites known to be investigated by high densities of nest sites searching bumblebee. Therefore it is likely that such areas might provide more suitable locations for trialling artificial domiciles and should be used for any future studies aiming to do this.

There is also a possibility that uptake rates of artificial domiciles could be enhanced by the utilisation of odour baits. Work presented in this thesis suggests that nest site searching queens of *B. terrestris* can be attracted by odours associated with aged bank vole nest material (chapter 4). This potential attraction may reflect a method used by queens to aid location of suitable nest sites since the abandoned small mammal homes often used as nest sites by this species (chapter 5). However, the conditions under which the experiment were carried out were not adequate to draw firm conclusions regarding this hypothesis. For example, the storage conditions of the old nest material trialled was not representative of conditions under which aging of the material would occur in the field and this may have influenced the results of this study. It would be useful to trial a range of fresh, and more naturally aged small mammal nest materials with bumblebee queens of several different species in order to confirm the effect observed here and to ascertain any potential of the material to be used as bait for artificial domiciles. Similarly, the conditions under which the queens were expected to perform were very artificial and behaviour observed may not have been representative of nest site searching behaviour under field conditions. More extensive field trials may solve this problem. If nest site searching bumblebee queens could be confirmed to demonstrate an attraction response to small mammal nest material, this would have profound implications for our understanding of the nest site searching behaviour of bumblebee queens.

A nationwide bumblebee nest survey presented in this thesis produced an extensive database of the characteristics of wild bumblebee nests found by members of the public across the UK. These data demonstrated species-specific differences in nest site choice in the urban environment and the propensity of different species to use the abandoned homes of other animals as nesting sites (chapter 5). The success of this study demonstrates the power of 'citizen science' for answering ecological questions and contributing to conservation efforts. There has been a general increase in public awareness of global declines in biodiversity and as a result, many are keen to contribute to scientific programs that will help to protect local species. The internet can now be used to reach a wide range of people and to harness this enthusiasm for the generation of useful datasets allowing simultaneous collection of many data points across a wide geographic range. Although there can be issues relating to the ability of recorders to correctly identify species or to follow protocols correctly, these problems can generally be resolved, for example, by asking for photographs to accompany records and for descriptions of how recorders implemented the methodology laid out. Public surveys are of particular value for large-scale monitoring projects and should be considered by organisations and researchers wishing to carry out such studies.

9.3 Potential declines of common bumblebee species

Although artificial domiciles for bumblebees have yielded low success rates in recent studies, more historical studies often report relatively high uptake rates (chapter 2). The effective population sizes of bumblebee populations are not easy to estimate due to difficulty in locating nests, and as a result, evidence for bumblebee declines comes mainly in the form of observations of range restrictions (Williams, 1986). However, a general decrease in uptake rates of artificial domiciles over time may suggest that species that were formerly found inhabiting domiciles are now less abundant than they once were. This is a concerning prospect since many of the species that were regularly reported to inhabit artificial domiciles (such as *B. lapidarius* and *B. terrestris* – Sladen, 1912) are still commonly observed and are therefore not considered to be threatened by the same processes that have caused declines in some rarer species (Williams et al., 2009).

Further evidence to suggest that currently common bumblebee species may also be showing signs of decline can be observed by comparing results of the public bumblebee nest survey conducted here with those presented in similar studies carried out previously (chapter 5). Comparing nest records from 1989-1991 (Fussell and Corbet, 1992) to those collected in 2004 (Osborne et al., 2007) and in 2007-2008 (chapter 5), the proportional representation of the ‘browns’ division of the colour group system used in previous studies (for which most observations represent *B. pascuorum*) has decreased by ~10% whilst other species have increased or remain the same in proportional representation, suggesting that this species may declining in abundance in the urban environment. Since this species is the only species of medium tongue-length in the UK, its decline might have important implications for the pollination of several plant species.

However, care must be taken when interpreting the results of comparisons among surveys. Since the exact geographic distribution of records from the initial survey is unknown, it is possible that these differences represent biases associated with geographic differences rather than the outcome of temporal change. Additionally, each study represents a temporal snapshot such that data may be heavily influenced by chance variations in environmental conditions, pressure from natural enemies and other short-term factors.

Since there are good historical distribution data for bumblebee species in the UK, British species tend to be designated as of conservation concern if they have demonstrated range restrictions. However, few long-term data are available on species abundance and there is therefore no way to quantify changes in bumblebee abundance over time. If the suggestion of declines in the abundance of common species is to be validated, there is a need for long-term quantitative data in order to build up a picture of long-term trends. Such data will also allow comparison of the yearly success of different species with variables such as weather conditions, providing further insights into the effects of environmental factors on bumblebee populations. A long-term UK-wide regular transect monitoring scheme such as that currently being trialled by the Bumblebee Conservation Trust (D. Goulson, pers. comm.) should be an ideal method for providing regional data on species abundance over time.

9.4 A possible role of climate change in bumblebee declines

The drop in the proportional representation of *B. pascuorum* between the 1989-1991 bumblebee nest survey and more recent surveys has been accompanied by a shift in the position in which nests of this species tend to be discovered. In the historic survey, 'browns' were more frequently observed nesting on the surface of the ground (the known preference of *B. pascuorum* – Sladen, 1912), however there is a greater propensity for nests of this species to be discovered below the ground in the more modern surveys (though see caveats above). This suggests either a change in habit of this species, or perhaps a change in the fate of colonies sited in different positions. Since the recent surveys have been carried out in years with greater rainfall than those in which the original survey was conducted, it is possible that the pattern observed reflects a greater tendency of nests built on the surface of the ground to succumb to poor weather. With many climate change models predicting increasingly wet weather in the UK (e.g. Jones and Reid, 2001; Ekstrom et al., 2005), surface nesting species such as *B. pascuorum* may suffer increasing yearly losses as a result of this fate. Similarly, *B. lapidarius* has been observed to be particularly susceptible to poor weather (Sladen, 1912), and this is supported by an almost 10% drop in observations of this species between 2007 and 2008, coinciding with a much greater rainfall in the latter year (http://www.metoffice.gov.uk).

Many bumblebee species coexist with one another despite very similar forage usage and the mechanisms of resource partitioning among such species are little known (Goulson et al., 2008b). In New Zealand, where bumblebees are not native but were introduced from Britain (Hopkins, 1914), niche overlap for forage is artificially high due to the relatively low diversity of bumblebee forage plants. In this situation, there is strong evidence for temporal niche partitioning as a result of differences in diel activity patterns among the species present (chapter 7). The most notable difference is between *B. hortorum* and *B. ruderatus* which are known to have extremely similar dietary preferences even within their native range. The former was found to show greater activity in the mornings and evenings when temperatures are relatively low and humidity is high whilst the latter is more active in the middle of the day when it is warm and dry. The native range of *B. hortorum* is more northerly than that of *B. ruderatus* and this suggests that these patterns might reflect differences in climatic adaptation between the two species. Similar differences in diel activity among species have been observed by Hasselrot (1960) in Sweden suggesting that these mechanisms may be widespread. If such partitioning is determined by the environmental tolerances of the species involved, increases in average temperatures as a result of climate change may cause earlier emergence of more southerly species, pushing these into competition and causing mechanisms of coexistence to break down.

However, it must be borne in mind that the situation in New Zealand is not representative of native bumblebee assemblages. Further research is required to establish how widespread this method of niche partitioning may be. Dawn until dusk surveys of foraging activity in different bumblebee communities would provide a method of doing this. Where possible, it would be particularly interesting to compare situations in which inter-specific competition for forage resources would be expected among two species, to situations in which each of the two species existed in the absence of the other. If daily activity were different among these different community types, this would provide strong evidence that differences in diel activity patterns do indeed represent niche partitioning among species.

9.5 Consideration of genetic factors in bumblebee conservation

Genetic diversity is often important for the persistence of healthy communities since its loss can lead to a reduced ability to adapt to changes in environmental conditions and to inbreeding depression (Frankham et al., 2004). Small populations are also likely to suffer from the fixation of deleterious alleles as a result of genetic drift, although in bumblebees, this effect may be reduced as a result of partial purging of the genetic load through haploid males (Antolin, 1999; Werren, 1993). Evidence suggests that some bumblebee species are well able to cope with low levels of genetic diversity (Schmid-Hempel et al., 2007), but whether this is sustainable in the long-term remains to be seen. A genetic comparison of bumblebees introduced into New Zealand at the turn of the last century (Hopkins, 1914) and UK bumblebee populations of the same species suggest that *B. hortorum* and *B. subterraneus* in New Zealand retain reduced genetic variation compared to that likely to have been found in the original UK populations, probably as a result of a population bottleneck upon their introduction into New Zealand (chapter 8). These populations have survived for over 100 years suggesting little or no impact of these losses in genetic diversity. However, recent data suggest that *B. subterraneus* may be declining in New Zealand. Whilst this has been attributed to reductions in forage availability as a result of changes in land use (Goulson and Hanley, 2004) it is possible that negative genetic effects associated with low genetic diversity also play a role in this process.

B. subterraneus is now extinct in the UK but a reintroduction attempt is underway with the aim of using New Zealand *B. subterraneus* to recolonise suitable habitat in southern England (Howlett et al., 2009). A comparison of allele frequency at microsatellite loci between museum specimens of *B. subterraneus* from the original UK population and individuals from the current New Zealand population demonstrate that the New Zealand population exhibits dramatically reduced genetic diversity compared to the original UK population (chapter 8). The comparison also reveals that the New Zealand population is highly differentiated from the original UK population. These findings raise doubt as to the suitability of the New Zealand population as a source population for reintroduction into the UK. Since healthy populations of this species can be found elsewhere in Europe, it is suggested that the inclusion of individuals from healthy populations of the same species within Europe be considered as a means of increasing the genetic diversity contained within the reintroduced individuals.

9.6 Management recommendations

1. Given current levels of understanding of nest site choice in bumblebees, artificial domiciles are unlikely to provide a realistic solution for ensuring adequate nest site availability for bumblebees. The provision of relatively undisturbed areas of natural or semi-natural habitat in areas where nest sites may be scarce is likely to be a more successful and sustainable method of increasing nest site availability for bumblebees and will also give conservation benefits across many other taxa.

2. The species-rich grassland prescription investigated here did little to provide spring forage for bumblebees. Continued investment in developing prescriptions

promoting forage availability, with a particular focus on providing resources throughout the spring and summer, would be beneficial.

3. Infrequent, low-intensity disturbance of semi-natural habitat can benefit many colonising plant species and as a result, can provide an economic source of spring forage for bumblebees. Conservation management focused on providing resources for bumblebees should consider the potential role of this type of disturbance in management prescriptions for these species.

4. Data presented here suggest that bumblebee declines may not be limited to those species currently recognised to be of conservation concern. There is a strong need for regular and continuous monitoring programs that can produce the quantitative data required to assess changing patterns of abundance of different bumblebee species.

5. Evidence presented here and elsewhere demonstrates that different bumblebee species are active at different times during the day and this may provide a mechanism of niche partitioning among species. Dawn until dusk studies carried out in a variety of bumblebee communities would further test this hypothesis and could produce valuable insights on patterns of coexistence and factors that may influence these.

6. Reintroduction attempts should always be followed by careful monitoring of the released population. Given the findings of this research, it would be particularly interesting to conduct careful long-term monitoring of reintroduced *B.*

subterraneus in the UK to assess survival and success in light of the genetic background of the source population. If the reintroduction is not successful, the supplementation of the introduced population with *B. subterraneus* of European origin may be beneficial.

9.7 Conclusions

Bumblebees are keystone species providing a pollination service for a very wide range of plant species throughout their range including many crops on which we rely. Bumblebee declines are therefore of ecological and economic concern. Whilst this is recognised, several aspects of bumblebee ecology remain understudied and several facets of their requirements are routinely ignored in attempts to understand their declines and to conserve rare species. This thesis draws together several important aspects of bumblebee ecology, contributing to our understanding of species interactions in bumblebee communities and genetic processes relating to small population sizes in bumblebee populations. It also highlights the need for further research into the nesting ecology of bumblebees and the potential impacts of climate change on bumblebee populations and emphasises the importance of provision of habitat for spring bumblebee queens, providing a basis for future work in these areas. Above all, this work demonstrates that an integrated approach to bumblebee conservation is absolutely vital if we are to conserve these important and charismatic pollinator species.

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