The Nesting Ecology of Bumblebees

Stephanie Alexandra O’Connor

2013

A thesis submitted for the degree of

Doctor of Philosophy
Institute of Biological & Environmental Sciences
School of Natural Sciences
The University of Stirling

Summary abstract

Bumblebees have undergone dramatic declines both in Britain and further afield during the last century. Bumblebees provide a crucial pollination service to both crops and wild flowers. For these reasons, they have received a great deal of research attention over the years. However, the ecology of wild bumblebee nests and the interactions between nests and other species, particularly vertebrates has been somewhat understudied. This is largely due to the difficulty in finding sufficient nests for well replicated study and a lack of appropriate methods of observation.

Here, methods for locating bumblebee nests were trialled. It was found that a specially trained bumblebee nest detection dog did not discover nests any faster than people who had received minimal instruction. Numbers of nest site searching queens provide a reliable indication of suitable nesting habitat (i.e. places where nests are more likely to be found).

In order to investigate aspects of bumblebee nesting ecology wild nests were observed by filming or regular observations by either researchers or members of the public. Some *Bombus terrestris* nests were collected and all the bumblebees were genotyped to identify any foreign individuals. A review of British mammalian dietary literature was conducted to identify those that predate bumblebees.

Great tits (*Parus major*) were filmed predating bumblebees at nests and it was clear from the literature and observations that badger (*Meles meles*), pine martens (*Martes martes*) and hedgehogs (*Erinaceus europaeus*) predate bumblebee nests, as well as the wax moth

Aphomia sociella. No evidence for predation by any other vertebrate species was found. Behaviours recorded included parasitism by *Psithyrus*, apparent nectar theft and possible usurpation by true bumblebees, egg-dumping by foreign queens and drifting and drifter reproduction by foreign workers. These events may cause harm to colonies for example, through horizontal transmission of pathogens, or exploitation of the host nest's resources). Alternatively where for example, usurpation by true bumblebees, egg-dumping or drifting is successful, these alternative reproductive strategies may increase the effective population size by enabling a single nest to produce reproductives of more than one breeding female. These data found that wild *B. terrestris* nests with a greater proportion of workers infected with *Crithidia bombi* were less likely to produce gynes than those with fewer infected workers. Gyne production also varied dramatically between years.

There is a growing body of evidence that a class of frequently used insecticides called neonicotinoids are negatively impacting bumblebees. An experiment was conducted using commercial colonies of *B. terrestris* which were fed pollen and nectar which had been treated with the neonicotinoid imidacloprid at field realistic, sub-lethal levels. Treated colonies, produced 85-90% fewer gynes than control colonies. If this trend is representative of natural nests feeding on treated crops, for example, oilseed rape and field beans or garden flowers, then this would be expected to cause dramatic population declines.

In this thesis methods for locating bumblebee nests have been tested, new behaviours have been identified (for example, egg-dumping by queens and predation by great tits) and estimations for rates of fecundity and destruction by various factors have been provided.

Doubt has been cast over the status of some mammals as predators of bumblebee nests and estimates for gyne production, nest longevity, etc, have been given. More work is needed, especially observations of incipient nests as this is when the greatest losses are thought to occur.

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.

SA O'Connor

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Stephanie Alexandra O'Connor

Acknowledgements

I consider myself incredibly fortunate to have been given the chance to research bumblebees and especially bumblebee nests which have enthralled me for years. This fantastic opportunity and experience has been entirely due to Dave Goulson. I have enjoyed my time working with you immensely and I can't imagine my project ever getting this far without your unfailing enthusiasm, support, optimism, and crucial "it'll be fine" mantra! I can't thank you enough for all your help.

Kirsty Park has also provided a great deal of support during my project and her no-nonsense comments on my experimental design and manuscripts has been invaluable. I would also like to thank Luc and Matt for their input, particularly during my earlier years.

Thanks are due to my many field assistants without whom this work would not have been possible, (I am of course delighted that I have been able to give the gift of 'finding a bumblebee nest' to so many)! I would like to say a huge thank you to the hundreds of people who reported and watched nests and to the land owners who allowed me to run experiments on their ground, as well as interfere with and occasionally abduct their bumblebees. In particular I would like to thank Iain MacFarlane, John Cross and the Muirhead Clan. Alison and Rowan Muirhead deserve a further mention for putting up with Toby and I as lodgers for several years. I like to think both have developed something of a love for bumblebees because of us!

I would like to thank all staff in the department, both past and present for making my time at Stirling a true pleasure. There was always someone on hand to offer advice, plenty coffee room banter and engrossing seminars. In particular, Lynn for handling and explaining project accounts, Scott for his instruction on all computer related issues and Ronnie, James and Willy for assisting with various vague and urgent construction projects. I would also like to thank Juliet Osborne at Rothamsted Research for assistance with one chapter and also thanks to Gordon Port and Mark O'Neill at Newcastle for getting me interested in bumble bees all those years ago!

The presence of the Bumblebee Conservation Trust was a key factor in my preference to become part of Stirling's bumblebee research team. Through the BBCT and the University's media team, I have had the opportunity to some of my work to public audiences on several television and radio programs. This has been both a great experience and useful in reminding me that there are many people in the real world, outside of academia who care deeply about the environment and bumblebees.

Thank you to my wonderful husband Kerr, to Kay and all of the Cessford family for supporting me during my final years and not being too cross with my references to 'intensification of agriculture' or stirring up issues with insecticide legislation.

My parents have always encouraged me to follow my love of animals and nature. I am grateful to them for supporting me during my first degree, keeping me on track through my time at Stirling and allowing me to collect plenty of pets while growing up! My brother James has been a source of constant support, and has always taken a keen interest in my work (which I like to think is because he sold out his science career for accountancy). As

well as being good humoured, James inflicts an enraging brand of motivation by his refusal to acknowledge any defeatist or negative attitude, upon those around him. A far more sympathetic ear could always be found from my dear friend Claire Stead. Thanks to all of the bumblebee research team and other PhD. students who have assisted and supported me. Special thanks go to Nicky, Dani, Jenny, Jess, Penelope and of course Gillian. I wouldn't have made it without you all and I certainly wouldn't have such fond memories: (Terrifying midnight hose reels; getting stung in the 'bee rearing-room', dog walking and training, sweltering greenhouses and sitting out storms under hedgerows). I treasure every experiment that Gillian and I attempted; from the construction of 400 pointless bumblebee nest-boxes to the delights of queen collection in Ullerpool and surprisingly bee-free transects in the Scottish borders. Thank you for your constant help, advice and friendship, your confidence in me has been overwhelming throughout.

Things didn't go entirely to plan... Toby didn't find as many nests as I'd hoped, the camera system employed was not the first one tested and my attempts at rearing nests from queens didn't go too well. I mention these things because it wasn't easy, and the people whom I have thanked in the above *really* had their work cut out. The completion of this project is as much of a credit to them as it is to me, so thank you all once again.

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Publications arising from this thesis

O'Connor, S., Park, K.J. and Goulson, D. (2012) Humans versus dogs; a comparison of methods for the detection of bumble bee nests. *Journal of Apicultural Research* 51, 204-211.

O'Connor, S., Park, K.J. and Goulson, D. (2013) Worker drift and egg-dumping by queens in wild *Bombus terrestris* colonies. *Behavioral Ecology and Sociobiology* **67**, 621-627

Whitehorn, P.R., O'Connor, S., Wackers, F.L. and Goulson, D. (2012) Neonicotinoid pesticide reduces bumblebee colony growth and queen production. *Science* 336, 351-352.

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Chapter 1

General introduction

1.1 General introduction

Bumblebees are social hymenopterans (Suborder: Aculeata, Superfamily: Apoidae:) which predominantly occur in the northern hemisphere. Approximately 250 species have been recorded globally and of these, 22 species currently occur in Britain, including both true bumblebees and six species belonging to the subgenus *Psithyrus* (Benton, 2006). British bumblebees have suffered from agricultural intensification, largely through loss of favourable habitat (Alford, 1975; Williams, 1982; Williams, 1986; Robinson and Sutherland, 2002; Goulson et al., 2008; Williams et al., 2008).Three bumblebee species have already gone extinct from the UK; *Bombus pomorum* and *Bombus cullumanus* have not been recorded since the late 1800's and early 1900's respectively (Sladen, 1912; Alford, 1975) and *Bombus subterraneus* was observed at several sites in the south of England as recently as the 1960's but has since gone extinct in the UK (Alford, 1975; Williams, 1982; Goulson, 2010), although if recent reintroduction attempts prove successful, it may be present in Britain once again. Only six species of true bumblebee are considered common and widespread throughout Britain*; Bombus terrestris*, *Bombus lucorum*, *Bombus lapidarius*, *Bombus pratorum*, *Bombus hortorum* and *Bombus pascuorum*. The ranges occupied by several species have dramatically contracted over the past 100 years; for example, *Bombus distinguendus* was detectable across much of the UK in the 1940-1950's but is now limited to the far north of Scotland and associated islands (Hebrides, Orkney and Shetland), notably remaining in areas which have undergone agricultural intensification to a lesser extent (Alford, 1975; Redpath et al., 2010). Other pockets of bumblebee diversity exist, for example, Salisbury plain has escaped intensification, due to designation as a military training ground, and is an area rich in bumblebee species (Carvell, 2002; Goulson and Darvill, 2004).

One species has recently arrived in Britain; *Bombus hypnorum* was first recorded in the South of England in 2001 (Goulson and Williams, 2001) and is now well established (Benton, 2006; Lye et al., 2012).

Bumblebees are important pollinators of wildflowers (Corbet et al., 1991; Fussell and Corbet, 1992a; Osborne and Williams, 1996; Biesmeijer et al., 2006; Winfree, 2010; Thakur, 2012), and have been considered useful bioindicators and a keystone group of species (Chapman and Bourke, 2001; Goulson et al., 2002a; Pearce et al., 2012) because their pollination service influences the ecosystem disproportionately to their abundance. Many plants are pollinated by bees, and some in particular by bumblebees, for example, *Digitalis purpurea* (foxglove) is almost entirely pollinated by bumblebees (Broadbent and Bourke, 2012) and rarer flowering species and spring flowering plants such as *Ribes sanguineum*, (flowering current) *Erica* spp. (heathers) and orchids (Corbet et al., 1991; Osborne and Williams, 1996) rely on bumblebee pollination. Bumblebees also contribute to the pollination of a large number of agricultural crops; over two-thirds of crop species require insect pollination to attain maximum yields (Williams et al., 1987; Kremen et al., 2002; Greenleaf and Kremen, 2006; Klein et al., 2007). In some cases, bumblebees provide a superior pollination service compared to *Apis mellifera* for example, bumblebees were able to pollinate more than three times more *Vaccinium corymbosum* (blueberry) blossoms per minute than honey bees at farms in Oregon, USA (Daly et al., 2013) see also; *Trifolium pratense* (clover), *Medicago sativa* (alfalfa), *Vicia faba* (broad beans) and *Lycopersicon esculentum* (tomato) (Corbet et al., 1991) and *Rubus idaeus* (raspberry), (Willmer et al., 1994). Many members of the Fabaceae family are preferential food plants of bumblebees

(Pywell et al., 2005). For example, bumblebees were introduced to New Zealand from Britain in the late 1800's to early 1900's in order to achieve satisfactory pollination of clover fields in order to provide adequate forage for sheep and cattle (Hopkins, 1914; Cumber 1953; Alford 1975; Edwards and Williams, 2004). Bumblebees pollinate species from a range of families, in particular, those of Boraginaceae, Ericaceae, Iridaceae, Lamiaceae, Orchidaceae, Solanaceae and Fabaceae (Carvell, 2002; Edwards and Williams, 2004; Goulson 2010). Members of the Fabaceae family in particular are preferred forage plants of bumblebees, due to the high proportion of protein and essential amino acids of Fabaceae pollen (Carvell, 2002; Goulson and Darvill, 2004; Goulson et al., 2005). To appreciate the extent of the ecosystem service provided by bumblebees, one must consider the position that Fabaceae plays in agriculture. For example, clovers and alfalfa among others are used as high protein animal feed (Cumber 1953). Species of the Fabaceae family are also essential as 'green manure' and ley crops which are used in less oil dependent agricultural systems (Goulson 2010). With this in mind, understanding bumblebee ecology is critical to halting species declines and developing sustainable agricultural practices. A lack of understanding of the nesting ecology of bumblebees may negatively affect conservation efforts to mitigate their decline (Edwards and Williams, 2004).

1.2 Overview of bumblebee nests

1.2.1 Life cycle

The majority of bumblebee species have an annual lifecycle. Inseminated queens emerge from hibernation from February to June, depending on their geographical location and species (Alford, 1975). Initially they consume pollen and nectar which enables their depleted fat reserves to be replenished and their ovaries to develop. Young queens then spend several days or weeks locating a suitable nest site (Free and Butler, 1959) and furnish it with a marble sized ball of pollen and a single wax cell which they fill with nectar. The queen lays her first clutch of eggs, typically 6-16 (Sladen, 1912; Free and Butler, 1959; Goulson, 2010) in the pollen ball and incubates them with her body heat. The eggs develop into larvae, which grow through four instars before pupating approximately 3-5 weeks after the eggs are laid (Alford, 1975; Duchateau and Velthuis, 1989; Duchateau et al., 2004, pers. obs.). The newly eclosed workers begin to assist the queen with foraging and nest care, for example, incubating and feeding the brood, removing dead or defective individuals, etc. Successive batches of workers are reared and nests may grow large, containing up to several hundred workers depending on the species. Thriving *B. terrestris*, *B. lapidarius* and *B. lucorum* nests may rear 300-400 workers (Free and Butler, 1959), whereas nests of other species rarely contain more than 100 workers at their peak of maturity (Sladen, 1912; Free and Butler, 1959).

At some point, the nest switches to producing reproductives i.e. males or females destined to become queens, or more accurately, gynes (i.e. a queen bumblebee that has not yet founded a nest). Males leave and do not return, but gynes frequent the nest for several days (Sladen, 1912; Alford, 1975), during which time they must increase their fat stores and mate. Males and gynes have species-specific mate location/selection criteria. Males in the majority of species patrol a route of several hundred metres and scent mark at prominent landmarks along this route (for example, trees or clumps of vegetation, (Sladen, 1912; Alford, 1975; Svensson, 1979)). In contrast, males of other species, for example, *Bombus muscorum* and

B. hypnorum congregate outside nests and attempt to mate with gynes as they exit or enter the nest (Alford, 1975; Benton, 2006; Darvill et al., 2006).

Microsatellite analysis of queen spermatheca and nest mates has demonstrated that most of the species of bumblebees that have been examined are monandrous (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000). An examination of colonies of three North American species, *Bombus perplexus* (n=24), *Bombus occidentalis* (n= 23) and *Bombus terricola* (n=21) revealed all, except for two of the *B. perplexus* colonies, were singly mated (Owen and Whidden, 2013). In European bumblebees, monandry is also prevalent, for example, Schmid-Hempel and Schmid-Hempel (2000) found only monogamy in *B. terrestris*, *B. lucorum*, *B. pratorum*, *B. lapidarius*, *B. hortorum* and *B. pascuorum* (for 17, 12, 5, 11, 5, and 6 nests of each species respectively). However, *B. hypnorum* was polygynous; four of seventeen *B. hypnorum* queens had mated twice (Schmid-Hempel and Schmid-Hempel, 2000). Similarly, when looking at *B. terrestris*, *B. lapidarius*, *B. pratorum*, *B. hypnorum* and *B. lucorum*, Estoup et al. (1995) reported only *B. hypnorum* to be polygynous; in two of the three nests analysed, the founding queens had mated twice and four times respectively). Monandry was also found in 32 *B. terrestris* queens (Lopez-Vaamonde et al., 2004).

Following mating, male bumblebees insert a mating plug into the queens' reproduction tract (the bursa copulatrix), which is thought to prevent or reduce backflow of sperm, and reduce the success of any subsequent copulation attempts (Duvoisin et al., 1999). Sperm plugs last for approximately two days in mated B. terrestris queens (Duvoisin et al., 1999), but only for a few hours in *B. hypnorum* queens, suggesting that *B. hypnorum* may have adapted to allow multiple mating (Brown et al., 2002). However, while European studies of wild *B. terrestris* have only found evidence for monogamy, multiple mating of *B. terrestris* queens has been reported in the laboratory (Duvoisin et al., 1999) and in feral populations in Japan. Colonies of *B. terrestris* were imported into Japan for the purpose of crop pollination. Escapees of these colonies established a feral population (Matsumara et al., 2004; Inari et al., 2005) and in an analysis of the spermatheca of nine feral *B. terrestris* queens, one was found to contain sperm from at least two matings (Inoue et al., 2012).

The rate of polyandry may differ according to geographical region. Records from France identified polygamy in two of three *B. hypnorum* colonies, whereas similar studies revealed it in two of fourteen colonies in Switzerland (Estoup 1995, 2000) and in seven of fourteen colonies from Sweden (Paxton et al., 2001). Even in the laboratory, when offered a choice of mates in flight cages, *B. hypnorum* queens do not always mate multiple times, for example, of 72 *B. hypnorum* queens, only 16 mated again the following day (Brown et al., 2002). The reasons for variation in likelihood of multiple mating remain unclear (Paxton et al., 2001; Brown et al., 2002).

Mated queens dig a hibernaculum, a tunnel about 10-15 cm long in which they will overwinter, typically in a northern facing slope with sandy soil (Alford, 1975). Queens remain in their hibernaculum until the following spring. A few species may complete two lifecycles within a single year; most notably *B. pratorum* which emerges early and has small, short lived colonies of typically fewer than 100 workers.

A few species of bumblebees are thought to be capable of rearing multiple (two, but theoretically more) broods in a single season under favourable conditions (Alford, 1975). For example, *Bombus pratorum* in the south of Britain during warm summers is thought to 'double brood'. However, evidence for this phenomenon consists mainly of observations of nests or fresh queens in late summer. These records may be the result of nests which have been delayed or persisted longer than typical *B. pratorum* nests rather than evidence for 'double brooding'. In addition, since the year 2000, *B. terrestris* queens and workers have been increasingly recorded in winter months, mostly in southerly cities such as London (Goulson, 2010; Stelzer et al., 2010). Artificially reared and colonies of *B. terrestris* were able to forage effectively on ornamental plants in gardens and glasshouses such as *Mahonia spp.* and maintain the nest throughout the winter. It seems likely that where nests are able to persist through winter months, two broods of reproductives may be reared.

Arctic bumblebees such as *Bombus polaris* have adapted to the brief summer of the region and typically rear only one brood of workers before initiating the production of gynes and males (Heinrich, 1993; Goulson, 2010).

In this thesis a nest is defined as any attempt from a queen to establish a nest, regardless of whether workers or reproductives were produced (after Donovan and Wier, 1978; Pomeroy, 1981). Evidence for attempted nest establishment consisted of a queen (i) entering the same hole more than once, (ii) carrying pollen in her corbicula (pollen baskets). Queens exhibiting nest-site seeking behaviour (flying in a zigzag pattern, investigating holes, etc,) were not regarded as nest attempts.

1.2.2 Haploid-diploid sex determination

Bumblebees have a haplo-diploid system of sex determination (Cook and Crozier, 1995). Unfertilised eggs, containing half of the mother's genome (and therefore, haploid) develop into males. Female bumblebees are diploid, inheriting DNA from both of their parents and develop from fertilised eggs (Cook and Crozier, 1995). The mechanism behind this sex depends upon an individual being homozygous or heterozygous at the complimentary sex determining locus (Duchateau et al. 1994; Gadau et al., 2001). Bumblebees that are homozygous for the sex locus develop into males where as bumblebees which are heterozygous at sex loci develop into females. It is possible for diploid bumblebees to inherit from each of their parents the same alleles for the sex loci and such diploid bumblebees develop into males. Diploid males have been detected in more than forty hymenopteran species (Zhishan et al., 2003) and have been found in bumblebees in laboratory inbreeding mating, for example, sibling crosses (Duchateau et al. 1994; Whitehorn et al., 2009). Diploid males are reared by the colony but do not assist with nest duties such as foraging, they are therefore considered to be a costly form of inbreeding depression. It is also possible for diploid males to produce triploid daughters, and both diploid males and triploid females have been detected in wild in populations suffering from inbreeding, for example, in fragmented populations of *Bombus muscorum* and *Bombus jonellus* on Hebridean islands (Darvill et al., 2010), fragmented populations of Bombus sylvarum (Ellis et al., 2006) and amongst a feral population of *B. terrestris* in Japan (Nagamitsu and Yamagishi, 2009).

Worker bumblebees do not mate, only queens mate and are therefore capable of producing either male or female offspring (Free and Butler, 1959; Alford, 1975). Workers are able under certain circumstances to develop their ovaries and lay unfertilised eggs which may develop into males (Duchateau and Velthuis, 1989). This only happens where the queen’s dominance is reduced or absent (for example, through death). Queens dominate their workers through physical contact and a pheromone known as ‘queen substance’ which suppresses the workers’ ovarian development (Duchateau, 1989; Cnaani et al., 2000; Alaux, 2004). When the queen begins to lay fertilised eggs destined to become gynes, the level of ‘queen substance’ that she produces is reduced. This is thought to be essential to enable the gynes to develop fully. However, this action also causes a reduction in the queen’s dominance over her workers and some of them begin to develop their ovaries and attempt to reproduce directly. The point at which the first male egg is laid by a worker is known as the competition point (Duchateau, 1989) and female eggs laid after this time will typically develop into queens (Cnaani et al., 2000). Subsequent to this a breakdown of the social order within the nest ensues, typically with many workers attempting to reproduce. Worker bees and the queen endeavour to limit eggs laid by others by eating them, a process known as egg policing (Zanette et al., 2012). This conflict of interests may lead to direct confrontation and the queen may be killed or driven out by her workers. The proportion of males produced by workers varies between species and condition of the nest. In most studies of queen-right bumblebee nests, fewer than 5% of males produced are worker derived, (Duchateau and Velthuis, 1989; Alaux et al., 2004; Lopez-Vaamonde et al., 2004; Takahashi et al., 2010), where the queen dies prematurely (Alaux et al., 2004; Takahashi et al., 2010) this figure can be much higher. Van-Honk et al. (1981b) reported over 80% of males were worker derived after observations of a *B. terrestris* colony, whereas of 233 males derived from 11 *B. hypnorum* nests, no worker reproduction was detected (Paxton et al., 2001).

1.2.3 Nest site preferences

Bumblebees generally establish colonies in the disused nests of other animals; in particular, those of small mammals such as mice (e.g. wood mouse; *Apodemus sylvaticus*), shrews (e.g. common shrew; *Sorex araneus*), and voles (e.g. bank voles; *Clethrionomys glareolus*, and field voles; *Microtus arvalis*) (Sladen, 1912; Fussell and Corbet, 1992b; Lye et al., 2012). Species vary in their preferences for locations of nest sites (Sladen, 1912; Holm 1966; Alford, 1975; Lye et al., 2012). The majority of *B. terrestris*, *B. lucorum* and *B. lapidarius* nests are subterranean, whereas carder bees such as *B. pascuorum* tend to be on the surface, amongst tussocks of vegetation and other species are more plastic in their nest site selection, such as *B. hortorum*, *B. pratorum* and *Bombus sylvarum* (Free and Butler, 1959). Britain's newest species, *B. hypnorum* commonly utilises bird nests in shrubs and trees (Alford, 1975; Benton, 2006). It has been suggested that *B. hypnorum* have a preference for sites close to human dwellings (Løken, 1973), but the other common six species of bumblebees (*B. terrestris, B. lucorum, B, lapidarius, B. hortorum, B. pratorum and B. pascuorum*) have also adapted to foraging and nesting in gardens (Gaston et al., 2005; Lye et al., 2012). Species-specific habitat preferences and, in some cases, micro-site preferences, such as the degree of shade or shelter have been described (Alford, 1975; Svensson et al., 2000; Kells and Goulson, 2003).

1.2.4 Chemical Ecology of Bumblebee Nests

Chemical composition of bumblebee nests has long been hypothesised, since Sladen (1912) stated that he could differentiate between the nests of different bumblebee species based on smell. Free and Butler (1959) also expected that bumblebees within a nest carried a similar chemical signature to one another and this allowed differentiation between fellow nest mates and intruding bees. Such claims have now been substantiated as technological advances have enabled analysis of the chemicals emitted from bumblebee nests. Specifically, gas chromatography-mass spectrometry of dissolved wax and samples of the headspace of worker bumblebees from four *B. terrestris* nests has been used to identify different chemicals emitted (Rottler et al., 2012). The majority of the 76 volatile chemicals detected in nest samples were hydrocarbons, and fifteen of these in particular presented in varying proportions in the four nests and could be used to differentiate between the four nests (Rottler et al., 2012). Chemical signatures of individual bumblebees have also been analysed e.g. Oldham et al. (1994) and Sramkova et al. (2008) described similar patterns of volatile chemicals from cuticle waxes and glands of bumblebees. Martin et al. (2010) investigated species-specific variation in the volatile chemicals from spring-caught queens of 14 species by analysing cuticle hydrocarbons and those secreted from Dufour's gland. Species could be distinguished according to their groups of alkene isomers and this trend was stable over a large area, (samples collected in Finland and UK) (Martin et al., 2010). In addition, cuticle hydrocarbon composition of five psithyrus species was found to either closely mimic the target host bumblebee species, or instead present dodecyl acetate, which has been shown to be strongly repellent to worker bumblebees and is thought to deter attack from natal bees and allow psithyrus queens to enter and take over nests (Zimma et al., 2003). The composition of chemicals emitted from queen bumblebees also varies according to biological stage (Kreuter et al., 2012). Volatile chemicals of *B. terrestris* queens at three biological stages of were analysed: pre-wintered virgins, nest-seeking and breeding as well as breeding queens of the Psithyrus species, *Bombus bohemicus*. In total, 136 volatile compounds were detected in the queens. The bouquets from *B. terrestris* queens, during both pre-breeding stages (virgins and nest seeking) were comparable with one another, but distinct from the volatiles emitted by queens in the breeding stage. In addition, there was a convergence of volatiles between the breeding queens of both species (Kreuter et al., 2012). This study also demonstrated that breeding queen *B. bohemicus* that were in direct contact with *B. terrestris* workers were able to suppress the worker's ovarian development (Kreuter et al., 2012), as has been found for other psithyrus and their host workers (Goulson 2010). However only a small number of nests (four) were examined and these were bred from a commercial, captive line of *B. terrestris*. Such lines are known to have been produced by selecting traits from crosses using *B. terrestris* originally collected from sites throughout Europe. Therefore, it is possible that the chemical composition of wild/local *B. terrestris* colonies may be different to those found in this study (Kreuter et al., 2012).

Workers appeared to be capable of using olfactory cues to identify their natal nest, in a choice-test study by Rottler, et al. (2012). Worker bees were presented with and arena, where they could move to an area flushed with scent from; their natal nest, a foreign nest or a control without nest scent. Worker bees were significantly more likely to move to and remain in areas where scent from their natal nest passed. This strongly indicates that that worker bees are able differentiate between their own and foreign nests based on the combination of volatile chemicals (Rottler et al., 2012).

Bumblebees leave traces of hydrocarbons from their feet, this is thought to assist bees with foraging i.e. flowers with recent (concentrated) footprint scents are likely to have depleted nectar and pollen (Goulson et al., 2000; Saleh et al., 2007). Similar scent marks are left at nest entrances as bumblebees come and go (Saleh et al., 2007). These scent marks, along with any bouquet emanating from the nest are thought to be used by psithyrus queens to locate host bumblebee nests (Kreuter et al., 2010). It may be that other enemies of bumblebee nests such as badgers also locate nests in this way, but there is no data to suggest this.

1.3 Determinants of nest success

A colony of bumblebees must prevail for at least several weeks (given that a brood of workers requires approximately three to five weeks to develop from egg to adult) if it is to succeed in producing males and gynes. There are many factors that can cause a nest to fail. Some such as flooding or fire are stochastic abiotic events; biotic factors are considered here.

1.3.1 Predation

Bumblebee nests in Britain suffer predation from several species. Wood mice (*Apodemus sylvaticus*) and common shrews (*Sorex araneus*) attack incipient nests before the first brood of workers emerges (Sladen, 1912; Pouvreau, 1973). Other mammals such as European mole (*Talpa europaea*), stoat (*Mustela nivalis*), badger (*Meles meles*) and red fox (*Vulpes vulpes*) are also thought to depredate nests (Sladen, 1912; Free and Butler, 1959; Pouvreau, 1973; Alford, 1975; Benton, 2006). Individual bees may be taken by a limited number of bird species in Britain; red shrike (*Lanius collurio*) target queens and workers, (Witherby et al., 1958; Pedersen et al., 2012), great tits (*Parus major*) attack bees drowsy from cool weather or drugged on the nectar from lime tree (*Tilia* spp.) or *Rhododendron* spp. (Sladen, 1912; Alford, 1975; Benton, 2006) and spotted flycatcher (*Muscicapa striata*) may take occasionally take small workers (Davies, 1977).

One of bumblebee's most important invertebrate enemies is the wax moth, *Aphomia* s*ociella*. The larvae consume entire bumblebee nests, including brood, wax, pollen and nectar stores (Sladen, 1912; Pouvreau, 1973; Alford, 1975). Crab spiders such as *Misumenia vatia* ambush foraging worker bumblebees on flowers (Alford 1975; Morse 1986; Benton, 2006), but are not thought to attack nests. Robber flies (*Asilidae* spp.) may take worker bumblebees (Bouseman and Maier, 1977; Benton 2006).

1.3.2 Parasitoids

Bumblebee parasitoids include dipterans such as *Conopids* spp. and *Physocephala* spp. and hymenopterans such as *Syntretus splendidus*, *Melittobia* spp. and *Monodontomerus montivagus* (Benton, 2006). A female parasitoid will lay one or more of her eggs either upon the host bumblebee or oviposit her clutch beneath the tergites of the bumblebee, within the abdominal cavity. Host bumblebees may be males or females, but a preference for workers has been found for Conopid flies within *Physocephala* and *Sicus* genera (Schmid-Hempel and Schmid-Hempel, 1990). Once inside the cavity, the larvae subsist on the haemolymph of the host and grow. When they are ready to pupate they leave the host. At this point, the internal organs of the bumblebee host may have been almost entirely consumed by the parasitoid and such bees are noticeably lethargic (Pouvreau, 1974; Alford, 1975; Goulson, 2010; pers. obs.).

In some years a large proportion (over 70%) of worker bumblebees may be infected with Conopid flies (Schmid-Hempel and Durrer, 1991; Muller and Schmid-Hempel, 1992).

1.3.3 Internal Parasites

Nests may be reduced or completely fail due to the queen or workers hosting internal parasites (Macfarlane et al., 1995). The nematode, *Sphaerularia bombi* infects queens during hibernation and inhibits nest founding. The queens instead appear to search for hibernation sites until they expire, shedding infection to the following years' hibernating queens (Alford, 1975).

Protozoan parasites such as the microsporidian *Nosema bombi*, flagellate trypanosome *Crithidia bombi* and neogregarinid protozoan *Apicystis bombi* range widely in the severity of harm to bumblebees. *Crithidia bombi* is common, and shows minimal sub-lethal effects (Brown et al., 2000), but these become more apparent during times of stress, for example, during diapause, or starvation, (Schmid-Hempel and Schmid-Hempel, 1998). A heavy *C. bombi* burden also reduces foraging efficiency by increasing bumblebee flower handling time (Gegear et al., 2005). The sublethal effects of *N. bombi* are somewhat more severe and infected queens have shown reduced fecundity (Macfarlane et al., 1995; Otti and Schmid-Hempel, 2007). Apparent infections of *A. bombi* (detectable by dissection) lead to destruction of the bumblebee's fat body and death (Durrer and Schmid-Hempel, 1995), but covert infections only detectable using molecular markers can be common and cause little harm (Arbetman et al., 2012).

1.3.4 Brood Parasitism

Nests may also be parasitized by *Psithyrus* queens, a group of obligate parasitic bumblebees (Sladen, 1912). *Psithyrus* cannot carry pollen and do not have a worker caste, therefore, they must locate a nest of the appropriate species of bumblebee, dominate or kill the queen and enslave the workers which are required to rear a brood of reproductive *Psithyrus* (Van-Honk et al., 1981a; Vergara, 2003; Dronnet, 2005).

Social parasitism may take place between true bumblebees, typically of the same species. Queens have been reported entering nests in early summer and may act as *Psithyrus* queens, by enslaving the workers and usurping the nest (Carvell et al., 2008; Barron et al., 2009). It is also possible for workers to enter foreign nests and either steal nectar (Andrews, 1969; Alford, 1975) or occasionally lay eggs (Birmingham and Winston, 2004; Lopez-Vaamonde, 2004); such bees are known as drifters.

1.3.5 Suitable food resources

Suitable forage plants, providing good quality pollen and sufficient nectar are needed for successful nest founding (Holm, 1966). Intensive agricultural systems, typically large arable fields with minimal semi-natural/unfarmed patches between fields provide fewer resources for bumblebees than more diverse habitats, and the numbers of bumblebees and other pollinating insects has been found to decline with intensification. For example, Steffan-Dewenter et al. (2002) found that the number of bumblebees visiting patches of flowers depended on the area of semi-natural habitat within approximately 750m; Lye et al., (2009) noted that increased ‘semi-natural areas’ on farms were beneficial to numbers of nest site-searching queens, and (Kremen et al., 2004) found effective pollination of *Citrullus lanatus* (watermelon) by wild pollinators (including bumblebees) depended on the availability of semi-natural upland within 1-2.5km. Such semi-natural areas, typically contain an assemblage of arable weeds and wild flowers which provide a suitable succession of pollen and nectar to bumblebee nests. Lack of suitable forage may result in reduced fecundity or failure of nests (Macfarlane et al., 1994; Génissel et al., 2002). Where forage is severely lacking, species of bumblebees may be lost from entire regions (Carvell et al., 2006; Goulson et al., 2006). Underfeeding developing workers results in smaller adults (Sutcliffe and Plowright, 1988); smaller bumblebees appear to have shorter foraging ranges than larger bumblebees (Greenleaf et al., 2007) and smaller bumblebees are able to carry less pollen (Goulson et al., 2002a). It seems likely that a stunted workforce may be detrimental to the colony, although this has not been empirically tested. Gynes in particular require adequate pollen in order to develop fully, and colonies of *Bombus ternarius* and *Bombus impatiens* which were experimentally provisioned with additional food produced a larger number of gynes and males than colonies without extra provisions (Pelletier and McNeill, 2003). Smaller gynes are less likely to survive diapause than larger specimens (Beekman et al. 1998), although following diapause, no negative affect on survival or colony growth has been detected (in a laboratory study of reared colonies; Duchateau et al., 2004). Adult gynes continue to feed from the nest's stores for several days to increase their fat store before leaving to mate and hibernate, although they will supplement this feeding with foraging trips outside the nest (Alford, 1975). Unlike *Apis mellifera,* bumblebees store pollen and nectar sufficient for only a few days (Sladen, 1912; Alford, 1975), therefore, prolonged periods of inclement weather may prevent effective foraging and so result in the starvation of the nest (Sladen, 1912; Alford, 1975; Macfarlane et al., 1994).

The drive to increase food production after the introduction of the 1945 Agriculture Act led to development and widespread use of agrochemicals (Robinson and Sutherland, 2002). Herbicides have reduced forage availability by decreasing arable weeds in crops and field boundaries (Corbet et al., 1991). Insecticides such as organophosphates and pyrethroids target insect pests directly and have been pivotal in the limitation of insect damage and facilitated increased crop yields (Oerke and Dehne, 2004). Insecticides have had detrimental effects on non-target wildlife species resulting in the banning of the most hazardous from being used in Britain and elsewhere, for example, perhaps the best known, dichlorodiphenyltrichloroethane or D.D.T. (Carson, 1962). More recently, a class of insecticides known as neonicotinoids have been developed. Neonicotinoids act by blocking the nicotinergic neuronal pathway of insects (Bonmatin et al., 2005). They are highly toxic to insect pests, but have a low toxicity for mammals and birds because the nervous systems of such vertebrates do not rely upon nicotinergic neuronal pathways. Neonicotinoids are systemic, meaning that they are present in all parts of the plant and a crop may be effectively treated from a single dose applied to seeds prior to planting (Bonmatin et al., 2003). Alternatively, neonicotinoid insecticides may by sprayed on crops in the conventional manner, or included in irrigation water. The ease and flexible modes of application and effectiveness have led to neonicotinoids being widely used in agriculture, amenity (e.g. golf courses) and domestic gardens (Bonmatin et al., 2005; Cresswell, 2011).

Neonicotinoid insecticides are also present in both nectar and pollen in treated plants. They can persist in plants for many months, in soil for over a year, and in plants subsequently grown in contaminated soil (Bonmatin et al., 2003). There is an increasing body of evidence that neonicotinoid insecticides have a negative effect on both honey bee and bumblebee colonies (Franklin et al., 2004). Bees feeding on pollen and nectar from such treated plants may have reduced immunity and foraging ability, whilst nests may face reduced fecundity or failure (Tasei et al., 2000). Poor adherence to application protocol has in some cases also increased the impact on bees. For example, poorly coated seed or planting in windy conditions allows neonicotinoid to drift and has caused harm to honey bee hives (Tapparo et al. 2012) and failure to correctly irrigate turf after application of imidacloprid resulted in decreased nest vitality in colonies of *Bombus impatiens*, resulting in fewer honey pots, brood cells, etc., (Gels et al., 2002). Experiments modelling neonicotinoid effects upon bumblebee colonies have often suffered from a lack of replication, and most have been carried out in laboratories, where bees are either provided with pollen and nectar directly or in small flight cages (Tasei et al., 2000; Morandin and Winston, 2003). There remains a gap in our understanding of the effects of neonicotinoids on colonies of bumblebees under field conditions (Cresswell, 2011), especially as there is evidence that foraging bees are exposed to a range of neonicotinoids at varying sub-lethal doses and bees treated with sublethal doses of imidacloprid appear to be more susceptible to *Nosema* infections (Alaux et al., 2010).

1.3.6 Competition

If any of the above resources are limited then either intraspecific or interspecific competition may occur. Competition for forage between bumblebees is expected, although evidence remains scarce (Pelletier and McNeil, 2003). Bumblebees differ in their requirements for floral resources to some extent, e.g. they have tongues of varying lengths, which allows some species to access flowers of some plant species that others are not adapted to pollinate.

For example, mean tongue length of *B. hortorum* workers are 12.9 mm, allowing them to utilise different flowers compared to *B. terrestris* and *B. lucorum* workers which have shorter tongues (mean length 7.9 mm; Goulson and Darvill, 2004). However, Goulson and Darvill (2004) discovered that several species have similar tongue lengths and coexist in the same area suggesting that competition for forage is not necessarily a strongly selective force.

Competition for nesting sites is also difficult to determine. One study of parklands in San Francisco found evidence that numbers of nest sites may limit bumblebee populations. McFrederick and Lebuhn (2006) found that numbers of bumblebees were found to positively correlate with numbers of rodent holes. Although it should be noted that this trend could indicate another feature in the parks from which both rodents and bumblebees benefited (for example increased areas of semi-natural habitats or a reduced insecticide/pest control regime).

Evidence for competition between queens for nest sites has been suggested. For example, dead queens have been found in queenright nests (e.g. Sladen, 1912), Carvell et al. (2008) found 30 wild *B. terrestris* queens in 18 of 48 laboratory reared *B. terrestris* colonies that had been placed in the field and Lye et al. (2009) caught 103 queens in 49 artificial domiciles baited with small mammal nest material. These incidences may be indicative of a lack of local nest sites, or it may just be chance, that these bees investigated the domiciles/nests for suitability and then were unable to leave. Multiple queens entering prospective nest sites or incipient nests might also indicate attempted usurpations, which may or may not be a result of competition (Alford, 1975; Carvell et al., 2008; Goulson, 2010).

Introduced species, for example *B. terrestris* have been imported into countries across the globe to pollinate flowering crops (Alford, 1975; Goulson, 2003; Matsumara et al., 2004; Torretta et al., 2006; Kanbe et al., 2008) and escapees have established themselves in the wild as feral populations. There are concerns that they may out-compete native species of bumblebees, where bumblebees were previously present (Ings et al., 2005; 2006), hybridise with native bees (e.g. *B. terrestris* can hybridise with native Japanese bumblebee *Bombus hypocrita sapporoensis*, and UK sub species, *B. terrestris audax* may hybridise with European forms of *B. terrestris terrestris*; Kanbe et al., 2008; Goulson, 2010).

1.4 Why study bumblebee nests?

In order to understand the effects of land use on bumblebees and to inform conservation efforts, population estimates are highly valuable (Osborne et al., 2008a; Goulson et al., 2010). The majority of bumblebees are non-reproductive female workers, with each colony representing a single breeding pair (Alford, 1975). This means that the population in a given area cannot be reliably estimated by counts of bees (typically foraging), as they may represent many individuals from a single nest or fewer individuals from many nests (Carvell et al., 2004; Goulson et al., 2010).

The sociality of bumblebees means that they have a lower effective population size than could be expected based on simple counts of individuals in the environment. It may be possible to count many foraging bumblebees in an area, but as the majority of these are workers which will not reproduce, the colony is the reproductive unit and represents a single breeding pair. Where populations are small or have become fragmented, lower effective population size may become a cause for concern as populations may be at increased risk if inbreeding depression (Whitehorn et al., 2009). For example, *B. terrestris* populations in the Canary Islands have reduced genetic diversity compared to mainland populations (Widmer et al., 1998) or due to habitat fragmentation such as the now rare British populations of *Bombus humilis* and *Bombus sylvarum* (Connop et al., 2011). It has been speculated that the haplodiploid sex determination of bumblebees may present an addition susceptibility to inbreeding as the male can only contribute half of the usual compliment of DNA (Chapman and Bourke, 2001; Zayed and Packer, 2005). Haploid males have also been suggested as a system of purging deleterious mutations from the gene pool, as they reduce the opportunity for harmful genes to be carried by heterozygote's and passed to offspring (Zayed and Packer, 2005). It seems likely that bumblebees are able to cope with a high level of inbreeding given their success as invasive species, despite being introduced in limited numbers (Goulson 2003; Lye et al., 2011).

Our understanding of bumblebee nest ecology is somewhat lacking and largely based upon qualitative studies carried out several decades ago (Sladen, 1912; Cumber 1953; Hobbs et al., 1962; Alford, 1975). For example, the interaction between bumblebees and mammalian species remain largely unknown and there is almost no quantitative data on those that are assumed to interact in some way, for example mice or badgers (Goulson, 2010). We have little understanding of the average nest longevity for different species or rates of reproduction.

1.5 Methods and barriers to the study of wild bumblebee nests

Bumblebees tend to nest in the burrows and nests of other animals (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Fussell and Corbet, 1992b; Lye et al., 2009). These are generally either underground or beneath vegetation such as clumps of grass, bushes, etc. Some bumblebee species such as *Bombus hypnorum* frequently commandeer birds' nests and all the common British species may adapt to an assortment of places in gardens and outbuildings such as compost heaps (Lye et al., 2009). The concealed nature of bumblebee nests and the infrequent worker traffic (compared to the constant stream of traffic seen at *Apis mellifera* hives of several thousand bees) make bumblebee nests difficult to locate by sight and means that they have been largely understudied (Suzuki et al., 2009; Carvell; 2008; Osborne et al., 2008a). Researchers wishing to study aspects of bumblebee nest ecology have employed a range of techniques.

1.5.1 Use of volunteers

Due to the costly nature of such lengthy searches in the field, members of the public have assisted researchers by reporting nests that they have happened upon (Fussell and Corbet, 1992b; Lye et al., 2012) or by volunteering to follow a given protocol for searching for nests (Osborne et al., 2008a). This allows large amounts of data to be collected (typically numbering several hundred) but spurious results may be obtained. For example, using a follow up questionnaire, Osborne et al. (2008a) found that a proportion of respondents had 'taken part' because they wanted to report a nest that they had already found, rather than following the given protocol. Identification of bumblebee species beyond basic colour patterns is difficult and so "citizen science" surveys have simplified identification. Even with colour identification guides, detailed knowledge and examination, some species remain impossible to distinguish without genital examination or genetic analysis (for example, *Bombus lucorum*, *Bombus cryptarum* and *Bombus magnus*) and so in most cases, members of the public have been asked to identify the bees to colour pattern group only (Fussell and Corbet, 1992b; Osborne et al., 2008a). This limits our ability to determine species-specific behaviour, such as nest-site preferences. More recently, digital photography and the widespread use of the internet and emails have enabled photo identification by an expert (Lye et al., 2012).

1.5.2 Spring queen counts

The number of nest site searching queens apparent in the springtime has been used as a proxy for nests (Svensson et al., 2000; Kells and Goulson, 2003; Lye et al., 2009). This method has revealed interesting information about habitat preferences exhibited by different species and the effect of land management on the availability of nesting sites. There remains a concern that the basis for this assumption may be flawed; an area with many nest site searching queens may be indicative of poor nesting habitat, forcing queens to search for longer periods.

1.5.3 Commercial or laboratory reared nests

Demand for sufficient pollination of agricultural crops such as raspberries and tomatoes has resulted in commercial enterprises rearing bumblebees (Goka et al., 2001; Benton, 2006; Lye et al., 2011b). Researchers have also reared their own colonies from captured spring queens, for example, Sladen (1912) collected nests in a specially adapted shed for study at his convenience. Laboratory or commercially reared nests which can be studied within the laboratory or placed in the field have been used extensively to investigate aspects of life history such as fecundity and *Psithyrus* invasion (Müller and Schmid-Hempel, 1992; Frehn and Schwammberger, 2001; Carvell et al., 2008), effects of internal parasite load (Otti and Schmid-Hempel, 2008) and rates of worker reproduction and drifting (Lopez-Vaamonde et al., 2004). However, results from such nests may be unrepresentative of wild situations as for example, colonies are typically maintained at a constant climate and fed ad. lib. during their early stages whereas wild queens have to forage extensively and cope with weather changes in order to establish nests. In addition, such experiments typically house nests in boxes and place them above ground. This positioning may reduce in unrealistically high rates of attack from *A. sociella*, *Psithyrus* and usurpation attempts from other true bumblebees (Goulson, 2002b; Carvell, 2008; Lopez-Vaamonde et al., 2004).

1.5.4 Artificial domiciles

Researchers wishing to study bumblebee nests have constructed artificial domiciles in the hope of attracting queens (Sladen, 1912; Donovan and Wier, 1978; Richards, 1978; Carter 1992; Lye et al., 2009). The success of such techniques is still very variable and whilst artificial domiciles can have good uptake rates in some countries, for example, New Zealand (Barron et al., 2000), in others the method has proved less successful (Lye et al., 2011b) and rates of occupation of <5% are typical in Britain. This technique may also still fail to represent natural nests; it has been suggested that nests in nest boxes are more likely to be destroyed by *A. sociella* (Free and Butler, 1959). In addition, nests may suffer from mammalian predation, invasion by *Psithyrus* or usurpation at varying rates due to nest positioning, entrance tunnel length, etc.

1.5.5 Microsatellites

Highly variable microsatellite markers have been used to investigate species' foraging ranges (Darvill, 2004; Knight, 2005), population structuring and inbreeding (Estoup et al., 1996; Ellis, 2006; Darvill et al., 2010); nest density and survival (Knight et al., 2009; Goulson et al., 2010), queen dispersal (Lepais et al., 2011) and mating systems, such as worker reproduction (Lopez-Vaamonde, 2004; Huth-Schwarz, 2011). Previous efforts to measure aspects of bumblebee ecology, such as foraging ranges had relied upon 'mark and recapture' studies, which over a scale of several square kilometres prove difficult to implement (Alford, 1975). In addition, researchers were unable to identify sisters, so could not estimate the numbers of nests contributing to the pollinating workforce purely from counting worker bees on flowers (Goulson, 2010). Microsatellite analysis enabled sister workers to be reliably identified and foraging ranges, nest densities, etc., for different species to be estimated, which developed our understanding of bumblebee ecology rapidly (Goulson, 2010). However, molecular studies have their limitations, nest densities rely heavily upon foraging range estimations for example, when estimating nest density of *B. pascuorum* using numbers of sister workers collected in an area, Knight et al. (2005) gave means of 177 nests km^{-1} or 35 nests km^{-1} depending upon foraging ranges of 449 m or 1000 km respectively. Foraging ranges vary considerably between species, so each species requires investigation in order to calculate nest density estimates (e.g. from approximately 450m for *B. pascuorum* and *B. lapidarius* to 758 m for *B. terrestris* (Knight et al., 2005) to

Bombus vosnesenskii, which was found foraging up to 2,783m from the nest (Jha and Kremen, 2012)). Foraging ranges for the same species also differ between studies and the method employed to measure it (Westphal et al., 2006; Osborne et al., 2008b; Wolf and Moritz, 2008; Osborne et al., 1999; Walther-Hellwig and Frankl, 2000; Hagen et al., 2011, Greenleaf et al., 2007).

The outcome of nests may not be predictable as floral availability varies through time and landscape, so bumblebees from a nest may be detectable during spring on a patch of useful forage, but not appear in later sampling of the same area and falsely assumed to have failed (Goulson et al., 2010). Molecular studies have provided valuable insights into some areas of bumblebee ecology. However their use is limited in the qualitative information that they can provide, for example, information regarding the causes of a colony failure.

1.6 Thesis aims and objectives

As the study of wild bumblebee nests has been hindered by researchers' inability to locate sufficient numbers, this study will initially assess methods for locating wild bumblebee nests (Chapters 2 and 3). Using wild bumblebee nests, the relationships between bumblebees and other species (in particularly; vertebrates, the wax moth *A. sociella* and internal parasites) will be investigated (Chapter 4 and 5). The prevalence of alternative reproductive strategies will be examined using genetic techniques (Chapter 6). Finally, the effects of a neonicotinoid insecticide on bumblebee gyne production will be investigated (Chapter 7).

Chapter 2

Humans versus dogs; a comparison of methods for the detection of bumblebee nests

This chapter has been published as:

O’Connor, S., Park, K.J. and Goulson, D. (2012) Humans versus dogs; a comparison of methods for the detection of bumblebee nests. *Journal of Apicultural Research* **51**, 204-211.

2.1 Summary

This study investigates alternative approaches to locating bumblebee nests for scientific research. We present results from three trials designed to assess: 1. The comparative efficiency of two detection dogs; 2. The ability of a dog to locate nests when carrying out repeat searches of agricultural habitats through the season; 3. The efficiency of a dog compared with human volunteers at finding nests in woodland, with the human volunteers using two methods: ‘fixed searches’ and ‘free searches’. The two dogs varied in their efficiency in finding buried portions of bumblebee nest material (62.5 % and 100 % correct indications). Searching for real nests in rural habitats, a detection dog located nine nests of four bumblebee species, in a range of habitats, at a rate of one nest for 19 h 24 min of searching time. A comparison of ‘free searches’ using human volunteers and the dog in woodland found that they located nests at similar rates, one nest for 1 h 20 min of searching time. Fixed searches located nests more slowly (one nest for 3 h 18 min of searching time), but probably provide a reliable estimate of nest density. Experienced volunteers performed no better than novices. Given the investment required to train and maintain a detection dog, we conclude that this is not a cost effective method for locating bumblebee nests. If the aim is to estimate density, then fixed searches are appropriate, whereas if the aim is to find many nests, free searches using volunteers provide the most cost effective method.

2.2 Introduction

Bumblebee nests are difficult to find due to their small size (relative to honey bees or social wasps) and their tendency to be located in relatively inconspicuous places such as the burrows and runs of small mammals (Sladen, 1912; Cumber, 1953; Free and Butler, 1959; Fussell and Corbet, 1992b; Kells and Goulson, 2003). The difficulty associated with finding bumblebee nests has hampered studies of numerous aspects of bumblebee biology. For example, little is known about rates of colony success and the relative importance of different mortality factors such as parasitism, predation and resource availability for bumblebee colony survival in wild populations (Goulson, 2010; Goulson et al., 2012). Artificially reared colonies have been used to investigate many aspects of bumblebee biology, e.g. homing range and flight distances (Goulson and Stout, 2001; Greenleaf et al., 2007), nest growth rates in different habitats (Muller and Schmid-Hempel, 1992; Goulson et al., 2002b; Carvell et al., 2008), effects of inbreeding (Whitehorn et al., 2009), longevity and reproductive output (Beekman and van Stratum, 1998; Lopez-Vaamonde et al., 2009) usurpation and resource availability (Carvell et al., 2008), drifting of workers (Lopez-Vaamonde et al., 2004), inter colony variation in learning abilities (Raine et al., 2006) and interspecific competition (Thomson, 2004). Such experiments, whilst providing a valuable insight, may however, not be representative of natural nests. For example, strains that have been bred in captivity for many generations may display altered susceptibility of parasitic infection; allowing *ad libitum* feeding in the early stages of nest founding may produce a nest which has an advantage over wild nests founded at a similar time; and setting out nests inside artificial boxes may make them easier for usurping queens of *Bombus* species or *Psithyrus*, to locate (Frehn and Schwammberger, 2001; Goulson et al., 2002b; Carvell et al., 2008).

Many bumblebee species have shown dramatic declines in recent decades which are thought to be due primarily to changes in agricultural practices (Williams and Osborne, 2009). Most attempts to quantify the effect of conservation management strategies on bumblebees have focused on counts of workers (Carvell et al., 2004; Walther-Hellwig et al., 2006; Redpath et al., 2010). In social Hymenoptera such as bumblebees, the effective population size is the number of colonies rather than individuals, since a colony represents a single breeding pair (Chapman et al., 2003). Population estimates, and the effects of environmental change and of conservation management practices ought therefore to be based on nest densities, rather than counts of individual foragers in the field. Recent studies have attempted to estimate nest density by using microsatellite analysis to identify nest mates amongst foraging workers (Knight et al., 2005). This technique is, however, expensive and constrained by its dependency on foraging range estimates to infer the actual location and density of the nests. Foraging range probably varies between species, nest size and location and is itself hard to quantify accurately (Osborne et al., 1999; Walther-Hellwig and Frankl, 2000; Westphal et al., 2006; Greenleaf et al., 2007; Wolf and Moritz, 2008; Hagen et al., 2011).

The development of a technique for detecting large numbers of bumblebee colonies would be a valuable tool for the conservation of these important pollinator species. Bumblebee colonies can be located by intensive observation of fixed areas, but the rate at which nests are detected is low (Cumber, 1953; Harder, 1986; Osborne et al., 2008a). Dogs are many times better at detecting scents than people and detection dogs have been trained by law enforcement agencies to recognise and respond to a wide range of odours, such as explosives, narcotics or missing persons (Helton, 2009). There is a long history of the use of detection dogs as a tool for ecological and conservation studies. In the late nineteenth century, a dog was trained to locate endangered kakapo, *Strigops habroptilus*, and kiwi, *Apteryx australis*, which were then relocated to an island free from the introduced predators that threaten them on the mainland (Hill and Hill, 1987). Since this time, detection dogs have been used in many countries to assist in conservation efforts, to find endangered or invasive species of a wide range of taxa including mammals such as black footed ferrets, *Mustela nigripes*, (Reindl-Thompson, 2006), reptiles such as desert tortoises, *Gopherus agassizii*, (Cablk and Sagebiel, 2008) and invertebrates such as termites, Isoptera, (Brooks et al., 2003).

In 2006 a male springer spaniel was trained to detect bumblebee nests. The dog was subjected to trials to ascertain the efficacy of this technique (Waters et al., 2010). As described by Waters et al. (2010), this dog was found to be 100 % effective at finding hidden bumblebee nest material in trials, and located 33 wild bumblebee nests of four different species when searching plots of various habitat on the island of Tiree, Scotland. This detection dog was retired in 2007 due to unforeseen circumstances and so in the same year, a second, male springer spaniel, was trained in order to investigate this approach further.

Here, we compare the rate at which nests are located by human volunteers using two different methods with the rate at which the dog located nests in the same habitat. We also compare the abilities of the two dogs, and assess the current dog’s ability to find nests in various farmland habitats. The aim of this study is therefore to determine which methods for locating bumblebee nests are most cost effective.

2.3 Materials and methods

The detection dog was trained to locate fragments of commercially reared *Bombus terrestris* nests at the Melton Mowbray Defence Animal Centre, UK. The dog was trained by the same team of professional dog trainers who trained the previous bumblebee sniffer dog, following the same positive reward procedures as used by Waters et al. (2010). Approximately 10g of frozen bumblebee nest was hidden in a wooden box within a secure room. The dog was fitted with a harness and given the command “Fetch” before being allowed to explore the room. When he happened upon the novel scent of the bumblebee nest a reward (a tennis ball) was given. This process was repeated over several weeks until the dog learned that the harness and command “Fetch” required him to search for bumblebee nest which was hidden in progressively more difficult places e.g. amongst dense vegetation, within rabbit warrens, under turf, etc. The dog would indicate presence of a nest sample by remaining stationary, facing the target, approximately 20-40cm from the entrance. Nest samples were handled with gloves and forceps and kept in bags to avoid contamination with human scent. Reinforcement training using pieces of bumblebee nest was carried out by the handler several times each week.

2.3.1 Detection dog efficiency

Between 18 February and 5 March 2010, trials were carried out to test the dog’s ability. Five 200m x 50m areas within grassland (n=4) or woodland (n=1) were chosen and five cylindrical plastic pots buried randomly within each area by an independent party in the absence of both the dog and handler. Pots were 5cm in height, 3.5cm in diameter and had six 5mm diameter holes drilled in their lids. Approximately 7g of bumblebee nest material was placed inside the test pots. A commercially available 'bulb planter' of diameter 7cm was used to remove a core of soil to create a hole of a standard depth (10cm). One of the pots was placed into the hole and the turf section of the core was then replaced. For each of the trials, one pot was buried empty as a control, whilst the other four contained nest material from one of the following species; commercially reared *Bombus terrestris*, wild *B. terrestris*, wild *B. pascuorum* or *B. hypnorum* (Linnaeus). All pots were kept in separate plastic bags and handled using gloves. The method followed the trial carried out in 2007 testing the abilities of the previous nest detection dog, except that Waters et al. (2010) used material belonging to *B. muscorum* and *B. distinguendus,* rather than *B. pascuorum* and *B. hypnorum*. In order to avoid the possibility of the dog locating natural nests during the trials, and such indications being regarded as false positives, trials were carried out at a time when no natural nests were likely to be present, again following Waters et al. (2010). Temperature during the trials varied from -3 to +7°C.

The dog searched the plots after a period of at least 24 hours had elapsed. This interval enabled the escape of volatiles from the buried pots and minimised the effect of 'detectable disturbance' as dogs are prone to preferentially investigate disturbed ground (Dutch Mulholland, Defence Animals Centre, pers. comm.). The dog was worked using the standard search technique (see Waters et al., 2010). Numbers of positive finds, missed pots and false positives (either finding the control pot or indicating at some other inappropriate item) were recorded. The accuracy of a detection dog can be described as: Proportion of Correct Detections = Hits/(Hits + Misses) according to (Helton, 2009). The term 'Misses' included undetected positive samples and incorrect indications on controls or other objects.

2.3.2 Nest density in the rural environment

In the spring and summer of 2008, the detection dog and his handler were deployed in farmland near Stirling, Scotland, UK. Six habitats were selected in order to represent a range of typical habitat types and features found in the rural environment which bumblebees are known to utilize for nesting (Alford, 1975; Carvell, 2002; Osborne et al., 2008a). These were hedgerow, fence-line (within one metre of the fence), bank (i.e., steeply sloping earth bordering lanes and ditches), long grass (>15cm), short grass (<10cm) and woodland edge (within 10 metres of the woodland edge). For each habitat type, 10 replicates of 1000m^2 were selected at random (Appendix I). All areas were searched for 25 minutes, seven times, once fortnightly from 26 May to 29 August 2008. The standard search technique was used as described above. Searches were carried out between 08.00 h and 20.00 h.

2.3.3 Effectiveness of detection dog searches versus human searches for locating bumblebee nests.

In order to compare the effectiveness of searches conducted with the detection dog against those using human volunteers, trials were carried out in open deciduous woodland (a habitat favoured by the detection dog) at the campus of the University of Stirling (OS *Grid Reference* NS 8096 and 8196) between 15 July and 29 August 2009. Trials were conducted between 08.00 h and 19.00 h in dry conditions. Forty volunteers were asked to complete a brief questionnaire in order to ascertain their knowledge of bumblebees. They were specifically asked whether they were able to distinguish a bumblebee from other flying invertebrates. If they were unable to do so or were unsure of their ability, they were shown ten colour photographs of common species of bumblebee, five dead specimens and live bumblebees as available in the field, before the experiment started. If volunteers had never previously seen a bumblebee nest and could not identify bumblebees to species they were deemed ‘unfamiliar’ with bumblebees. Had they either seen a nest previously or were able to identify bumblebees to species, they were classed as being ‘familiar’. Many of the volunteers were students and staff of the University of Stirling. They were aged between 18 and 70, representing both sexes (18 males and 22 females).

Each volunteer carried out two surveys, a ‘fixed search’ and a ‘free search’, each lasting for 20 minutes. The order in which these took place was randomised. Volunteers were accompanied by a single guide (S.O.). The guide explained that bumblebees tend to nest in holes in the ground, beneath leaf litter or in clumps of vegetation, and that a bumblebee flying into or out of such an area would be likely to indicate the presence of a nest. As male bumblebees were commonly seen carrying out patrolling behaviour in similar sites, this behaviour was also described to the volunteers. The guide ensured that the protocol was correctly followed and looked for bumblebee nests simultaneously.

2.3.3i Fixed search

The ‘fixed search’ methodology was adapted from that used by Osborne et al. (2008a) in which volunteers were asked to observe a fixed area of ground for a set period of time. In this study, each volunteer conducted a fixed search in one of 40, 6 x 6m arenas in woodland clearings that were free from large shrubs such as *Rhododendron* spp. or other dense undergrowth, in order to maximise the likelihood of nest detection. Arenas were marked out with flags and volunteers were asked to remain on the perimeter of the marked arena for the duration of the survey, observing the entirety of the plot for 20 minutes. Osborne et al.

(2008a) argued that any nest present within the area is likely to be detected within this period of time. If a volunteer discovered a nest before the end of the 20 minute survey, they were asked to continue watching the plot and advised that there could be more than one nest within the arena. Whilst volunteers were surveying the plot, the guide also looked for bumblebee nests.

2.3.3ii Free search

During free searches, volunteers were asked to search for bumblebee nests in any way that they chose. This generally resulted in volunteers moving through an area of woodland at their own pace, searching for activity that might indicate the presence of bumblebee nests. Volunteers were accompanied by the guide who remained behind or to one side. Flagged arenas for the fixed search were not included in the free search.

2.3.3iii Dog search

The detection dog was used after each volunteer had carried out their free search, in a nearby area of woodland for the same amount of time. A total of 40 x 20 minute searches were carried out by the detection dog using the standard search technique. This provided an equal search effort to that used by the human volunteers in their ‘free searches’. During the free volunteer and dog searches, the guide recorded the approximate route so that the approximate area searched could subsequently be calculated, assuming a 5m radius detection area (within this distance, volunteers readily noticed bumblebees). Areas were plotted and calculated using ArcGIS software. A binary logistic regression was used to determine variables influencing the likelihood of a volunteer finding a nest during their free search.

Covariates used were date and time of search (all times were rounded to the nearest hour in which the search took place). Factors included in the model were volunteer age (three categories were used, 18-30, 31-45 and 46-70), sex, and prior knowledge (unfamiliar or familiar). Variables that did not contribute significantly to the model were removed in a backwards, stepwise fashion (α = 0.05). The analysis was conducted using SPSS version 1.5.

2.4 Results

2.4.1 Detection dog efficiency

The dog located 79 % of pots containing bumblebee nest, (i.e. 15 out of a total of 19) but also gave five false positive indications (Table 2.1). Three of these were directed at control pots, one at a patch of bare ground with no evidence of a previous nest, and one where the independent party had attempted to dig a hole but had failed to achieve the required depth due to the ground being frozen. This represents a percentage of correct detections of 62.5 % (Helton, 2009; see Methods).

Table 2.1. Results from trials in 2010 with a bumblebee detection dog (□= indication, X = no indication). In the first search, the pot containing wild *B. terrestris* was removed by a wild animal prior to the search and so was discounted from the trials. An indication at an empty control pot is a false positive.

Habitat	**Commercial *B. terrestris***	**Wild *B. terrestris***	**Wild *B. hypnorum***	**Wild *B. pascuorum***	**Control**	**False Indications**
Grassland 1	□	Removed	□	□	□	1
Grassland 2	□	□	X	□	X	0
Grassland 3	□	□	X	□	□	0
Grassland 4	□	□	□	□	□	1
Woodland 5	X	□	□	X	X	0

2.4.2 Nest density in the rural environment

Nine bumblebee nests were located by the dog during the searches conducted on agricultural land; three were located in woodland edge habitat and three within hedgerows, and one was found in each of short grass, long grass and bank habitats with none detected along fences. The nests of four species of bumblebee were found; three each of *B. terrestris* and *B. pascuorum,* two of *B. lucorum* and one *B. hortorum*. For a summary of the details of nests located, please see table (Table 2.2.)

Table 2.2. Details of bumblebee nests located by dog during farmland searches.

Period nest located	**Species**	**Habitat Type**	**Details of location [Aspect of nest entrance]**	**Details of nest site**
Jun 6- Jun 20	*B. lucorum*	Hedgerow	Mixed thorn hedgerow, over 1m wide, beside farm track. [N.W.]	Small mammal tunnel
Jun 23 - Jul 4	*B. pascuorum*	Woodland edge	Under a beech tree, bordering a meadow. [N.]	Beneath small sheet of roofing corrugated tin, had been a mouse nest previous to bee occupation.
Jul 7 - Jul 18	*B. hortorum*	Long grass	Long grass occasionally grazed by sheep. [S.]	Rabbit hole (observed to be in use by rabbits).
Jul 21 - Aug 1	*B. terrestris*	Short grass	Short grass grazed periodically by sheep. [S.W.]	Rabbit hole (believed to be in use by rabbits, fresh faeces, dug soil and tracks at entrance).
Aug 4 - Aug 15	*B. terrestris*	Bank	Beside farm track [S.E.]	Small mammal tunnel below dense grasses.
Aug 4 - Aug 15	*B. lucorum*	Woodland edge	Deciduous woodland, sparse vegetation on ground. [W.]	Small mammal tunnel.
Aug 18- Aug 29	*B. pascuorum*	Hedgerow	Hedgerow bordered by grass margin (approx 2-3m wide) beside farm track. [E.]	Surface nest, among a pile of grass clippings from previous summer.
Aug 18- Aug 29	*B. terrestris*	Hedgerow	Hedgerow bordered by grass margin (approx 2-3m wide) beside farm track. [E.]	Small mammal tunnel leading into hedge.
Aug 18- Aug 29	*B. pascuorum*	Woodland edge	Beneath a holly tree. Ground cover absent, leaf litter only. [S.]	Small mammal tunnel leading ~30cm below the surface of the ground.

No nests were located during the first search, carried out 26 May to 6 June (Figure 2.1). The largest number of nests (three) found in any one survey period were found during the last search (18 August to 29 August). A total of 175 hours were spent searching for nests. This equates to a rate of one nest located for 19 h 24 min of searching time.

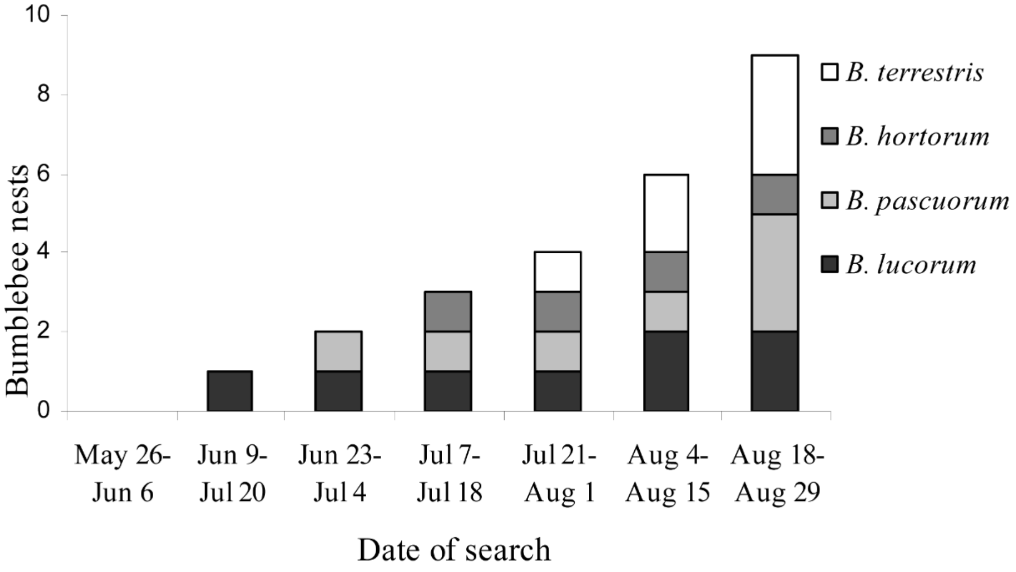

Figure 2.1. Cumulative bumblebee nests located by the dog in searches on farmland from May to August, separated by species.

2.4.3 Effectiveness of detection dog searches versus human searches for locating bumblebee nests.

2.4.3i Fixed search by humans

Four bumblebee nests were found by volunteers whilst carrying out fixed searches (three nests of *B. terrestris* and one *B. pratorum*). The total area of all the fixed search plots was of 1440m^2, giving a minimum nest density of 27.78 ± 13.33 nests ha^{-1} for this woodland habitat. This translates into a nest detection rate of one nest for 3 h 20 min of searching. The guide detected all nests identified by the volunteers but no additional nests.

2.4.3ii Free search by humans

Ten bumblebee nests were found during the free searches, translating into a nest detection rate of one nest for 1 h 20 min of searching (seven nests of *B. terrestris*, two *B. lucorum* and one *B. pratorum*). The mean area searched was estimated to be 1735.0 ± 376.6m^2. Hence the estimated nest density was 1.44 nests ha^{-1} (compared to 27.8 for fixed searches). Assuming the nesting density calculated from the fixed searches is a reasonably accurate approximation to the true number of nests, the free search resulted in the discovery of approximately 5.1 % of total nests, but found nests at a rate 2.5 times faster than the fixed search.

The likelihood of a volunteer in finding one or more nests during the free search was not affected by age ($\chi^2_{2,} = 1.544$, $p = 0.462$), sex ($\chi^2_{1,} = 0.876$, $p = 0.349$), familiarity with bumblebees ($\chi^2_{1,} = 0.875$ $p = 0.350$), date ($\chi^2_{1,} = 1.473$, $p = 0.225$) or time of day ($\chi^2_{1,} =0.440$, $p = 0.507$).

2.4.3iii Dog search

The dog located ten nests (seven nests of *B. terrestris*, one *B. lucorum*, one *B. hortorum* and one *B. lapidarius*) during his searches of the same area as the human volunteers. The dog searched a mean area of 1777.5 ± 266.5m^2 resulting in a nest density of 1.41 nests ha^{-1}, which is equal to volunteers carrying out the free search, resulting in an efficiency in terms of nests located per hour equal to that of volunteers.

2.5 Discussion

The current detection dog proved to be less effective than his predecessor during the artificial trial (62.5 % versus 100 % for the current and previous dogs, respectively; (Waters et al., 2010)). The previous bumblebee detection dog was used to search for bumblebee nests in the Western Isles, Scotland, and located 33 nests at a rate of one nest for 9 hr 5 min searching (Waters et al., 2010). These searches took place in August and September, the peak period for bumblebee activity in the Western Isles. The current dog found nests at a rate of one per 19 h 24 min in repeated searches of rural farmland sites, but found one per 1 h 20 min during searches of woodland on the University campus. The searches on rural farmland began in May, when nests are small and a few may not yet have been founded (none were found in the first search). They were also repeated seven times in the same area, which might explain the low efficiency in terms of nests located per hour.

The efficiency of detection dogs is known to vary (Helton, 2009). In the conservation literature, Engeman et al. (2002) reported success of approximately 63 % for trained snake detection dogs, and Reindl-Thompson et al. (2006) found that one dog trained to find black footed ferrets detected 100 % of the ferrets, whilst another only detected 57-71 % of them.

Despite being initially trained using only nest material collected from one bumblebee species (harvested from artificially reared colonies of *B. terrestris*), the detection dog located wild nests belonging to four different species. This supports the findings of the previous bumblebee detection dog, which detected nests of four different bumblebee species during field trials in the Hebrides, Scotland (Waters et al., 2010). Detection dogs used for conservation purposes have been shown to be able to generalise between similar target substances (Long et al., 2007) and this is considered an important attribute to their use. This is particularly important for bumblebee nest detection dogs, as nests of the rarer bee species are unlikely to be commonly available for training purposes.

The nest density across all farmland habitats resulting from the detection dog searches was 1.5 ha^{-1}, based on seven consecutive visits to the same sites. Based on estimates from Osborne et al. (2008a), nest density would have been 22.52 ha^{-1} for the same area of these habitats (not including bank which was not investigated in their study). The estimated density from free searches of woodland was 1.4 ha^{-1} (using either dog or human volunteers), whilst that from fixed searches in woodland was 27.8 ha^{-1}. Osborne et al. (2008a) reported a range of nest densities for different habitats, based upon volunteers performing fixed searches, which ranged from 10.8 ha^{-1} for woodland to 37.2 ha^{-1} for fence-lines. Our figures from fixed searches are therefore broadly similar, and in marked contrast to free searches. It would seem that fixed searches are necessary if the aim is to estimate nest density, since in free searches both volunteers and the detection dog failed to find an estimated 95 % of the nests present. Even with repeated visits to the same sites, the number of nests detected by the detection dog, and hence the estimates of nest density, are far below estimates from fixed searches. In contrast, if the aim is to find lots of nests for study, then free searches appear to be more efficient (approximately 2.5 times more efficient in the habitats used in this study) in terms of the number of nests detected per hour.

During fixed searches, volunteers found all nests observed by the experienced guide, confirming the findings of Osborne et al. (2008a) that this is probably a reliable way of detecting the majority of bumblebee colonies. The fact that nests were found regardless of the level of familiarity that volunteers have with bees (in both fixed and free searches) suggests that volunteers can provide a valuable tool for locating bumblebee colonies with minimal training. Whilst our detection dog can readily detect nests, in this study he performed no better than naive humans. Given the cost of initial training and subsequent maintenance training (several hours each week, all year round), and the need for a person to handle the dog in the field, simply employing a person to search for nests for the duration of the experiment would appear to be more cost effective, especially where keen members of the public are willing to volunteer their time.

2.6 Acknowledgements

We would like to thank the Leverhulme Trust for funding this research. Thanks to the two referees who provided improvements to a previous draft of the manuscript, Gillian Lye and Penelope Whitehorn for providing assistance with field work, and to Jenny Norwood and Dave Hollis for training the detection dog. We would also like to thank the farmers who allowed the dog onto their land and the forty people who volunteered their time to look for bumblebee nests.

Chapter 3

Location of bumblebee nests is predicted by counts of nest-searching queens

3.1 Abstract

Bumblebee nests are difficult to find in sufficient numbers for well replicated studies. Counts of nest-searching queens in spring and early summer have been used as an indication of preferred nesting habitat, yet high densities of nest-searching queens may indicate habitat with few nesting opportunities. As yet, the relationship between numbers of nest-searching queens and actual nests founded in a given area has not been established. From mid April 2010, queen bumblebees were counted along transects in grassland and woodland habitats in Central Scotland, UK. The number of inflorescences of suitable forage plants were also estimated at each transect visit. The area surrounding each transect was searched for nests in the summer. In total 173 bumblebees were recorded, and of these 149 were nest-searching queens. Searches subsequently revealed 33 bumblebee nests. The number of nest-searching queens on transects was significantly, positively related to the number of nests subsequently found. Floral abundance did not correlate with numbers of nest-searching queens or the number of nests found, suggesting that queens do not target their searching to areas providing spring forage. The data suggest that counts of nest-searching queens do provide a useful positive indication of good nesting habitat, and hence where bumblebee nests are likely to be found later in the year.

3.2 Introduction

Bumblebees nest in the dwellings of other animals, typically those of small mammals such as mice and voles but sometimes using other nests such as those of birds or rabbits (Sladen, 1912; Free and Butler, 1959; Alford, 1975; Fussell and Corbet, 1992b; Lye et al., 2012). These tend to be subterranean or under thick vegetation such as tussocks of grass.

Bumblebees have an annual life cycle and are founded in spring or early summer by a fertilised queen (Sladen, 1912). The queen rears an initial brood of 8-16 worker bees, which then assist in rearing successive broods (Plowright and Pendrel, 1977). The workforce increases to a maximum of several hundred workers (depending on species), which is small compared to hives of other social bees, for example *Apis mellifera,* which may contain many thousands of workers (Goulson, 2010). The result is a well concealed nest which may only be revealed by sporadic worker traffic to and from the entrance. A variety of approaches to locating wild bumblebee nests have been deployed, including training sniffer dogs (O'Connor et al., 2012; Waters et al., 2012), or recruiting volunteers to search for nests following a variety of protocols (Fussell and Corbet, 1992b; Osborne et al., 2008a; Lye et al. 2012). The most effective method is time-consuming diligent searches for worker bee traffic, although costs are reduced if volunteers can be recruited for this task (O'Connor et al., 2012). Because of the labour-intensive nature of this work, and the small numbers of nests found per hour, we still have a poor idea of the preferred nesting habitats of different bumblebee species, particularly for the less common species.

The relative suitability of different habitats as nest sites for bumblebees, and differences in nesting habitat preferences among bumblebee species can be studied indirectly using counts of nest-searching queens (Svensson et al., 2000; Kells and Goulson, 2003, Lye et al., 2009). In these studies, the abundance of nest-searching queens is used as index of the nesting suitability of an area. This approach has been used to demonstrate that bees tend to prefer linear features (for example hedgerows and fence-lines) to open ground, and in some cases have more specific site preferences. For example, more sheltered sites near forest boundaries may be preferred by *B. pascuorum* and *B. lucorum*. However, the use of such indices has rarely been tested, and it is possible that high numbers of nest-searching queens indicates poor habitat where nest sites are unavailable, leading to prolonged searching by queens. In the only test of this assumption to date, numbers of nest-searching queens of *Bombus ardens* were found to positively correlate with the presence of actual nests in Japan (Suzuki et al., 2009), but only six nests were detected.

Bumblebee queens in spring and early summer must have access to sufficient pollen and nectar to develop their ovaries, fuel their nest site searches and initiate a nest (Cumber, 1953; Stephen, 1955; Alford, 1975; Steffan-Dewenter and Tscharntke, 2001). Lack of forage causes slower colony growth and impacts on survival and fecundity (Plowright and Pendrel, 1977; Schmid-Hempel and Schmid-Hempel, 1998). One may therefore expect that locations with plenty of spring flowering forage plants would provide the most suitable nesting sites (Fye and Medler, 1954; Holm, 1966), and in support of this Suzuki et al. (2009) found a positive relationship between floral availability and number of nests with *Bombus ardens.*

In this study we aim to determine whether the number of nests in an area can be predicted by regular counts of nest-searching queens during the spring, testing the implicit assumption of Svensson et al. (2000), Kells and Goulson (2003) and Lye et al. (2009). If reliable, this would enable spring queen counts to infer suitability of habitat or land management for conservation purposes and allow researchers wishing to locate bumblebee nests to target resources to areas where greater numbers of bumblebee nests are likely to be found. We also examine whether nest locations are predicted by availability of spring forage.

3.3 Method

Bumblebees were counted and floral abundance estimated along transects of 100m in springtime. The first set of observations were carried out in the week beginning 19th April and the last transects took place on 4th June 2010. Transect walks took place in dry conditions between 08:30 and 19:30. The temperature ranged between 6 ºC and 22ºC. All transects were visited once a week, for seven weeks.

Twenty transects were selected; ten in woodlands and ten in grasslands as bumblebees of the six common species in Britain are known to utilise both of these habitats for nesting (Alford, 1975; Osborne et al., 2008a). Sites were either on the campus of the University of Stirling (Scotland, UK) or on nearby private estates. It was important that sites were accessible to researchers, and so areas with thick undergrowth, (e.g. *Rhododendron* spp., *Rubus fruticosus* (flowering current), *Urtica dioica* (stinging nettle), etc.), those on steep slopes or prone to becoming water logged were avoided. Woodlands were dominated by deciduous species such as *Quercus robur* (oak), *Fraxinus excelsior* (ash), *Fagus sylvatica* (beech) and *Betula pendula* (birch). Grasslands were long-established, tussocky swards (>10 cm) which receive minimal management. There were numerous signs of small mammal and rabbit activity and burrows in both habitats.

The transect protocol followed Lye et al. (2009). Each was 100m in length, and was walked at a slow, constant pace of approximately 2 miles per hour. Bumblebees were counted within 3m each side of the path walked by the observer. Bumblebees were identified to species, and their caste and behaviour at the time were also recorded. Bumblebee behaviours included ‘nest-searching’, ‘in flight’ or ‘foraging’ for nectar or pollen (as indicated by presence of pollen in pollen baskets). Nest-searching behaviour consisted of bees flying in a low, zigzag pattern and/or investigating holes in the ground, tussocks of vegetation, etc. Bees classed as 'in flight' were typically flying higher, on a straighter trajectory and not apparently investigating either potential nesting sites or flowers. In addition, plant species visited by foraging bees was noted. The amount of forage available to bumblebees was recorded during each visit. Species of plants and estimations of the number of inflorescences were estimated within 50m of each transect to provide an approximate measure of forage availability at the sites. Individual plants or small patches of inconspicuous flowering herbs may have been missed in these estimations, however, substantial resources such as flowering trees for example, *Salix* spp. (willow species), *Prunus* spp., (cherry species) etc, and patches of herbs, such as *Symphytum officinale* (borage) and *Hyacinthoides non-scripta* (common blue bell) were recorded.

An area of 0.5ha, surrounding each transect (i.e. within approximately 25m of the spring transect) was intensively searched for nests twice; initially for three man hours in early summer, in the period between June 9th and 18th and again in mid-summer for one man hour between July 20th and 28th (80 man hours in total). Searches were carried out in dry conditions between 08:00 and 20:00. Data from the two searches were pooled for analysis.

3.3.1 *Analysis*

Analysis was carried out in R Statistical Software Version 2.12.2 (R Development Core Team, 2011). A Generalised Linear Model with Poisson errors was used to test the association between the response (total nests detected) and covariates (numbers of nest-searching queens (all species pooled) and floral abundance (using the total number of inflorescences for all known bumblebee forage plant species within each site)) and the factor (habitat (woodland/grassland)).

The initial model included all explanatory variables, plus all two and three way interactions. The model was simplified by backwards, stepwise removal of explanatory variables using a P-value significance threshold of 0.05. Habitat preferences for the different species were examined using Chi-squared tests, where sufficient data were available. Minitab 15 Statistical Software (2006) was used to carry out a Mann-Witney U to test to assess the difference in floral abundance between sites of the two different habitats.

3.4 Results

In total, 174 queens were observed. Of these, 19 were foraging and 6 were in flight. A total of 149 nest-searching queens were recorded (Figure 3.1). The peak of queen nest-searching activity may have occurred before the beginning of the experiment as *Bombus terrestris* and *Bombus pratorum* numbers were at their highest in the first week of recording (week beginning 19th April). *Bombus pascuorum* activity peaked later, during the 5th week of the experiment. No workers were seen in weeks 1-4, the first (*B. pratorum*) was recorded during the 5th week. In the 6th week, there were a further four workers recorded foraging (a *B. pratorum* and three *B. pascuorum*) and in the 7th week 18 workers were recorded (nine *B. pratorum*, three *B. pascuorum,* four *B. hortorum* and two *B. terrestris*).

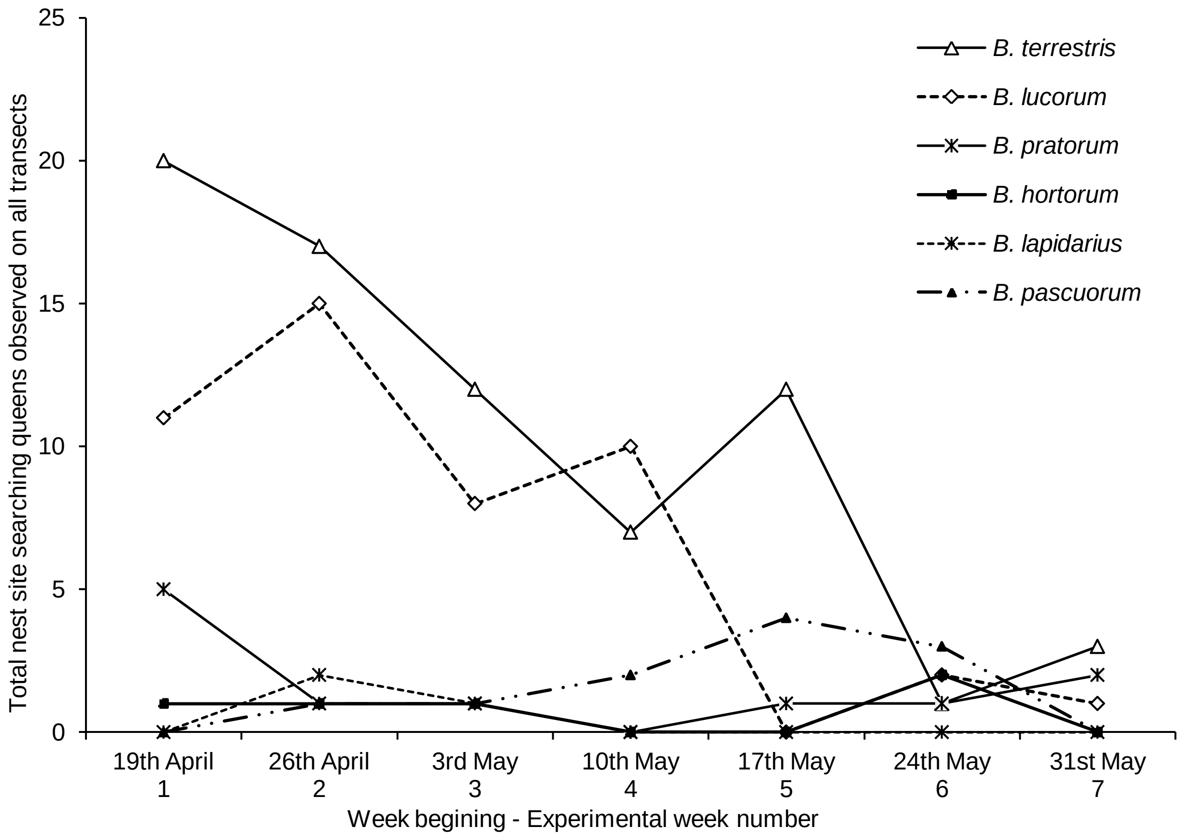

Figure 3.1. Total nest-searching bumblebee queens (n=149) recorded on all transects during the seven survey periods, separated by species.

In total 33 nests were found; 18 in grassland and 15 in woodland. Nest density across all ten sites of each habitat (5 ha total area) was calculated as 3.30 nests ha^{-1} (3.60 nests ha^{-1} and 3.00 nests ha^{-1} for grassland and woodland sites respectively).

There was no interaction between numbers of nest-searching queens and floral abundance on transects. There was a significant, positive association between numbers of nest-searching queens on transects and number of nests subsequently found at sites ($\chi^2_{\text{D.F. 1}} = 6.61$, p = 0.010; Figure 3.2). Habitat and floral abundance had no effect on the number of nests and were removed from the model (habitat: $\chi^2_{\text{D.F. 17}}$ $p = 0.157$, $p = 0.692$, floral abundance $\chi^2_{\text{D.F.}}$

$_{18}$ = 1.56, *p* = 0.212). Foraging queens (n=18) were recorded on too few transects (n=3) to allow further analysis of plant species preferences, bumblebee species, habitat, etc.

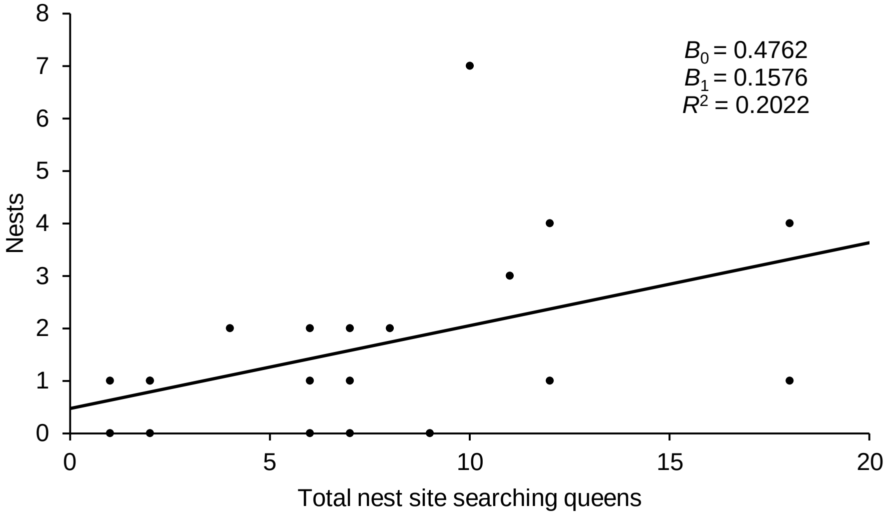

Figure 3.2. Total nest-searching queens observed during transects correlated with bumblebee nests at sites.

On average, there were more nest-searching *B. terrestris* queens in woodland than grassland sites (median of four and two queens in woodland and grassland sites respectively) but this difference was only marginally significant ($\chi^2_{\text{D.F. 1}}$ = 3.56, *p* = 0.059; Figure 3.3), however numbers of nests in the two habitats was very similar ($\chi^2_{\text{D.F. 1}}$ = 2.25, *p* = 0.007). *Bombus pascuorum* queens were significantly more likely to be recorded in grassland ($\chi^2_{\text{D.F. 1}}$ = 7.36, *p* = 0.007), and no preference was found for queens of *B. lucorum*, ($\chi^2_{\text{D.F. 1}}$ = 0.0213, *p* = 0.884) or *B. pratorum* ($\chi^2_{\text{D.F. 1}}$ = 0.818, *p* = 0.376). There were too few data to test for habitat preferences of nest-searching queen *B. hortorum* or *B. lapidarius* or for differences in numbers of nests for any species other than *B. terrestris*.

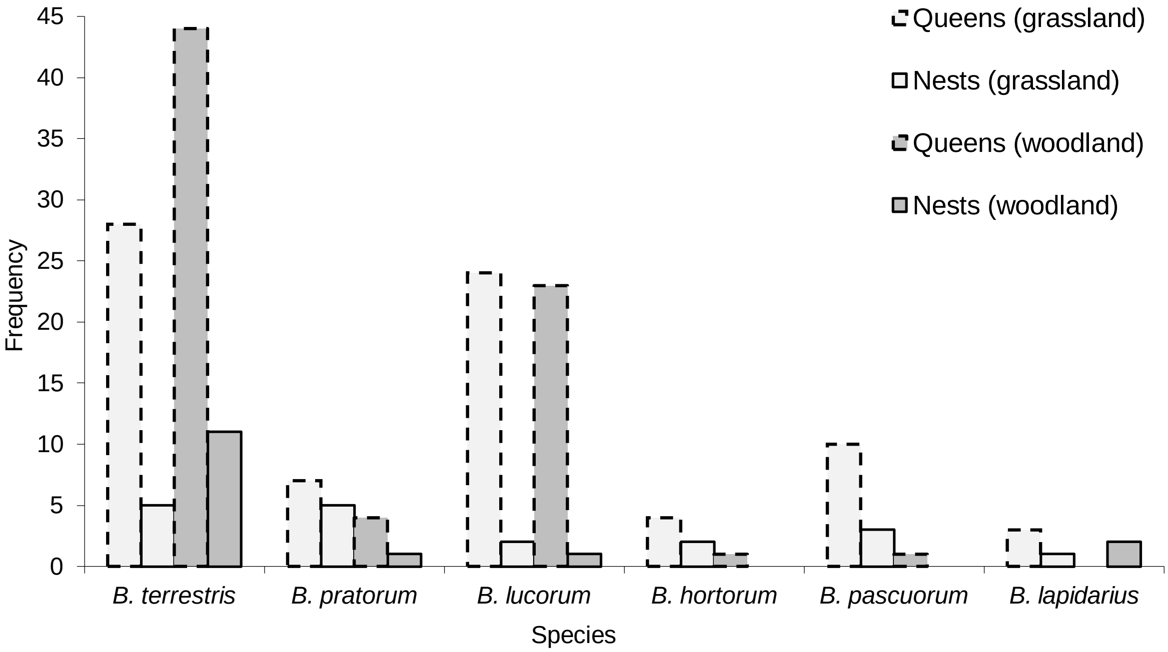

Figure 3.3. Total nest-searching queens and nests, separated by species and habitat.

3.4.1 Comparison of efficiency between searches of favourable and unfavourable sites.

During the first round of searching (3 h), 24 nests were found, followed by 9 additional nests in the second (1 h). This equates to 2h 25 min of searching per nest across the twenty sites. Each 100m transect took approximately 5 minutes to complete (35 mins in total across seven visits). Had nest searches only taken place at ‘favourable sites’ with median to high nest-searching queen abundance (n=11; 7 to 18 total nest-searching queens), 25 nests would have been found (2.27 nests per site) compared with 8 nests (0.89 nests/site) at the nine sites where fewer than seven nest-searching queens were observed. Had only these 11 ‘favourable sites’ been searched, nests would have been located at a rate of 1h 36 min per nest. Inclusion of the time required to walk transects and count queens during spring in this measure of efficiency reduces the rate to 2h 08 min of ‘man hours’ per nest.

3.4.2 Floral availability and habitat variation

There was no significant difference between floral abundance in the different habitats (Figure 3.4). Floral availability varied widely between transects. Floral resources were absent from seven sites, whereas seven of the other sites averaged over 1000 inflorescences (mean across all visits). Of the foraging bees (queens and workers) 80.5% (n=33) were recorded at a single grassland site which was also one of the most florally rich sites (mean over seven transects ~600 (± 253 S.E.) inflorescences).

Floral abundance had no effect upon numbers of nests found at sites. The ten sites with greatest floral availability (100 to >4500 mean inflorescences) yielded 13 nests, whereas 14 nests were found the ten sites with poorest availability of spring flowers (>40 mean inflorescences). It may also be noted that the seven sites devoid of floral resources yielded nine bumblebee nests.

Queens were recorded foraging on just five plant species; eight on *Vicia cracca* (tufted vetch), seven on *S. officinale*, two on *Rhododendron* spp., one each on *H. non-scripta* and *Taraxacum* sp. Within the wider area, flowering trees such as *Prunus* spp. and *Salix* spp. were common and accounted for much of the floral resources (61.51% inflorescences), along with occasional shrubs such as *Rhododendron* sp., *Ribes sanguineum* and herbs including those found on transects and also *Prunella vulgaris*, *Pentaglottis sempervirens* and *Borago officinalis.*

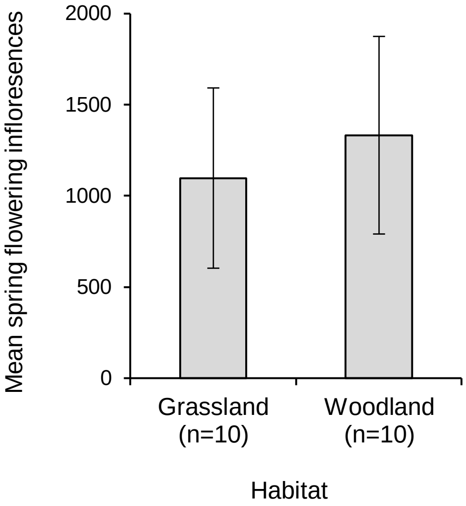

Figure 3.4. Mean floral abundance at grasslands and woodland sites. A single mean was calculated for each transect from the seven visits, and these means used to calculate an overall mean and standard error across the 10 replicates. Number of inflorescences varied widely between sites, and there was no significant difference between habitats (Mann-Witney U test; W = 110.0 *p*=0.728).

3.5 Discussion

Data presented here demonstrate that the density of nest-searching queen bumblebees does positively predict nest density later in the year, thereby confirming the underlying assumption of previous studies which have used queen abundance to infer nesting habitat (Svensson et al., 2000; Kells and Goulson, 2003, Lye et al., 2009).

The density of floral resources, did not predict the density of nest-searching bumblebee queens. This is in accordance with Lye et al. (2009), who found that floral availability of agricultural field margins was not correlated with abundance of nest-searching queens. In contrast, floral resources have been found to predict nest-searching queens and actual nests of *B. ardens* (Suzuki et al., 2009). However in this study, floral abundance was assessed at a much greater scale, ($2.5km^2$). Bee foraging ranges vary between species and size of bee (Darvill et al., 2004; Knight et al. 2005; Greenleaf et al., 2007). Bumblebee workers rarely forage immediately outside their nest, tending to fly in excess of 100m before beginning to forage (Dramstad, 1996; Dramstad et al., 2003; Osborne 1999). Although no data exists for queen foraging ranges, it is possible that the scale of the forage survey was inappropriate. In addition, the survey provides only a crude estimate of available forage, as inflorescences of all those species surveyed are not equal in terms of the quantity and quality of pollen they provide and their preferred use by bumblebees (Carvell, 2002; Goulson and Darvill, 2004; Goulson et al., 2005). Regardless of these limitations, our data strongly suggest that the availability of high densities of floral resources close to nests (within 100m) is not necessary for nest establishment. It must also be considered that this study was limited to only two habitat types (and specifically woodland that was open and accessible) and a range of common species of bumblebee. Other species of bumblebee may have different requirements, for example, some of the species of bumblebees that have suffered declines have longer tongues and are more suited to different plants than those observed in this study (Williams and Osborne, 2009). Workers of some rarer species of bumblebees forage over a smaller area (Connop et al., 2011). If this trend is the same for queens of these species, available forage within 100m may be essential for successful nest establishment.

The study site comprised a mosaic of grassland and woodland habitats which bordered one another. This means that most species of forage plants (such as *Prunus* spp. and *Salix* spp.) were typically within 50-200m of sites of either habitat and this prevented meaningful differentiation between sites based upon floral abundance of particular plant species.

It appears from data that the woodland sites provided a similar level of floral resources as grasslands, although the sites were managed for wildlife, shooting, etc and are unlikely to be representative of all woodlands, for example, commercial coniferous plantings. This is likely to only be the case in deciduous woodlands, where plants of the herb layer such as *H. non-scripta*, *Anemone nemorosa* (wood anemone) and *Lamiastrum galeobdolon* (archangel), flower in spring before the canopy closes, although woodland clearings may provide summer forage, for example, *Digitalis purpurea* (fox gloves) which are almost entirely pollinated by bumblebees (Broadbent and Bourke, 2012). Flowering trees represent a substantial resource to bumblebees, *Salix* spp., *Prunus* spp. and later flowering trees such as *Tillia* spp. (lime) and *Aesculus hippocastanum* (horse chestnut) may provide a succession of forage. Land managers may utilise such flowering trees to support beneficial pollinator populations where resources for more traditional agri-environmental schemes (e.g. set-aside, wildflower margins, etc,) are limited.

Svensson et al. (2000) reported that *B. terrestris* and *B. lapidarius* queens displayed a preference for open ground and field boundaries, whereas in this study, *B. terrestris* and *B. lucorum* queens and nests were equally likely to be recorded in woodland. *B. terrestris* is fairly plastic in its habits and woodland nests are not uncommon in the UK (Alford, 1975; O’Connor et al., 2012). These data are in accordance with earlier studies which suggest that established grassland is the preferred nesting habitat of some bumblebees including *B. pascuorum* (Alford, 1975; Svensson et al., 2000; Kells and Goulson, 2003).

Queen counts in spring have the potential to increase the efficiency of nest searches later in the year. Nest discovery rate could have been increased from 2h 25 min per nest when all sites are searched to 1h 36 min per nest if only favourable sites were searched (i.e. those with median to high queen abundance). Nest detection rate is reduced to 2h 08 min per nest when transect assessment time is included. The researcher's time is often a limiting factor in field experiments and for experimental purposes it may be desirable to locate nests rapidly so that observations of fecundity, predation, etc, or experiments can begin with a cohort of nests, rather than being staggered over several weeks as nests are located and added to the sample. If this is the case, it may be sensible to allow a few hours for transect walks in spring in order to increase the rate of nest location in the busier summer months. In addition, volunteers and assistants become disillusioned and frustrated if they do not find nests after several hours of searching (pers. obs.). With this in mind, any improvement in the rate of nest location is considered very useful. Nests in this study were found more slowly than previously, where the same method detected nests at 1 h 20 min per nest in woodlands (O'Connor et al., 2012). This disparity may be explained by the date at which searches took place; nests would have been larger and more detectable in mid to late summer when volunteers were recruited in O'Connor et al. (2012).

Nest density across both habitats was 3.30 nests ha^{-1}. This is comparable with molecular studies which have estimated nest density for four common British bumblebee species. Estimates for *B. pascuorum* nests have ranged from 193 nests km^{-1} (Darvill et al., 2004), 26 nests km^{-1} Knight et al., (2005), 35-173 nests km^{-1} (depending upon foraging ranges of 449 or 1km respectively; Knight et al., 2009). *Bombus terrestris* nests are less common, 13 nests km^{-1} (Darvill et al., 2004) and 29 nests km^{-1} (Knight et al., 2005). Knight et al. (2005) estimate densities for nests of *B. lapidarius* and *B. pratorum* 117 and 26 nests km^{-1} nests respectively. 1km^2 is equal to 100 hectares, therefore these estimates are 1.06 nests ha^{-1} for *B. pascuorum* (mean of four estimates), 0.21 nests ha^{-1} for *B. terrestris* (mean of two estimates). 1.17 nests ha^{-1} for *B. lapidarius* and 0.26 nests ha^{-1} for *B. pratorum*. Molecular Across all four species, this is approximately 2.70 bumblebee nests ha^{-1} for these four common British bumblebee species. There is no molecular estimate for nest density of *B. hortorum* or *B. lucorum*, but assuming they nest at comparable densities to those other species and taking the mean of the estimates for the four species (i.e. 0.67 nests ha^{-1}), we could perhaps expect around 4.05 nests ha^{-1}. However, this figure and nest density found in this study are lower than the numbers of nests found when areas of ground are exhaustively searched Osborne et al. (2008a) recorded nest density at 14.6 nests ha^{-1} and 10.8 nests ha^{-1} for long grassland and woodland respectively and O'Connor et al. (2012) estimated woodland nest density at 27.8 nests ha^{-1}. Molecular studies can be expected to provide lower estimates as they consist of a mixture of habitats including those that are unfavourable for nesting such as ploughed fields. Osborne et al. (2008a) used satellite imagery and GIS software to estimate the areas of habitats observed in their study (such as woodland, gardens, hedgerows, etc,) for an area of Hertfordshire (UK) and estimated that there were approximately seven nests ha^{-1}.

It was not expected that all nests would be found in this study as fixed searches (where a person observes a small area for twenty minutes) are required to locate the majority of nests (Osborne et al., 2008a; O'Connor et al., 2012). Had fixed searches been carried out for each

0.5 ha site, more than 23 hours of observations would have been required per site. Whilst searches of the intensity and duration used in this study are sufficient for locating some nests and estimating comparative nest densities between sites, considerably more effort is required to adequately estimate actual density.

Counts of nest-searching queens on transects in spring are a useful measure of suitability of nesting habitat and predict the location of nests later in the year, demonstrating that such counts do provide a useful tool in studies of bumblebee nesting ecology.

3.6 Acknowledgements

I would like to thank my field assistants and the volunteers who assisted with nest searches and also the land owners and farmers who kindly allowed me to carry out this study on their ground. Thanks are due to the Leverhulme Trust who partly funded this work.

Chapter 4

The impacts of predators and parasites on bumblebee colonies

4.1 Abstract

The study of wild bumblebee nests has been hindered by the difficulty in locating and observing them. Here, 47 wild bumblebee nests were located during 2010 and 2011 in rural locations around Stirling, central Scotland, UK, and the entrances to 32 were filmed using movement sensitive camera recorders in order to identify successful nests (those which produced new queens, termed gynes), vertebrate species interactions and in particular predators. Faecal samples were taken from workers from each nest and examined for presence of *Crithidia bombi, Nosema bombi* and *Apicystis bombi* to enable assessment of their impacts upon gyne production.

Of the 47 nests, 71.4% and 21.1% produced gynes in 2010 and 2011, respectively. A total of 39 vertebrate species were filmed at nest entrances, although the majority did not interact with the bumblebee nest. Great tits (*Parus major*) depredated or attempted to depredate bees on 32 occasions and were also recorded waiting at entrances in an additional 17 events. European hedgehogs (*Erinaceus europaeus*) and carrion crows (*Corvus corone corone*) investigated or attempted, but failed, to access nests. Shrews (*Sorex* spp.), wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), field vole (*Microtus arvalis*), and occasionally rabbits (*Oryctolagus cuniculus*) and stoats (*Mustela erminea*) were recorded accessing entrances to bumblebee nests, but whether they predated the bumblebees was not known. Two nests were visited several hundred times by wood mice, apparently transporting leaf litter into the entrance within a single night, after which bumblebee traffic ceased.

The faeces of 1,179 *B. terrestris* from 29 nests were screened for internal parasites. *Crithidia bombi* was the most prevalent of the three recorded parasites, apparent in 49.0% of samples, and at least one bee from all nests surveyed was infected. Bumblebees with increased wing wear (a sign of age) were significantly more likely to be infected than those with less wing wear. Nests with a high prevalence of *C. bombi* infection among workers were less likely to produce gynes, the first evidence for a direct impact of this common parasite on bumblebee colony reproduction in wild nests.

4.2 Introduction

Bumblebees have many mammalian enemies in Britain, for example, small mammals such as wood mice (*Apodemus sylvaticus*) and shrews (*Sorex* spp.) are thought to enter and predate nests before the first brood of workers have emerged (Darwin, 1906; Sladen, 1912; Cumber, 1953; Pouvreau, 1973), or they may be excavated and eaten by larger mammals such as badgers (*Meles meles*) (Sladen, 1912; Pouvreau, 1973; Alford, 1975) and foxes (*Vulpes vulpes*) (Benton, 2006; Goulson, 2010). Bumblebee nests can also fall victim to the larvae of the wax moth *Aphomia sociella* which consume the entire nest; destroying comb and brood (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Goulson, 2010). A large proportion of our understanding of bumblebee nest predators originates from the extensive work of Sladen (1912). Whilst this book underpins bumblebee research, the author occasionally neglects to describe methods in sufficient detail for them to be replicated or to quote sources of information. For example Sladen (1912) writes that ‘moles and weasels also destroy nests’ yet later states that he has found ‘no evidence for predation by any vertebrates other than mice and shrews’. Similarly in a study of the life histories of 80 *Bombus pascuorum* (formally *Bombus agrorum*) nests, Cumber (1953) documented that 17 were ‘destroyed by rodents, badgers, etc’ and 25 ‘died out prematurely’, but no details on the data collection is given. It is therefore unclear how rodent predation was deduced as the cause of death, or what proportions of failed nests were due to the different predators. Darwin (1906) quoted Col. Newman’s estimate that ‘Two thirds of bumblebee nests are destroyed by field mice’ but again, methods for assigning mice as the cause of failure are not given. Further clarification of the predators of bumblebee nests and quantification of the rates of their destruction is needed in order to advance understanding of bumblebee nest ecology and enable suitable conservation strategies (Goulson, 2010; Winfree, 2010).

The study of wild bumblebee nests has been somewhat neglected as locating nests remains challenging (Osborne et al., 2008a; Suzuki et al., 2009; Kells and Goulson, 2003) due to infrequent worker traffic early in the year and the tendency for nests to be concealed under vegetation or in the burrows of small mammals (Alford, 1975; Fussell and Corbet, 1992b; Lye et al., 2012). In addition, relatively infrequent observations may not provide sufficient information to be certain of a nest’s fate. For example, deducing whether the nest produced males, gynes or was visited by small mammals or succumbed to *A. sociella* during the observer’s absence may not be possible. Most vertebrate predators are likely to modify their behaviour if a human observer is present.

Aspects of bumblebee ecology and behaviour have been studied using nests reared from wild caught inseminated queens in the laboratory or obtained from commercial bumblebee rearing companies (Schmid-Hempel and Schmid-Hempel, 1998; Imhoof and Schmid-Hempel, 1999;

Goulson and Stout, 2001; Goulson et al., 2002b; Carvell et al., 2008; Whitehorn et al., 2012). Such colonies have been either kept in the laboratory or exposed to field conditions according to experimental protocols. The outcomes of these experiments, whilst valuable, may not always provide an accurate representation of wild bumblebee nests as they are not subjected to the same conditions faced by wild nests. For example, nests may be given unrestricted nectar and pollen, maintained at constant climatic conditions, removed from competition, etc. and therefore have an 'advantage' over wild nests which they are meant to represent (e.g. Carvell et al., 2008). Alternatively, confinement of colonies may have a detrimental effect on nests; for example, increase transmission rates of internal parasites (Otti and Schmid-Hempel, 2008). In addition, artificially reared bumblebee nests placed in the field tend to be housed in constructed domiciles raised above the ground and with entrances that are apparent (Lopez-Vaamonde et al., 2004; Carvell et al., 2008). It is conceivable that parasitism by *A. sociella*, (Goulson et al., 2002b) and *Psithyrus* or usurpation by other true bumblebees (Carvell et al., 2008) may be more likely where nests are above ground and easily located, rather than camouflaged amongst vegetation and with entrance tunnels made by other animals.

Rates of gyne production from wild nests are largely unquantified. Data on wild nests in Britain is limited to a study by Cumber (1953) who found 23 (28.8%) of 80 *B. pascuorum* nests produced gynes. Experiments using artificially reared nests find varying levels of reproduction. For example, of control colonies fed *ad. lib.* in the laboratory for two weeks before being placed in the field to forage freely, 25 commercially reared *B. terrestris* colonies resulted in a mean of 13.7 gynes per nests (± 5.7) from 14 (56%) of nests

(Whitehorn et al., 2012). Of 36 laboratory reared *B. lucorum* nests, 5 (13.9% of nests) produced gynes, ranging from 1 to 125 per nest and totalling 250 (Müller and Schmid-Hempel, 1992), and in another study of 32 *B. lucorum* nests, 21.9% produced gynes (Imhoof and Schmid-Hempel, 1999). Others reported lower success; for example none of 14 laboratory reared *B. terrestris* colonies placed in the field produced gynes (Otti and Schmid-Hempel, 2008). It has been hypothesised that the majority of nest failures occur in the very early stages when the founding queen is solely responsible for establishing a nest (Sladen, 1912; Free and Butler, 1959; Alford, 1975), so figures obtained from laboratory reared nests or those followed in the wild after the first brood have hatched are likely to be overestimates.

Internal parasites have been shown to have varying levels of effects upon individuals and laboratory reared colonies. For example, *Nosema bombi* is a microsporidian parasite of bumblebees that has been shown to have a negative impact on bumblebee colonies of *B. terrestris* in the laboratory (Otti and Schmid-Hempel, 2007). Infection of the flagellate trypanosome *Crithidia bombi* is less harmful than *N. bombi* (Brown et al., 2000) while *Apicystis bombi* (a neogregarinid protozoan) is severely detrimental to host bees (Durrer and Schmid-Hempel, 1995).

This investigation aimed to elucidate the relationships between wild bumblebee nests and British vertebrate species, and investigate the effect of wax moths and internal parasites, *A. bombi, C. bombi* and *N. bombi* on gyne production in wild bumblebee nests.

4.3 Methods

The work took place on the University of Stirling campus and nearby farmland in 2010 and 2011. A trained bumblebee nest detection dog and volunteers (mostly students of Stirling University) assisted in locating nests (O'Connor et al., 2012). Two habitats were searched, woodland and meadows. These areas were selected based on the likelihood of their yielding nests (Cumber, 1953; Alford, 1975; Svensson et al., 2000; Free and Butler 1959; Fussell and Corbet, 1992b). Woodlands included mature stands of oak (*Quercus robur*), ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) with deep leaf litter; planted mixed copses approximately 25 years old, with ground cover of grasses and herbs or mature deciduous/coniferous woods with an open canopy and extensive ground cover of nettles, ferns, etc, where light allowed. Grasslands were semi-natural, unmown and ungrazed and characterised by presence of tussocks of dead grasses and herbs. All sites had to be suitable for repeat visits and for use of recording equipment, therefore areas next to roads and paths were avoided to avoid risk of equipment theft or vandalism.

On occasions, nests were found which were deemed too close to paths, and some were reported by farmers in sheds, animal field shelters, etc. These were observed for a minimum of 20 min twice each week and parasite samples were taken but they were not filmed.

4.3.1 Internal parasites

Faeces from *B. terrestris* were screened for the internal parasites *Nosema bombi*, *Crithidia bombi* and *Apicystis bombi*. Faecal samples were collected from five bees twice weekly from each nest where possible. Bumblebees were collected at their nest in clean sample pots. They were released when they defecated or after 15 minutes. Faeces were collected from the pot using a microcapillary tube which was then sealed at both ends with PTFE tape, labelled and chilled on an ice block in the field before being refrigerated at 2-5 ºC. In addition, the same bumblebees were examined for signs of wing wear and assigned to one of four categories (after Carter,1992; see also Alford, 1975; Rodd et al., 1980; Müeller and Wolfmueller, 1993; Whitehorn et al., 2011): 0= no wing wear; 1, some minor indentations; 2, most of margin with minor indentations; 3, more than 5% wing surface missing. In the laboratory, samples were transferred to a haemocytometer within 24 hours and examined under a light microscope at x400 magnification. The presence of *N. bombi*, *C. bombi* and *A. bombi* was recorded, and numbers of each within 0.1μL on the haemocytometer grid was counted. Counts of *C. bombi* and *N. bombi* correlate with intensity of infection (Otterstatter and Thompson 2006; Otti and Schmid-Hempel 2008).

4.3.2 Cameras

Ten camera recorders were designed and manufactured by N. Butcher at the R.S.P.B. Headquarters, Sandy, UK. Each consisted of a black and white, waterproof camera, (Misumi, MO-R430G-C) with a resolution of 240 T.V. lines. Six infrared, no-glow bulbs were positioned around the camera to facilitate night filming. Infrared lighting was controlled by a digital timer, housed inside the weather proof box. A metal hood fitted over and around the camera (and infrared bulbs) and measured approximately 6 x 4 x 3 cm. This was connected to a metal stake 50cm in length. Both hood and stake were painted with a green and brown pattern to camouflage the camera. The metal stake was driven into the ground to hold the camera in position approximately 40-60 cm from the bumblebee nest entrance. The camera was connected via a 4 m cable to a 12 Volt battery and a MemoCam

Digital Video Recording unit, (Video Domain Technologies Ltd., Petah Tikva, Israel), which was housed inside a plastic weatherproof box (approximately 15 x 15 x 12 cm). The weather proof box and battery were wrapped in a rubble sack and buried inside a shallow pit, 4m from the camera. The turf from the excavation was replaced above the equipment to minimise disturbance and provide camouflage. The wire was also buried just below the surface of the ground.

The MemoCam software package is designed for surveillance operations and has been used for vertebrate observational studies (Bolton et al., 2007). The software allows the user to specify an area of the filmed image to be movement sensitive. In this case the nest entrance was selected. The software detected any movement at the nest entrance and recorded one frame before this movement and the following five frames. This ensured there was no time lag between the movement trigger and start of filming, as was found to be an issue with other commercially available wildlife camera traps. Sensitivity was set so that movement of anything greater than ~3mm in diameter would trigger recording (i.e. the very smallest bumblebees were filmed, but diminutive flies were unlikely to trigger recording). Footage was recorded onto 2 G.B. ‘mini’ S.D. memory cards. Batteries and memory cards were replaced every two to three days.

4.3.3 Video analysis

Footage was viewed at approximately x2 real time. Any events which were of interest were watched again at slower speed to establish their exact nature. The number of bees entering and leaving nests was recorded for one hour, from 12:00-13:00 hrs, each day and termed ‘midday traffic’. In some cases the nest was visited at midday by researchers, (changing batteries, S.D. cards, etc,) and in these cases, bumblebee traffic for the hour nearest to midday was used. A nest was deemed over/ended when hourly traffic reduced to fewer than four bees per hour. For every day that a nest was filmed, a seven-day running mean midday traffic was calculated. The greatest value of seven-day mean midday-hour traffic was termed ‘peak traffic’ and used as a proxy measure for the maximum size attained by each nest for statistical analysis.

All vertebrates and wax moths filmed within approximately 1m of the entrance were identified to species and their behaviour was recorded. Behaviours were categorised as: no interaction (where animals simply passed nest entrances); some interaction (sniffing at entrance, waiting at hole); attempted predation (widening entrance, chasing bumblebee foragers) and predation (bees killed); or entering or exiting the nest entrance. For each species, rates were calculated for attempted predation/predation or use of nest entrance by dividing the total number of events by the total number of days that the nest was filmed. Small mammals are more active at night, with very few records during later morning daylight hours. Their numbers were calculated for each 24 hr period beginning at 8am (instead of for example, midnight which would result in nightly visits being split over two days). For small mammals which entered the hole, we would expect a visit to consist of one record of entry followed by one record of exit, but this was not always the case (presumably because some holes led to underground tunnel networks with multiple exits). In this case, the number of entries or exits per 24 h period (whichever was the greater) was used.

4.3.4 Nest success

This study used gyne production as the key measure of nest success. Nests producing gynes invariably also produce males, and there is always a surplus of males (the average ratio has been estimated to be 1 gyne: 7 males; Goulson 2010 and references therein), the majority of which will not mate or contribute to the next generation. The numbers of colonies in the next generation depends entirely upon the numbers of gynes (Chapman and Bourke 2001). It is also more difficult to detect males as for *B. terrestris*, males are not distinctive, and they do not return, whereas gynes frequent the nest for several days before finally leaving.

4.3.5 Statistical analysis

Statistical analysis was carried out using R Statistical Software Version 2.12.2 (R Development Core Team, 2011). Assumptions for all tests were checked. Where possible, models were simplified by backward, stepwise removal of non-significant variables, using a *P*-value significance threshold of 0.05. Model fit was checked by visual examination of residuals. Over-dispersion in the data was assessed and any points with Cook's Distance of greater than 1 were removed from analysis due to disproportionate influence on the data set (Zuur et al., 2007).

Gyne production by nests

(i) *Gyne production in the different years*

A Chi-squared test was used to compare gyne production in 2010 and 2011 for all bumblebee species combined. This analysis included both filmed nests and those that were observed (minimum of bi-weekly).

A General Linear Model (GLM) with binomial distributions was used to assess the effect of 'peak traffic' and 'days filmed' on the likelihood of each nest producing gynes, using data from the filmed nests only.

Species interactions with bumblebee nests

(ii) *Factors influencing nest visitations by great tits, moths or small mammals.*

Four Generalized Linear Models were used to investigate the likelihood of wood mice, shrews, great tits and wax moth visiting nests, with each species requiring a separate model. The response for each of these models was the total number of visits from the species of interest to each nest, using 'year' as a fixed factor and 'peak traffic' as a covariate in the model. This was used in order to detect preference or avoidance of large nests. Models used quasi-Poisson distribution to account for over-dispersion in the data. Some data points were removed from the analysis (two nests each from wood mouse, shrew and great tit models and one nest from wax moth model) because these data were outliers and were overly influential as they had a Cook's Distance of >1 (Zuur et al., 2007). There were too few nests visited by bank voles (*Clethrionomys glareolus*) or field voles (*Microtus arvalis*) to allow statistical analysis (four and three nests respectively).

(iii) *Effect of great tit, moth or small mammal visits upon gyne production*

A General Liner Model with binomial distribution was used to assess the effect of visits from wood mice, shrews, great tits and wax moths upon gyne production (binary response), including 'peak traffic' as a covariate.

Internal parasites

(iv) *Factors affecting the likelihood of bee carrying protozoan infection*

Two Generalized Linear Mixed effects Models (GLMM) were carried to identify factors that influenced the likelihood of a *B. terrestris* worker bee carrying either a *C. bombi* or a *N. bombi* infection. The model used 'Presence of infection' (of either *C. bombi* or *N. bombi*) as the binary response, with the following potential explanatory variables: 'Year', 'Habitat', and 'Presence of other protozoan infection' (i.e. either *C. bombi* or *N. bombi,* whichever was not being used as response). As fixed factors in these two models, 'Nest' (i.e. the nest from which the worker was caught) was used as a random factor, and 'Day' (i.e. day on which the sample was taken; day one being the first day a nest was found in that year) as a covariate. The interaction between 'Year' and 'Day' was also included.

(v) *Impact of protozoan infections on gyne production of nests*

In order to assess the impact of a workforce infected with either *C. bombi* or *N. bombi* on nest success, i.e. gyne production. A General Linear Model with binomial distributions was used to assess the likelihood of *B. terrestris* nests producing gynes (the binary response), with the 'proportion of infected bees' for *C. bombi, N. bombi* and presence or absence of *A. bombi* as covariates.

4.4 Results

4.4.1 Gyne production

A total of 47 bumblebee nests were found. In 2010, 28 nests were located and 19 of these were filmed. In 2011, 19 nests were found and 13 were filmed (Table 4.1). The majority of the nests (34) were *B. terrestris*, with small numbers of other species; *Bombus hortorum* (4), *Bombus lapidarius* (3), *Bombus lucorum* (2), *Bombus pascuorum* (2) and *Bombus pratorum* (2).

(i) *Gyne production in the different years*

Across all 47 nests (i.e. all species and both filmed and observed nests gyne production was significantly greater in 2010 than 2011 with gynes successfully produced by 71.43% and 21.10% of nests in 2010 and 2011, respectively ($\chi^2_{\text{D.F. 1}} = 12.71$, $P < 0.001$; Figure 4.1a). Two nests (27 and 29; Table 4.1) failed on or soon after the day that they were found (i.e. >2 bees were seen to enter or leave the entrance, but thereafter, either no or very few (<5) bees were seen. It is highly unlikely that gynes could have been made by these nests, but as we have no estimations of peak traffic, vertebrate species visits, etc, these two nests were not included in statistical analysis of predator/moth visits etc.

Of the filmed nests suitable for analysis, (n=30) those with high ‘peak bumblebee traffic’ were significantly more likely to produce gynes ($F_{\text{D.F. 1}} = 40.26$, $P < 0.001$; Figure 4.1b). There was no difference in the duration of nest filming and likelihood of it producing gynes, therefore, data were collected equally for both nests that successfully produced gynes and nests that failed to produce gynes ($F_{\text{D.F. 1}} = 0.80$, $P = 0.379$; Figure 4.1c).

Table 4.1. Longevity, gyne production and the proportion of bees hosting *C. bombi* and *N. bombi* infections for filmed nests. *Nest which had failed prior to being filmed; >2 bees were seen to enter or and leave, but footage of entrances revealed few/no further bee traffic. These nests were excluded from predation/wax moth analysis.

Nest details			**Period of filming (dd/mm/yy)**			**Proportion of bees hosting an infection**		
Ref.	**Species**	**Habitat**	**Start**	**End**	**Gynes Produced**	***C. bombi***	***N. bombi***	**(n)**
1	*B. terrestris*	Woodland	19/07/10	17/08/10	Yes	0.46	0.05	39
2	*B. terrestris*	Woodland	27/07/10	17/08/10	Yes	0.74	0.03	35
3	*B. terrestris*	Woodland	15/06/10	26/07/10	No	0.92	0.08	26
4	*B. terrestris*	Woodland	09/08/10	18/08/10	No	1.00	0.50	2
5	*B. terrestris*	Grassland	29/07/10	16/09/10	Yes	0.48	0.05	65
6	*B. hortorum*	Grassland	25/06/10	05/08/10	Yes	0.43	0.11	37
7	*B. hortorum*	Grassland	19/06/10	25/07/10	No	0.62	0.12	34
8	*B. lapidarius*	Woodland	27/07/10	10/08/10	Yes	0.50	0.00	6
9	*B. pratorum*	Grassland	10/06/10	08/07/10	No	0.80	0.07	15
10	*B. terrestris*	Grassland	13/06/10	28/07/10	No	0.34	0.00	29
11	*B. terrestris*	Woodland	22/06/10	30/07/10	Yes	0.50	0.06	34
12	*B. terrestris*	Woodland	09/08/10	20/08/10	No	0.29	0.00	7
13	*B. terrestris*	Woodland	18/08/10	22/08/10	Yes	0.60	0.20	5
14	*B. lucorum*	Woodland	16/06/10	29/08/10	Yes	0.51	0.03	63
15	*B. terrestris*	Woodland	22/06/10	27/07/10	Yes	0.65	0.18	55
16	*B. terrestris*	Woodland	13/08/10	06/09/10	Yes	0.28	0.01	80
17	*B. terrestris*	Woodland	16/06/10	16/07/10	No	1.00	0.00	9
18	*B. terrestris*	Woodland	29/06/10	16/08/10	Yes	0.39	0.07	61
19	*B. terrestris*	Woodland	19/06/10	03/09/10	Yes	0.69	0.21	94
20	*B. pratorum*	Woodland	31/05/11	20/06/11	No	-	-	-
21	*B. terrestris*	Woodland	01/06/11	08/08/11	No	0.55	0.02	60
22	*B. terrestris*	Woodland	01/06/11	29/08/11	No	0.27	0.01	138
23	*B. hortorum*	Woodland	01/06/11	08/08/11	No	0.38	0.00	13
24	*B. terrestris*	Woodland	02/06/11	11/07/11	No	0.71	0.00	24
25	*B. terrestris*	Grassland	02/06/11	29/06/11	No	0.50	0.00	10
26	*B. terrestris*	Grassland	06/06/11	02/09/11	Yes	0.38	0.02	112
27*	*B. terrestris*	Woodland	09/06/11	15/06/11	No	-	-	-
28	*B. terrestris*	Woodland	14/06/11	23/06/11	No	1.00	0.00	1
29*	*B. terrestris*	Woodland	23/06/11	24/06/11	No	-	-	-
30	*B. terrestris*	Woodland	27/06/11	01/09/11	Yes	0.27	0.00	75
31	*B. terrestris*	Woodland	12/07/11	28/09/11	No	0.38	0.00	58
32	*B. terrestris*	Woodland	22/07/11	10/09/11	Yes	0.33	0.00	51

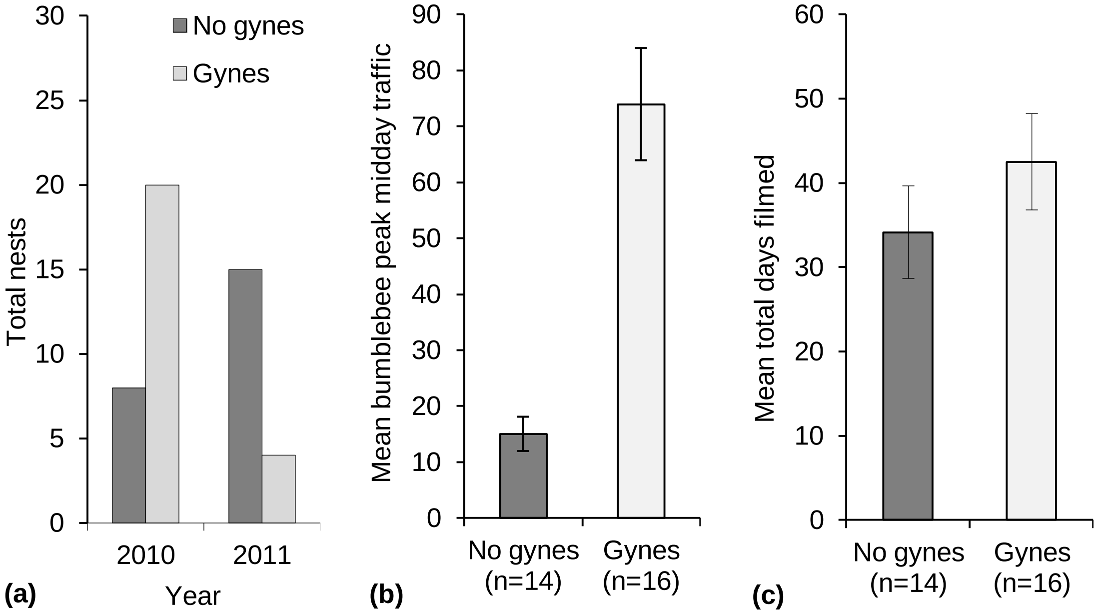

Figure 4.1. (a) Total nests and presence or absence of new gynes, for all species. (b) Mean bee peak of traffic for nests with and without new gynes (filmed nests only). (c) Mean of total days nests were observed for. (Error bars (b and c) show standard errors of means.)

4.4.2 Species interactions with bumblebee nests

Thirty-three vertebrate species were recorded at bumblebee nest entrances on at least one occasion (Table 4.2) in addition to the wax moth *A. sociella*. The majority of vertebrates filmed did not interact with the bumblebees or their nests.

Table 4.2. Interactions with animals observed on the cameras. Invertebrate observations were not recorded, with the exception of wax moths and their larvae.

Species (common name)	**Events**	**Nests**	**Summary of interactions with nests (n=events)**
Large mammals			
Vulpes vulpes (fox)	4	4	No interaction
Mustela erminea (stoat)	12	5	Enter and leave (1)
Erinaceus europaeus (hedgehog)	34	15	Attempts to gain access (7)
Sciurus carolinensis (grey squirrel)	157	22	Sniffed at or near entrance (32), displayed some interest, looked in hole or dug at nearby leaves (7)
Oryctolagus cuniculus (rabbit)	207	22	Sniffed at entrance (34), entered hole (1)
Lepus europaeus (hare)	7	3	No interaction

Species (common name)	**Events**	**Nests**	**Summary of interactions with nests (n=events)**
Capreolus capreolus (roe deer)	8	4	No interaction
Ovis aries (sheep)	1	1	No interaction
Felis catus (cat)	6	3	No interaction
Canis lupus familiaris (dog)	1	1	No interaction
Bos primigenius (Cow)	9	1	No interaction
Small mammals			
Clethrionomys glareolus (bank vole)	17	4	Enter and leave (8)
Microtus arvalis (field vole)	70	3	Enter and leave (21)
Apodemus sylvaticus (wood mouse)	1396	18	Enter and leave (837)
Unidentified small mammal	16	7	Enter and leave (16)
Sorex spp. (shrew species)	162	10	Enter and leave (56)
Reptiles and Amphibians			
Lacerta vivipara (common lizard)	1	1	No interaction
Rana tempora (frog)	7	5	No interaction
Bufo bufo (toad)	5	3	No interaction
Wax moth			
Aphomia sociella	19	8	Enter and leave (19)
Birds			
Anas platyrhynchos (mallard)	1	1	No interaction
*Columba palumbus (*wood pigeon)	8	3	No interaction
Corvus corone corone (carrion crow)	16	4	Pecking at hole and widening entrance (5)
Erithacus rubecula (robin)	29	12	Investigation/waiting at nest (5) possible attempted predation of worker (1)
Fringilla coelebs (chaffinch)	20	6	Looking at or waiting at hole (3) no bee chases or kills
Haematopus ostralegus (oyster catcher)	12	1	No interaction; Investigating entrance (1)
Turdus merula (blackbird)	28	13	Investigating/waiting at hole (5) Possible attempted predation of worker (1)
Turdus spp. (thrush other)	10	6	Entrance investigated (1), no traffic and no predation
Parus caeruleus (blue tit)	1	1	No interaction
Parus major (great tit)	60	10	Predations (10) attempted predations (22) 'stalking' (17)
Passer montanus (tree sparrow)	6	4	No interaction
Pica pica (magpie)	1	1	No interaction
Prunella modularis (dunnock)	22	4	Investigating/waiting at entrance (6) no bee chases or kills
Troglodytes troglodytes (wren)	11	7	No interaction

The most interactive vertebrates were great tits, hedgehogs, crows and small mammals (Table 4.2; Figure 4.2). Hedgehogs and crows were filmed investigating entrance holes and enlarging the entrance in what appeared to be deliberate access attempts on seven and five occasions respectively, but they were unable to penetrate any of the subterranean nests (Figure 4.2). Squirrels and rabbits were filmed around nest entrances often, and sometimes dug in leaf litter, but did not appear intent on gaining access nor did they attempt to interact with bees, and were observed carrying out similar behaviour away from nest entrances (Table 4.2).

Great tits depredated foragers, gynes and one male as they departed from or returned to the nest. On a total of 32 occasions at six nests, the birds pecked at walking bees, but also appeared to watch returning bees before they landed and occasionally pursued bees into the air. Great tits were also filmed exhibiting ‘stalking behaviour’ on 17 occasions at eight nests (i.e. remained at entrance holes, looked inside, removed leaf litter from the entrance, etc,) but no bees were present. Stalking, predation attempts or successful predations took place at ten nests, in both years, at sites approximately 4km apart.

Figure 4.2. (a) Great tit predating *B. terrestris* (nest 1; Table 4.1); (b) Hedgehog ‘rooting’ in leaves at nest entrance (nest 1; Table 4.1).

Small mammals (i.e. bank voles, field voles, shrews and wood mice) were frequently recorded (>1000 events) entering and leaving nest entrances. However, most of these events probably indicate shared occupancy of the burrow system rather than small mammal predation of bumblebee nests or direct interaction and this data is therefore difficult to interpret. However, at two nests, (ref 16 and 23; Table 4.1) wood mouse visits peaked during one night and no bumblebee traffic was seen thereafter (Figure 4.3). In these instances, mice carried leaf litter into the entrances and in one case (Figure 4.4) excavation of the tunnel revealed that the tunnel had been tightly blocked with leaf litter and more than 50 live but subdued adults and considerable amounts of pupae and larvae remained in the nest, suggesting that it ended prematurely.

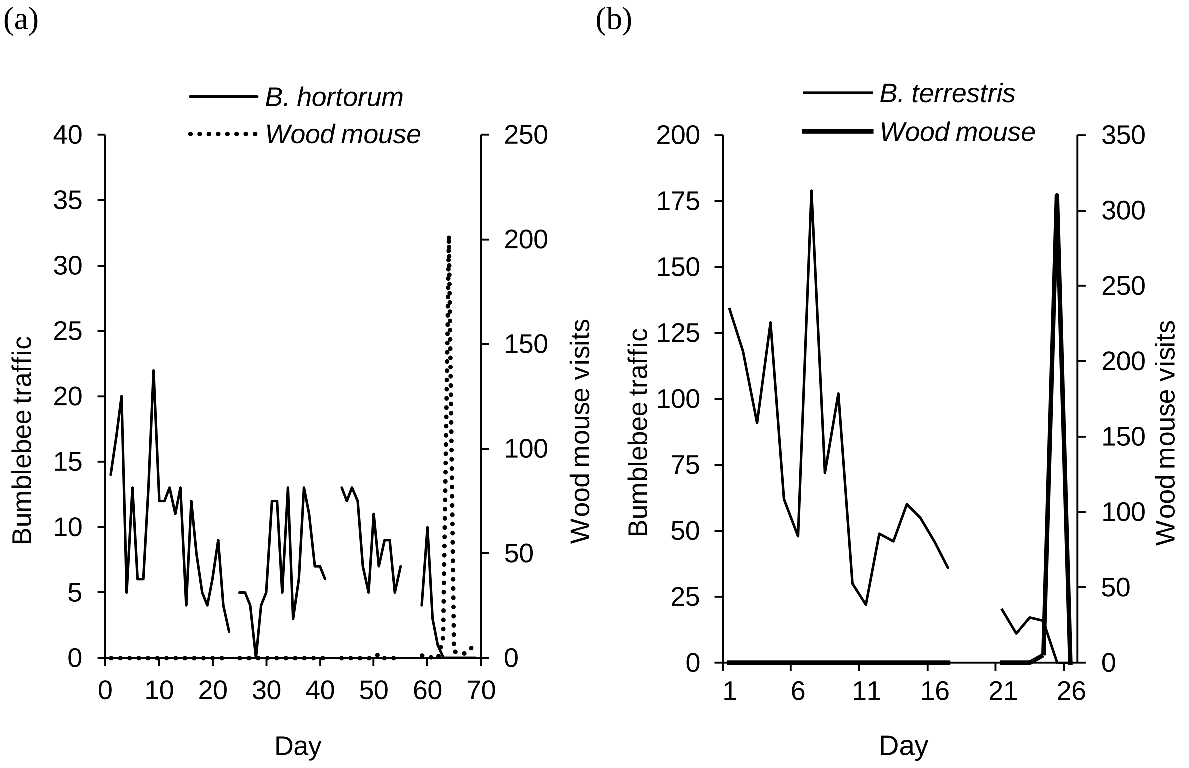

Figure 4.3. Wood mouse visits during 24hr and daily bumblebee midday hourly traffic at (a) nest 23 (*B. hortorum*) and (b) nest 16 (*B. terrestris*). Breaks in lines indicate loss of footage. Wood mice transported leaf litter into nest entrances during visits.

Figure 4.4. (a) Wood mice transported leaf litter into *B. terrestris* nest entrance (nest 16). (b) Nest tunnel and external entrance was blocked by leaves and sticks placed by wood mice several hours later. Bumblebee traffic ceased.

Wax moths were filmed entering/leaving 8 of the 30 filmed nests. There was one instance of a *B. terrestris* worker removing what appeared to be an apparently live *A. sociella* caterpillar outside the nest entrance (Figure 4.5). No other caterpillar removal events were recorded.

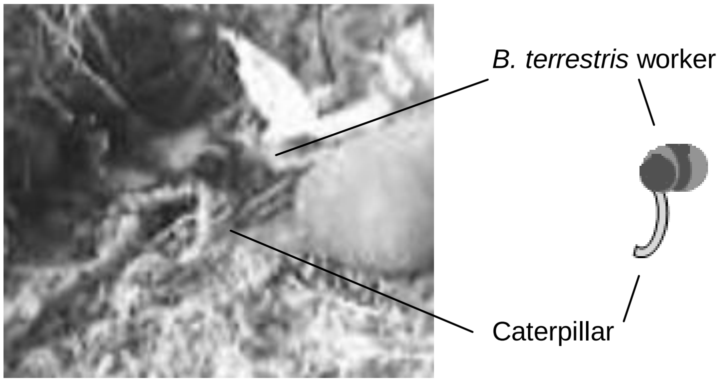

Figure 4.5. *Bombus terrestris* worker removing a possible wax moth caterpillar from the nest entrance (Nest 26; Table 4.1).

(ii) *Factors influencing nest visitations by great tits, moths or small mammals.*

Numbers of great tit predations were not significantly different in either year ($\chi^2_{\text{D.F. 1}}$ = 1.13, *P* = 0.470). There appeared to be a trend for great tits to target larger nests with greater peak bumblebee traffic than smaller nests with infrequent bumblebee traffic, but this trend was not significant ($F_{\text{D.F. 1}}$ = 7.94, *P* = 0.057; Figure 4.6).

Visits from wood mice to nests were not influenced by year, ($F_{\text{D.F. 1}}$ = 1.16, *P* = 0.291) or peak bumblebee traffic ($F_{\text{D.F. 1}}$ = 1.23, *P* = 0.276). Numbers of shrew visits to bumblebee nests differed significantly between years ($F_{\text{D.F. 1}}$ = 44.86, *P* < 0.001; Figure 4.7) but were not influenced by bumblebee traffic ($F_{\text{D.F. 1}}$ = 0.02, *P* = 0.890). There were too few nests visited by bank and field voles to allow statistical analysis.

The likelihood of wax moths entering or leaving a nest was not affected by the year ($F_{\text{D.F. 1}}$ = 0.92, *P* = 0.443) or peak bumblebee traffic ($\chi^2_{\text{D.F. 1}}$ = 1.92, *P* = 0.279).

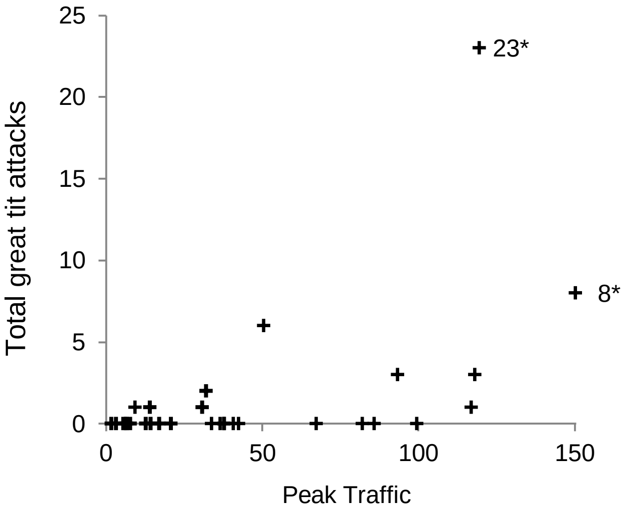

Figure 4.6. Total number of great tit attacks in relation to peak traffic of nests. Points 8 and 23 were removed from the analysis as they had Cook's distance greater than 1 (i.e. they were overly influential outliers; Zuur et al., 2007).

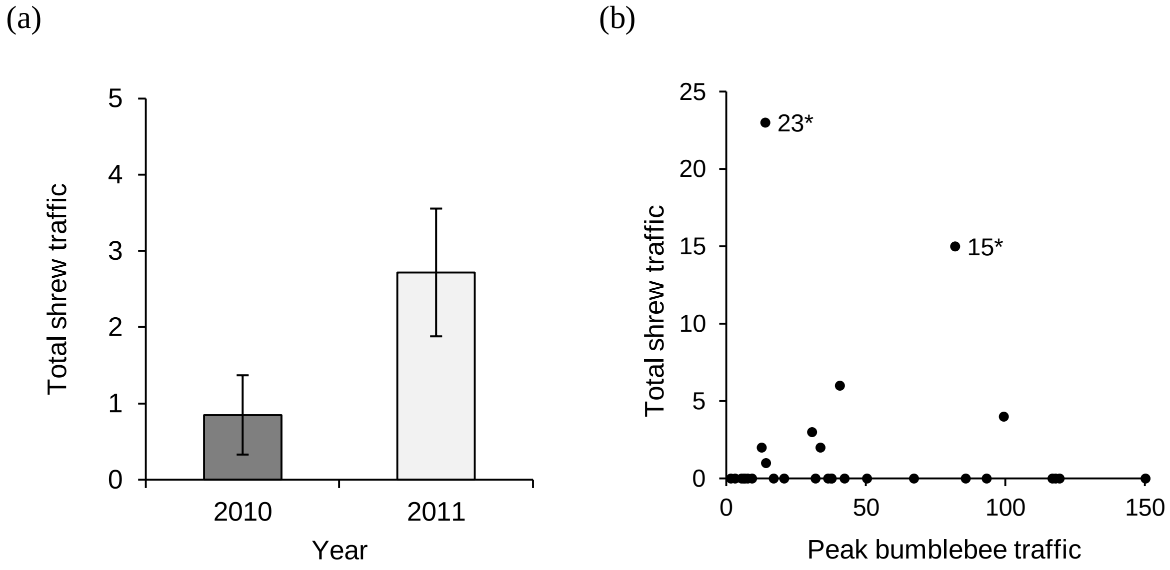

Figure 4.7. (a) More shrews were recorded visiting nests in 2011 than in 2010 (mean and standard errors); (b) There was no relationship between shrew visits and peak bumblebee traffic. *Points 23 and 15 were removed from statistical analysis as they were overly influential on the data set (Cook's distance of >1; Zuur et al., 2007).

(iii) *Effect of great tit, moth and small mammal visits upon gyne production*

Neither mouse nor shrew visits affected the likelihood of a nest producing gynes ($\chi^2_{\text{D.F. 1}} = 0.48$, $P = 0.485$ and $\chi^2_{\text{D.F. 1}} = 0.32$, $P = 0.571$, for wood mice and shrews respectively). Great tit attacks and wax moth visitations were positively correlated with gyne production ($\chi^2_{\text{D.F. 1}} = 5.47$, $P = 0.019$ for great tits (Figure 4.8a) and $\chi^2_{\text{D.F. 1}} = 3.88$, $P = 0.049$, for wax moths (Figure 4.8a). As gyne production is not dependent upon depredation by either of these species it seems more probable that longer lived nests were more likely to be targeted than shorter lived nests which were less likely to produce gynes. Results should be interpreted with caution due to sample size (wax moths were recorded at eight nests and great tits at ten).

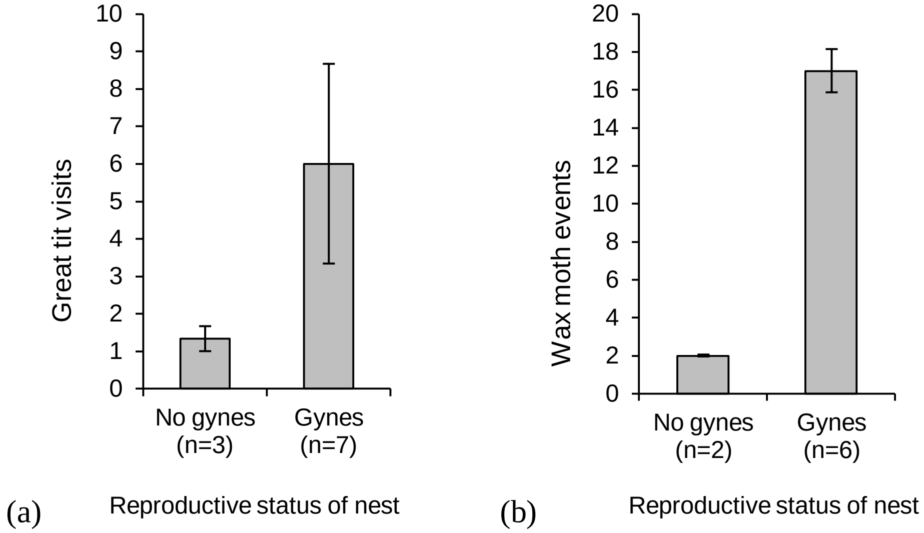

Figure 4.8. Mean great tit attacks (a) and wax moth events (b) to nests with and without gyne production (error bars show standard errors).

4.4.5 Psithyrus

A *Bombus sylvestris* queen was filmed exiting a *B. pratorum* nest 5th June 2011 (nest 20; Table 4.1). Within seven days the nest traffic was much reduced to 0-4 bees per hour. No other *Psithyrus* were observed.

4.4.6 Interspecific parasitism (other bumblebee species)

One *B. lapidarius* nest was visited by 14 *B. terrestris* or *B. lucorum* workers (Figure 4.9) over six days. None of the *B. terrestris* visitors were carrying pollen and so cohabitation of the burrow system seems doubtful. It seems more likely that the *B. terrestris* were ‘stealing’ nectar or pollen from the *B. lapidarius* nest.

A queen *B. terrestris* or *B. lucorum* entered a small *B. terrestris* nest on 8th July 2010 (nest 17; Table 4.1) and a queen exited the nest approximately twelve minutes later. The queen walked around the entrance of the nest for some time and it appeared unable to fly, eventually walking out of view. Whether this bee was the founding queen or the intruder is unclear as the nest was queenless eight days later when it was excavated. Subsequent genetic analysis of the remaining twelve workers (see Chapter 6) showed that they were sisters. Similarly, a *B. terrestris* or *B. lucorum* queen was filmed entering a *B. terrestris* nest (nest 10; Table 4.1) in early July, and genetic analysis of nest mates showed that there were unrelated individuals in the nest (see Chapter 6), but the foreign queen was not found.

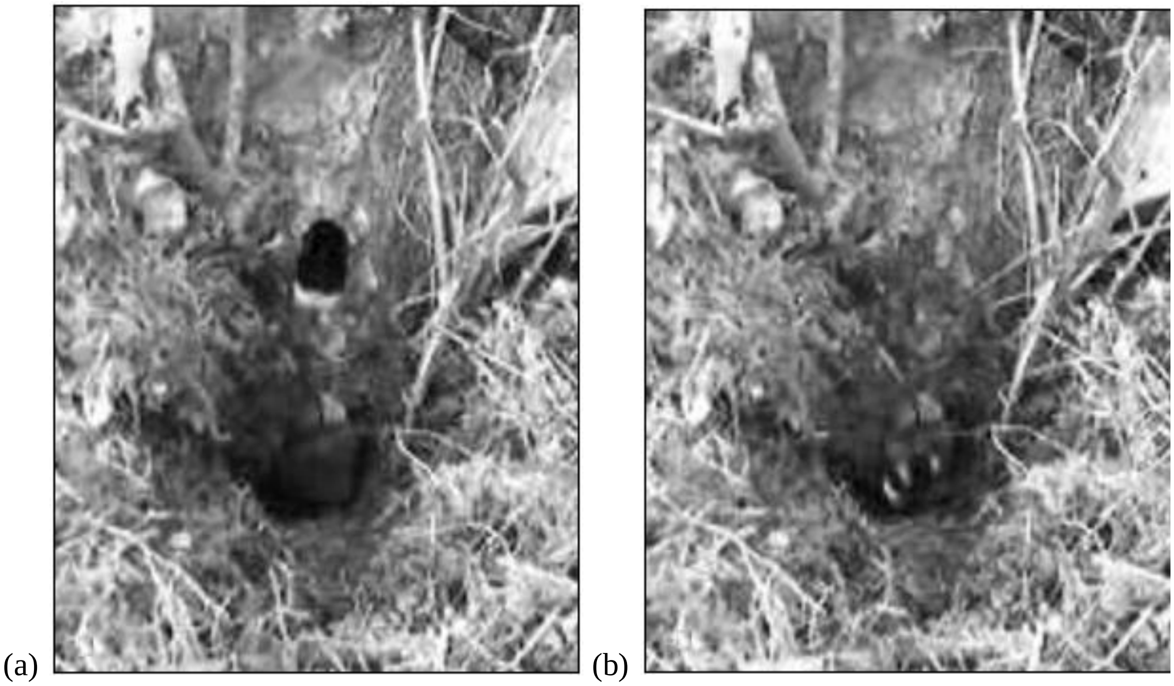

Figure 4.9. (*a*) *B. lapidarius* nest (*b*) visited by *B. terrestris* or *B. lucorum* worker. The footage allows identification from the different stripe patterns between some species. (The red tail of *B. lapidarius* appears white.)

4.4.7 Internal parasites

In total 1,179 faecal samples from *B. terrestris* workers were examined for infections of the three protozoan infections (682 and 497 collected in 2010 and 2011, respectively). *Crithidia* *bombi* was far more prevalent than *N. bombi* and only eight bumblebees were infected with *A. bombi* (bees from five nests, all detected in 2010).

(iv) *Factors affecting the likelihood of a B. terrestris worker presenting an infection*

Infections of *C. bombi* were detected more frequently in the faeces of *B. terrestris* with increased wing wear (assumed to be older bees) than unworn, younger bees ($\chi^2_{\text{D.F. } 3} = 60.89$, $P < 0.001$; Figure 4.10). There was a significant 'Year by Day' interaction; *B. terrestris* were less likely to present *C. bombi* infections towards the end of the summer and this decline was more marked in 2011 ($\chi^2_{\text{D.F. } 1} = 11.00$, $P < 0.001$; Figure 4.11). Infection with *N. bombi* was not significantly associated with *C. bombi* infection ($\chi^2_{\text{D.F. } 1} = 3.82$, P 0.051).

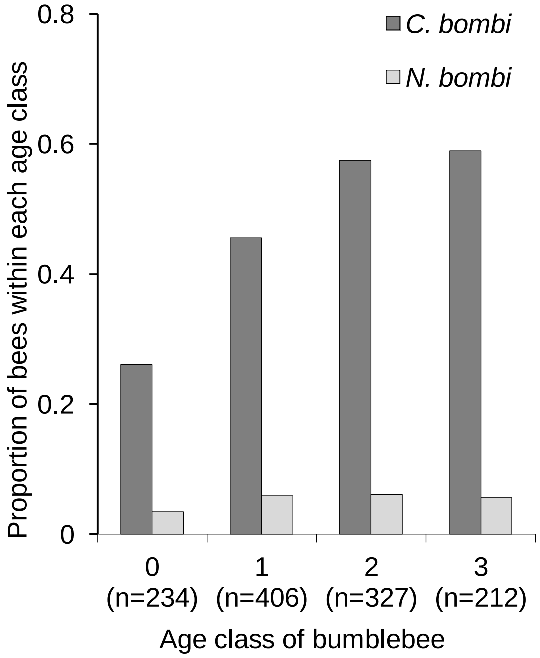

Figure 4.10. Proportion of bumblebees infected with *C. bombi* and *N. bombi* within each age class (0=no wing wear; 1=some indentations; 2=<5% of wing surface damaged; 3=>5%wing wear absent).

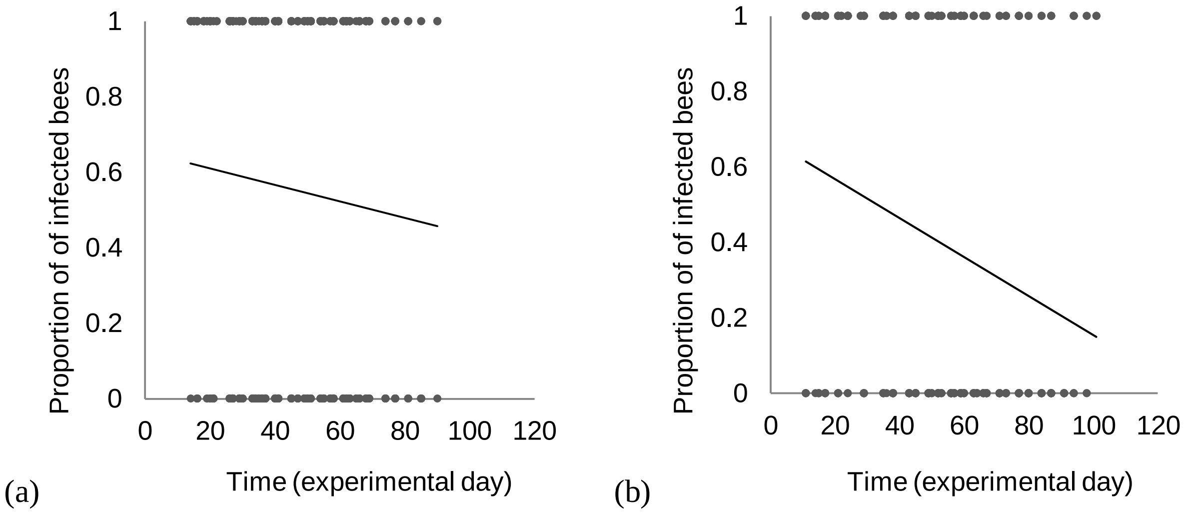

Figure 4.11. Proportion of worker *B. terrestris* infected with *C. bombi*, throughout the experiment in (a) 2010 and (b) 2011.

Crithidia bombi infections did not spread through all nest mates in wild *B. terrestris* nests (Figure 4.12 shows two typical examples of sampled nests). There were often uninfected and infected bees collected within the same sample, and intensity of infections varied greatly.

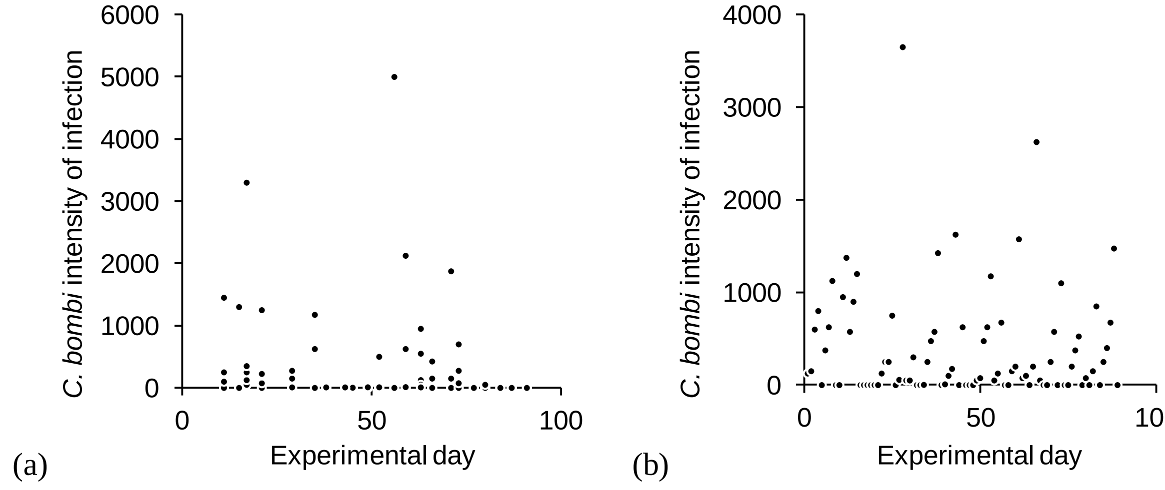

Figure 4.12. Intensity of *C. bombi* infections in *B. terrestris* from two typical nests (Nests 16 and 26; Table 4.1) for the duration of observations.

The likelihood of a *B. terrestris* worker presenting a *N. bombi* infection was significantly affected by 'Year' ($\chi^2_{\text{D.F. 1}}$ =15.16, $P < 0.001$) with a far greater proportion of *N. bombi* infections detected in 2010 (0.084 (62 bees) and 0.008 (4 bees) in 2010 and 2011 respectively). Bumblebees infected with *C. bombi* were significantly more likely to be infected with *N. bombi* ($\chi^2_{\text{D.F. 1}}$ =11.34, P =< 0.001). Unlike with *C. bombi* infections, the likelihood of a *B. terrestris* worker being infected with *N. bombi* was not associated with bee 'Age' ($\chi^2_{\text{D.F. 3}}$ =0.27, $P = 0.965$; Figure 4.10). 'Day' was not significant ($\chi^2_{\text{D.F. 1}}$ =0.1, $P = 0.750$) and there was no 'Day' by 'Year' interaction ($\chi^2_{\text{D.F. 1}}$ =0.23, $P = 0.630$).

(v) *Impact of protozoan infections on gyne production of nests*

The likelihood of a *B. terrestris* nest producing new gynes was not affected by presence of *A. bombi* in at least one worker ($\chi^2_{\text{D.F. 1}}$ =0.447, $P = 0.580$), nor by the proportion of workers infected with *N. bombi* ($\chi^2_{\text{D.F. 1}}$ =0.217, $P = 0.641$). The proportion of workers infected with *C. bombi* had a significant negative effect on the likelihood of a nest producing gynes, ($\chi^2_{\text{D.F. 1}}$ =7.433, $P = 0.006$; Figure 4.13).

Figure 4.13. Gyne production from each nest and mean proportion of *B. terrestris* workers infected with *C. bombi* from 29 nests (with interquartile ranges, maximum and minimum values are shown).

4.5 Discussion

The proportion of nests producing gynes varied between the two years of observations with more nests producing gynes in 2010 than 2011. No reason for this disparity was observed in the field (for example nests were not flooded) and the trend was not explained by rates of other species visitations to nests. The proportion of *B. terrestris* infected with *C. bombi* and *N. bombi* was appreciably lower in 2011, but this is unlikely to have caused any reduction in gyne production. There may have been more inclement weather in early summer 2011, (pers. obs.) and this may have been influential, but climatic data was not included in this experiment.

Great tits were previously known to predate vulnerable/walking bumblebees, including bees feeding on *Rhododendron* spp. (Free and Butler, 1959) or *Tilia* spp. (Sladen, 1912; Benton, 2006) which have an intoxicating effect on bees, at their overwintering sites and those infected with *Sphaerularia bombi* (Bols; quoted in Benton, 2006). This is therefore the first time that great tits have been found to predate ‘healthy’ bumblebees, and identifies them as a new predator of bumblebee nests. Some of the nests which were targeted by great tits produced gynes. Whilst some gynes were subsequently predated by the birds and the behaviour is extensive (almost a third of filmed nests were targeted by great tits) it seems unlikely that great tits are a limitation to populations. It would be useful for further filming of nests to be carried out elsewhere to establish if this behaviour is constrained to the region or common across Britain.

4.5.1 Small mammal traffic

All nests were found in networks of nests, runs and burrows which appeared to have been made by other animals, which is a well known trait of bumblebees (Sladen, 1912; Alford, 1975; Lye et al., 2012). The majority of these burrows were frequented by vertebrates at the time of bumblebee occupation, as indicated by video footage. Whilst not empirically tested, there seems to be no evidence that small mammals avoid burrows containing a bumblebee nest. Several mammalian species were recorded frequenting burrows where bumblebees were nesting, and their status as predators or cohabiters remains uncertain. Evidence in the form of continuing daily bumblebee traffic suggests that these species did not destroy nests, but they may have been predating some adult bees or brood, and footage of the nest itself would be needed to establish the actual relationships with these animals. Footage from within the nest would facilitate any interactions out-with the capacity of this experiment for example those of European moles (*Talpa europaea*). As most small mammal predation events are thought to occur when bumblebee nests are small, Sladen, 1912; Pouvreau 1973; Alford, 1975) before the first brood of workers, it would be extremely interesting to film incipient nests, as this is also the time at which most usurpations are thought to take place, and is the time of most nest failures (Alford, 1975). However, finding such nests in the wild poses a serious challenge.

4.5.2 Internal parasites

Infections of *C. bombi* and *N. bombi* are known to be spread horizontally between nest mates consuming contaminated nectar and pollen from stores in wax pots within nests (Otti and Schmid-Hempel 2008; Erler et al., 2012) or between foragers visiting flowers which have recently been contaminated by an infected bumblebee (Durrer and Schmid-Hempel, 1994; Rutrecht et al., 2007). As found here, rates of infections of *C. bombi* and *N. bombi* are known to vary greatly between species, populations and years (Popp et al., 2012) and *C. bombi* was by far more common than *N. bombi* or *A. bombi* (Otti and Schmid-Hempel, 2008) and is thought to be less harmful (Brown et al., 2000).

Older bumblebees are more likely to be infected and this is thought to be due to increased exposure and reduced immune response (Shykoff and Schmid-Hempel, 1991; Otterstatter and Thompson, 2006; Rutrecht et al., 2007). However, it should be noted that although commonly used as a measure of age, (for example, Müeller and Wolfmueller 1993; Collar et al., 2006; Whitehorn et al. 2011), wear to the wing margin does not always correspond accurately to the age of a bee, as was found in a mark-recapture study of *Euglossa cordata* (Lopez-Uribe et al., 2008).

In experiments of laboratory reared nests in which bumblebees were allowed to forage externally, it was found that infections of *C. bombi* were rapidly picked up (Imhoof and Schmid-Hempel, 1999) which is also in line with our findings as all nests sampled had at least one individual infected with *C. bombi*. In laboratory reared nests, *C. bombi* infections spread quickly among nest-mates (Otti and Schmid-Hempel 2008). However this trend was less obvious in this study as uninfected bees were recorded alongside infected nest-mates, several weeks after the first infected bees were recorded in the colony. This could be because foragers are able to supplement their nectar and pollen intake out-with the nest whereas in the laboratory colonies close confinement and the use of communal feeders may facilitate transmission (Imhoof and Schmid-Hempel 1999). Alternatively it may be an artefact of the detection method as there is known to be a delay of several days from infection until presentation in faeces (Shykoff and Schmid-Hempel, 1991) which will result in under recording of early infections. Faecal samples were also highly variable in their parasite load of *C. bombi* and this had been reported previously (Otterstatter and Thompson, 2006).

These data provide the first insight into the fate of wild nests infected with these three parasites. While infection by *N. bombi* and *A. bombi* was scarce and not clearly associated with nest performance, nests with a high prevalence of *C. bombi* infection among workers were less likely to produce gynes, the first evidence for a direct impact of this common parasite on bumblebee colony reproduction in wild nests. We did not study the impacts of this parasite on the fitness of queens produced from heavily infected nests, which would provide an interesting avenue for further study.

4.5.3 Wax Moths - Aphomia sociella

Wax moths are well known enemies of bumblebee nests (Sladen 1912; Free and Butler, 1959; Pouvreau 1973; Alford 1975; Goulson et al., 2002b), but there is little quantitative information on the rates of infestations. A quarter of filmed nests in this study were invaded by wax moths, a considerably lower rate than was found for commercially reared nests of bumblebees which have been set out in the field for several weeks (Goulson et al., 2002b; pers. obs.). This may support assumptions that subterranean nests are less likely to be affected than surface nests (Alford 1975) and again highlights the need to interpret results from experiments using artificially reared colonies with caution. Six of the eight nests entered by wax moths produced gynes, suggesting that at least sometimes, it is possible for nests to grow large and achieve some degree of reproductive success before the brood is destroyed. However, sample sizes are too small to draw firm conclusions.

Bumblebees are considered defenceless to wax moths (Free and Butler, 1959). However, a *B. terrestris* worker was filmed removing an apparently live *A. sociella* caterpillar outside the nest entrance, although the quality of footage does not allow confident identification of the caterpillar species. Whilst this was the only record of this phenomenon, it is possible that this is a more common occurrence. However, this is unlikely to have prevented the destruction of the nest given the large size already attained by the caterpillar and that infested nests typically contain around 100 *A. sociella* caterpillars (Alford 1975; Goulson 2010).

4.5.4 Psithyrus

Only one *Psithyrus* was observed, a single *B. sylvestris* queen was recorded exiting a *B. pratorum* nest. Nest traffic dwindled thereafter. It is not known if this was a rebuffed queen *B. sylvestris* or a newly produced gyne. If it were a gyne, then one would perhaps expect to have seen more than one *B. sylvestris* offspring leaving the nest, but a new generation of reproductive *B. sylvestris* are reported to emerge from the end of May onwards (Benton, 2006) so it may have been a new queen. We recorded no *Psithyrus* entering nests of *B. terrestris*, which is unsurprising given that *Bombus vestalis* although widespread in England, does not occur in Scotland (Benton, 2006). *Bombus bohemicus* does occur in Scotland and although *B. bohemicus* preferentially invades *B. lucorum*, it is sometimes considered to be a generalist nest parasite (Kreuter et al., 2010) and has enslaved *B. terrestris* colonies in laboratory trials (Vergara et al., 2003) and been observed in a *Bombus hypnorum* nest (Benton, 2006). No *B. bohemicus* were filmed in this study. Other studies have found *Psithyrus* more common. For example, in a study conducted in southern England of 48 commercially reared *B. terrestris* colonies which were transferred outside after their first brood of workers, 39 were invaded with 129 *B. vestalis* queens during May and June (Carvell et al., 2008). Whilst the authors realise the positioning of the colonies above ground level may have influenced the rate at which nests could be located by *B. vestalis*, this highlights the need to carry out filming elsewhere in UK in order to fully understand the varying pressures on bumblebee nests.

4.5.5 Interspecific parasitism (other bumblebee species)

The incidence of *B. terrestris* repeatedly entering a *B. lapidarius* nest is interesting. Although the footage provides no information on the activity of *B. terrestris* inside the *B. lapidarius* nest, it seem likely that they were stealing nectar as this has been reported in the literature (Free and Butler, 1959; Andrews, 1969). The *B. lapidarius* host colony had already produced gynes, so this social parasitism may have had little effect. It seems likely that if nests were invaded at an earlier stage, (i.e. when food was limiting or prior to gyne production) the effect could be detrimental to the host colony, either through reducing food stores or spreading disease.

It appeared that a failed usurpation attempt was recorded at one *B. terrestris* nest and possibly a partly a successful usurpation at another. Usurpation by true bumblebee queens is thought to occur early in the season, (Sladen, 1912; Alford, 1975; Donovan and Weir, 1978;

Paxton et al., 2001) whereas the potentially successful usurpation occurred later in the season. Usurpers are typically conspecifics, but the quality of the footage does not allow *B. terrestris* and *B. lucorum* females to be distinguished here, so it may be possible that these may have been non-specific usurpation. These were the only detected incidences of nest usurpation which is surprising considering the frequency of detection found in previous studies. For example, of 48 artificially reared *B. terrestris* nests which were placed in the field in spring time, 18 colonies were invaded by a total of 30 wild *B. terrestris* queens (Carvell et al., 2008).

4.5.6 Further work

This study highlights the broad range of animals associated with bumblebee nests, but leaves unanswered a number of important questions with regard to what impact many of these animals have on the nests. Filming within nests would be invaluable in this respect. Our study also focussed on the later stages of nest development, due to the practicalities of finding nests. Studies of nest founding and the earlier stages of nest development would be enlightening and pose an ongoing challenge to future researchers.

4.6 Acknowledgements

I would like to thank my field assistants and volunteers for their dedicated assistance. P. Whitehorn for instruction on internal parasite analysis, J. Struthers at Stirling University and other land owners for allowing me to carry out the experiments. Thanks also to N. Butcher at R.S.P.B. for manufacture of the camera recorders.

Chapter 5

Causes of colony mortality in bumblebees

5.1 Abstract

Bumblebee nests are thought to fall victim to a range of mammalian predators, yet rates of predation, nest survival and fecundity for wild nests are largely unknown. Here we describe data on the survival of 908 bumblebee nests. Cessation of traffic, production of gynes, males, or presence of any other species or alterations to the nest were noted. We also survey the literature on the diet of putative mammalian bumblebee predators, to aid in interpretation of nest predation events.

Overall, 75% of nests produced gynes. There was evidence for the failure or decline or of 100 nests (excluding those attacked by the wax moth, *Aphomia sociella,* which are considered separately). The main reported causes were excavated by large animal (n = 50) and human disturbance (n = 26). Nests above ground were more likely to be infested with *Aphomia sociella* than those below the surface (68.7% and 30.6% of nests infested above and below ground respectively).

A review of dietary analysis literature suggests that badgers are the most widespread predator of bumblebee nests in Britain, and was probably responsible for the majority of the 50 records of nest predation by large mammals. Hedgehogs and pine martens also consume bumblebee nests. No evidence for the consumption of bumblebees in Britain by foxes, stoats, weasels or moles was found. The status of wood mice, voles and shrews as predators of bumblebee nests in Britain remains largely uncertain. Bird nests boxes were frequently inhabited by bumblebees and at times this gave rise to interspecific competition for nest sites. The majority of these interactions resulted in bumblebees ousting the birds (often blue tits).

5.2 Introduction

In many social hymenopterans such as bumblebees, the majority of nest mates will not reproduce, meaning that a nest represents a single breeding female (Chapman and Bourke, 2001). It is therefore useful for population models to have reasonable estimates of nest density and fecundity for example, in order to interpret effects of altered land use, conservation schemes or climate change (Suzuki et al., 2009; Williams and Osborne, 2009; Goulson, 2010). Bumblebee nests are difficult to locate in sufficient numbers for well replicated study, and remain an area which we know comparatively little about (Osborne et al, 2008; Goulson et al., 2011). In a study of 80 *Bombus pascuorum* nests at a site in southern England, Cumber (1953) reported that 23 produced queens, (i.e. 28.8%) and this is the only direct estimate of fecundity in natural bumblebee nests. It is assumed that most nests fail to produce reproductives due to predators and parasites (Edwards and Williams, 2004). Nest survival has been estimated by calculating numbers of nests at the start and end of the summer using microsatellites to identify sister clusters (e.g. Goulson et al., 2010). However, such genetic estimates could be flawed as floral resources may change over time and so areas with plentiful spring forage (and many foraging bumblebees) may appear to have lost many of the colonies if it presents fewer floral resources later in the year.

A more common approach to studying the nesting ecology of bumblebees has entailed monitoring and manipulation of artificially reared nests which have been either maintained in the laboratory or placed in the field and allowed to forage. Rates of nest survival and fecundity, effects of internal parasites, *Psithyrus* invasions and usurpation attempts have been studied in this way (for example, Müller and Schmid-Hempel, 1992; Frehn and Schwammberger, 2001; Goulson et al., 2002b; Carvell et al., 2008; Otti and Schmid-Hempel, 2008). These studies have provided valuable information, but such colonies are unlikely to be entirely representative of wild nests, for example, reared nests are typically fed *ad. lib*. in a climatically controlled environment, whereas queens of wild nests must provision, incubate and care for incipient nests themselves. In addition, invasion by wax moths, psithyrus or foreign queens or workers may be more likely in reared colonies as such colonies are generally housed in weather proof boxes, often with very apparent entrances, with no ‘tunnel’. This is in contrast to wild nests which are typically camouflaged amongst vegetation and positioned within animal burrows sometimes accessed by several meters of entrance tunnels (Alford, 1975; Goulson, 2010). It seems plausible that such reared nests may be more easily detected and attacked by invaders than their wild counterparts.

The ecology of interactions between bumblebees and vertebrate species is an area that has been largely ignored. In addition, much of our understanding of the ecology of bumblebee nests (in terms of species reproductive rates, wax moth infestation rates, etc) is based upon observations carried out decades ago, (for example, Sladen, 1912; Cumber, 1953) and since then Britain has undergone extensive land use change, (Robinson and Sutherland, 2002), acquired a new species of bumblebee, *Bombus hypnorum* (Goulson and Williams, 2001), lost *Bombus subterraneus* and experienced notable range reductions in the majority of other species (Alford, 1980; Williams, 1982; Goulson, 2010).

5.2.1 Predators of bumblebee nests

Small mammals are thought to attack bumblebee nests, consuming the brood and pollen stores, particularly before the first brood of workers have emerged (Sladen, 1912; Free and Butler, 1959; Pouvreau, 1973; Alford, 1975). In New Zealand, mice were suspected of destroying 11 nests (in a study of 84 nests in artificial domiciles), and two of these were attacked after production of gynes (Donovan and Wier, 1978). Other quantitative measures of rates of mouse predation do not exist. Darwin (1906) quoted Col. Newman's claim that up to two thirds of nests might be destroyed by field mice, but no evidence or data is provided. Sladen (1912) attributed mice or shrews to the demise of several nests and developed secure mouse proof domiciles to avoid future depredations. Evidence for mouse or shrew predation consisted of: (1) nest remains destroyed and torn apart in the manner expected by a small mammal; (2) droppings of a small mammal present; (3) mouse nest found in the bumblebee nest remains; (4) Shrews captured in traps set in nests depredated the previous night (Sladen, 1912). Cumber (1953) located and observed 80 *B. pascuorum* nests throughout a summer at single site in southern England. He attributed the failure of 17 to rodents, badgers, etc., and listed a further 25 nests as having 'died out prematurely'. Unfortunately, methods for the collection of data and further breakdown of the rodent, badger predated nests are not provided. It is unclear how rodent predation was deduced as the cause of death, or if some of the 25 'prematurely failed' colonies may also have been the result of small mammal attacks. Benton (2006) quoted an account from C. Muller who witnessed a dormouse predate a bumblebee nest; 'it bit through all the thoraxes of bees and ate all brood' (Benton, 2006; pp. 126).

The destruction of nests caused by larger predators such as badgers is more obvious and this species is a well known predator of bumblebee nests (Pease 1898; Sladen 1912; Pouvreau, 1973; Alford, 1975; Benton, 2006). Badgers seek out bumblebee nests, excavate them and consume the entire comb seeming to ignore the bees' defensive efforts (Pease 1898). Badgers have also been blamed for depredating commercially reared bumblebee colonies during experiments investigating colony growth (Goulson et al., 2002b). Other mammals such as foxes, stoats, moles and hedgehogs are thought to predate bumblebee nests (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Benton, 2006, Goulson 2010). In some cases evidence for species predating bumblebee nests is limited, for example, Sladen (1912) saw a weasel ‘five yards’ from one of his nests which had been destroyed when he checked it the following day. He attributed this destruction ‘in all probability’ to ‘this animal or a shrew’ and weasels have been regarded as predators of bumblebee nests ever since this incident (Alford, 1975; Benton, 2006, Goulson, 2010).

In Britain, avian predators of bumblebees are limited to red-backed shrike (*Lanius collurio*) which is a rare species patchily distributed in Scotland (Owen, 1948; Witherby et al., 1958; Pedersen, 2012), spotted flycatcher (*Muscicapa striata*) which predate small bumblebees occasionally (Davies, 1977) and great tits (*Parus major*) which may take bumblebee queens or workers if they are impaired in any way, for example, queens parasitized by *Sphaerularia bombi* (Bols; quoted in Benton, 2006), or bees intoxicated from feeding on *Rhododendron* (Free and Butler, 1959) or *Tilia* spp. (Sladen, 1912; Benton, 2006).

5.2.2 *Psithyrus*

Nests may be invaded and parasitized by *Psithyrus* queens (Sladen, 1912; Free and Butler, 1959; Pouvreau, 1973). *Psithyrus* bumblebees do not have a worker cast and rely on ‘cuckooing’ a bumblebee nest into rearing their new gynes and males (Alford, 1975). *Psithyrus* queens typically attack strong, early nests prior to the emergence of the second brood of workers (Muller and Schmid-Hempel, 1992). They may reside in the nest for several days before attempting to kill the host queen and enslave the workers through physical contact and pheromone secretions which mimic those produced by host queens (Van-Honk et al., 1981a; Vergara et al., 2003; Martin et al., 2010). The *Psithyrus* queen lays her eggs in the nest and the *Bombus* workers of the host nest will rear a new generation of *Psithyrus* gynes and males. In Britain there are six species of *Psithyrus*, and most are host specific to a single true bumblebee species (Alford, 1975; Benton, 2006).

True bumblebees may also enter nests and usurp the resident queen or steal nectar (Sladen, 1912; Free and Butler, 1959; Carvell et al., 2008). Usurpation is thought to occur primarily at the beginning of the season, before the colony has produced more than one or two broods of workers (Alford, 1975).

5.2.3 *Aphomia sociella*

The bumblebee wax moth, *Aphomia sociella* is said to cause the demise of many nests each year (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Goulson et al., 2002b), yet we have little data on the actual rates of infestations by wax moths or the damage they cause to colonies (in terms of preventing reproduction). Wax moths are thought to target subterranean nests less frequently than surface nests (Alford, 1975). Alford (1975) also reports that he had never found a nest of *Bombus lapidarius* infested with wax moths, suggesting that bumblebee species may suffer at varying rates.

5.2.4 Aims

This study aims to estimate the duration of survival, rates of gyne production and causes of nest mortality of a large sample of natural bumblebee nests in Britain. Bumblebee consumption by British mammals will be investigated through a review of the published dietary literature.

5.3 Method

Nests were located for use in experiments between 2007 to 2011 using a trained bumblebee nest detection dog and deliberate human searches. The majority of these nests were located in rural locations around Stirling, in central Scotland. These nests were visited a minimum of fortnightly and observed for 20-30 minutes to ascertain if the nest was still active, if gynes or males were present, or if it had succumbed to a predator. The majority of nests were observed bi-weekly and the entrances to a subset of 32 nests were filmed (see Chapter 4). It was sometimes possible to collect or excavate nests. In this case, they were stored at -18ºC and later inspected to reveal invasion by wax moths and presence of gyne cells.

Bumblebee nests were reported by members of the public through the Bumblebee Conservation Trust, honey bee keepers and pest control agencies between 2010 and 2012. Those reporting a nest were asked to fill in a brief online questionnaire about the location of the nest and a subset were willing to record further information. Participants were asked to observe nests weekly for fifteen minutes and record worker activity, production of gynes and males and report any interesting activity with a photograph where possible, for example, no activity, dug up, swarms of bees at entrance, other animals present, etc. Some people were unable to participate in the weekly observations but were willing to submit occasional reports, or report if they noticed something unusual. In a few cases, nests were filmed by members of the public, usually in bird boxes, fitted with purpose made camera recorders.

Volunteers emailed photographs of bees so that the species could be verified. Occasionally volunteers preferred to post dead samples or record videos, and others were identified by experts (often survey coordinators of the Bumblebee Conservation Trust). In some cases species were verified through description alone. It is likely that rarer species will have been mistaken for more common species in this study as it is rarely possible to identify all morphological details through photographs (e.g. *Bombus jonellus* may have been mistaken for *Bombus hortorum*, or *Bombus ruderarius* for *B. lapidarius*). *Bombus lucorum* will include members of the complex of *B. lucorum, Bombus cryptarum, Bombus magnus* and may include *B. soroeensis*. Every effort was made to distinguish *Bombus terrestris* from *B. lucorum*, where this was not possible they were classed as unidentified. There were too few records (<4) of rarer species (such as *Bombus distinguendus*, *B. soroeensis* and *B. jonellus*, to include in species-specific analysis and these were grouped with unidentified nests.

Whilst some volunteers remained confused or unable to differentiate between sexes of bees, the majority of volunteers readily reported 'very big bumblebees' or 'different coloured bumblebees' which in all photographed cases were new gynes, males or *Psithyrus*. Where spurious results were received (for example, reports of many new gynes or males but no workers during their fifteen minute survey,) these records were not included in analysis but were used to establish longevity of the nest.

Gyneless nests were so determined only if no gynes had been observed during regular observations, lack of gyne cells at nest dissection or if nests were known to fail very prematurely (i.e. April-May). An additional method of gyne production was available for *B.*

hypnorum, where a 'swarm' of males could be seen at entrances to nests producing new gynes.

The remains of 113 nests were inspected. This allowed the presence or absence of wax moth caterpillars and their silk to be determined and in some cases presence or absence of gyne pupae cells could inform gyne production (in some cases volunteers were unable to identify cells, but photographs revealed this information).

5.3.1 Analysis

Chi-squared tests were carried out in R Statistical Software Version 2.12.2 (R Development Core Team, 2011). Using data for verified species only, a Chi-squared test was used to assess variation between numbers of nests that did or did not produce gynes for different species.

To investigate whether the claim that incipient nests are more vulnerable to failure than larger nests (e.g. Sladen, 1912; Cumber, 1953), a second Chi-squared test examined the likelihood of a nest which was detected when only the queen was present, producing gynes compared to nests which had already reared workers when detected. This test used data from nests which reported gyne/non-gyne production regardless of location, species or species verification.

A Chi-squared test was used to assess variation between rates of infestation of nests by wax moths depending on their position, above, upon or below the surface of the ground. Chi-squared tests were also used to determine if nests of *B. hypnorum* were more likely to be infested with wax moths than other species and if *B. hypnorum* are more able to produce gynes from nests infested with wax moths than other species.

Other statistics were carried out in Minitab 15 Statistical Software (2006). Three analysis of variance tests were used to investigate variation between dates when nests of different species (1) were found; (2) produced gynes and (3) ended. Fisher least significant difference post hoc tests were used to investigate variation between species.

A two sampled t-test assessed the hypothesis that gynes were more commonly produced from nests that were noticed earlier in the summer. A second t-test assessed the likelihood of nests on the surface and above the ground being found earlier than subterranean nests. Both of these tests excluded data from *B. hypnorum* as they this species is known to preferentially nest above ground, early in the season and male swarming may make their nests disproportionately easy to detect.

5.3.2 Mammalian predators of bumblebee nests

Studies of mammalian diets were reviewed to ascertain the likelihood of species predating bumblebee nests. Where possible, studies from the British Isles were used, (as species diets are known to vary with geographical location) and in particular those that identified insect prey items to family level. The aim was to accumulate a minimum of 1000 samples from each species, from British studies, but this was not always possible. Details from studies conducted outside Britain were considered where British studies were lacking and to inform of potential insect/hymenopteran predation tendencies. Literature documenting winter feeding habits were excluded as bumblebee nests are not expected to be readily available during this time, and so an absence of bumblebee remains in mammalian faeces or guts is not considered informative.

Studies identified and reported food items to varying specifications. However, studies were considered useful for indicating that species will consume (i) insects (in some cases, information for other invertebrates (such as earthworms) is given; (ii) hymenopterans and (iii) *Bombus* spp. The literature has been interpreted in this way as it is conceivable that for example, a species frequently reported to consume insects and in particular hymenopterans may be more likely to be an occasional bumblebee predator than a species which does not consume insects or invertebrates.

Where either bumblebees were not found or only values for hymenopterans, insects or invertebrates are given, this is summarised. A summary of the literature used is provided in Appendix II; Table 5.4.

5.4 Results

In total data for 908 nests were collated (135 nests were located by researchers and members of the public provided additional information for 773, of 3,956 nests initially recorded as part of a wider survey). The species of 640 of these were verified (i.e. from photographs, etc.) allowing this subset of data to be used to inform on interspecies differences. Members of the public could identify some species very readily such as *B. lapidarius* and *B. hypnorum*, whereas others were commonly confused, notably; *B. lucorum*, *B. terrestris* and

B. hortorum which are all striped, yellow and black with white/pale tails. *Bombus hypnorum* nests were the most frequently found, 211 had been verified by experts, and they were often located in above ground location (Figure 5.1). The six other species were all detected above, upon and below ground level in varying numbers.

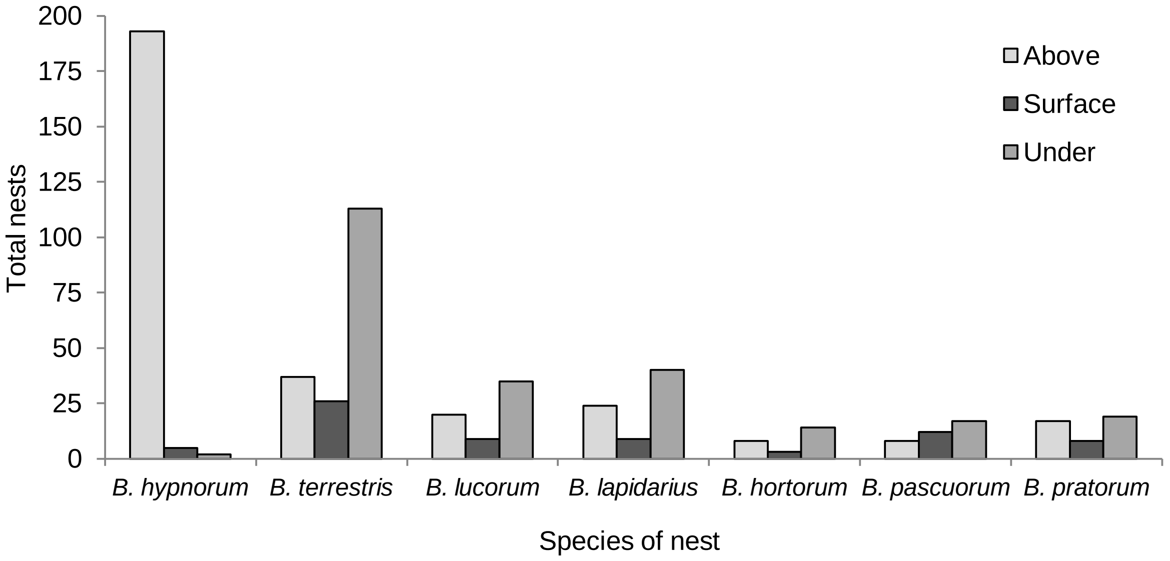

Figure 5.1. 619 nests of verified species for which locations were known; above the ground, on the surface or subterranean. (Note, location was not provided for 21 nests.)

Across records for all species, 76.19% of nests (which were under a suitable monitoring regime) produced new gynes (399 of 489). Discounting unverified/unknown species, 76.39% nests produced gynes (356 of 466 nests). This proportion varied between species, ($\chi^2_{D.F.6=}$74.51; $P < 0.001$) with a larger proportion of *B. hypnorum* nests produced gynes than any other species (Figure 5.2). Gyne production was lowest in both of the long tongued species, *B. hortorum* and *B. pascuorum*.

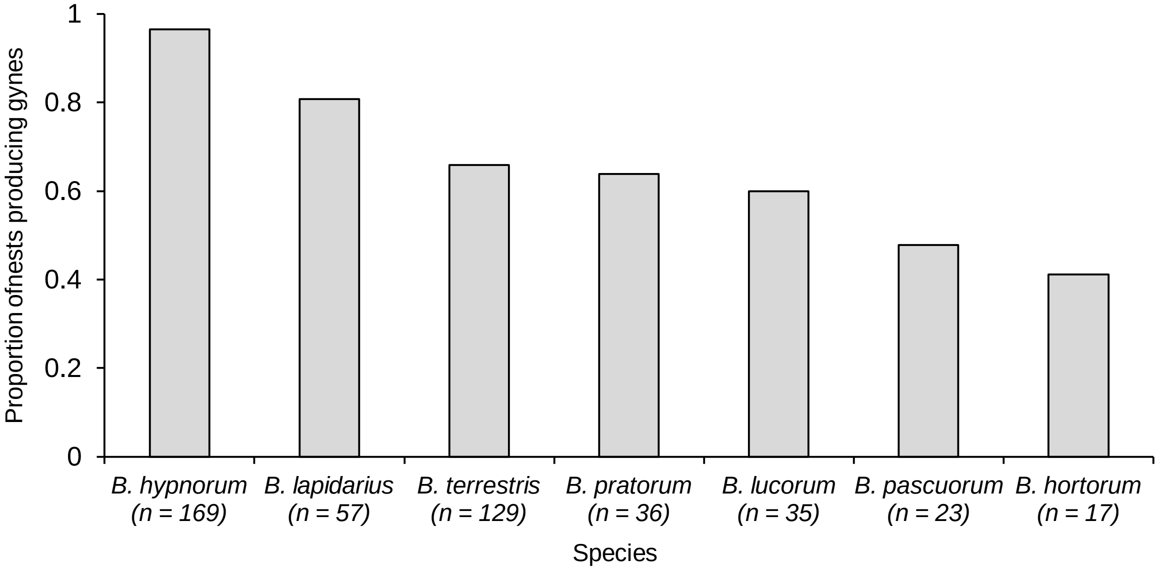

Figure 5.2. Proportions of nests producing gynes of different species (using data from known and verified nest reports only n =466).

Of 24 nests which were discovered when only the queen was present, only 54.2% produced gynes, compared to 76.1% of nests detected after emergence of workers (n = 465). However, there was no significant difference between these data ($\chi^2_{\mathrm{D.F.\ 1}} = 0.64$, $P = 0.422$). This result of non significance may be partially due to the skew in sample sizes (Zuur et al., 2007).

Dates of detection, gyne production and cessation of traffic varied between species: ($F_{\mathrm{D.F.}}=624$; $P < 0.001$; $F_{\mathrm{D.F.}}=275$; $P < 0.001$; $F_{\mathrm{D.F.}}=309$; $P < 0.001$, for detection, gyne production and cessation respectively).

Bombus hypnorum and *Bombus pratorum* nests were frequently found before other species (Figure 5.3). *Bombus pascuorum* nests were the latest to be detected. Similarly, gynes were detected in *B. hypnorum* and *B. pratorum* four to five weeks prior to those of other species and two months before those of *B. pascuorum*.

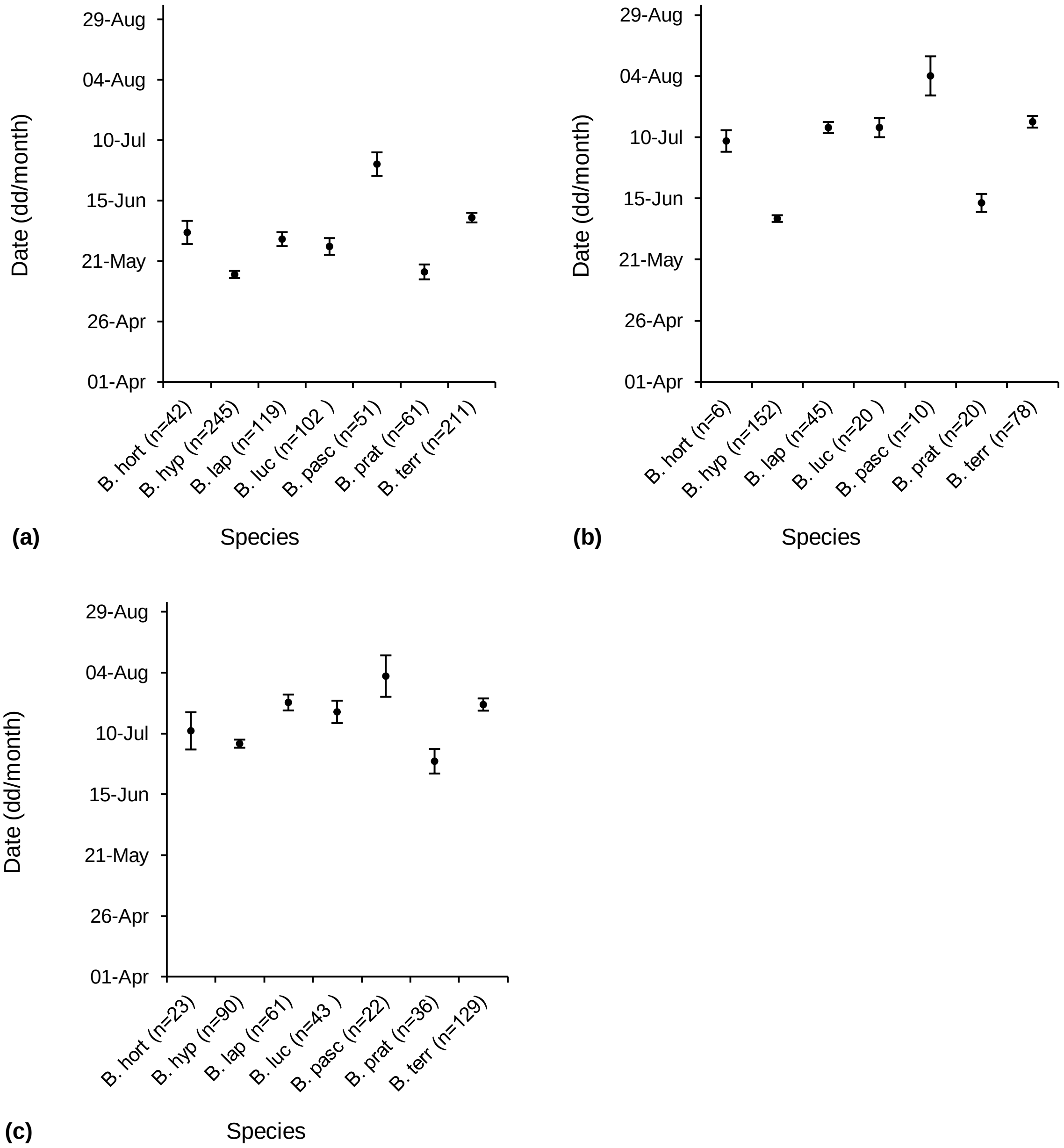

Figure 5.3. Mean dates nests were discovered (a); gynes first seen (b) and cessation of activity (c), with standard errors for the seven well represented species (abbreviated species names are listed in the following order: *B. hortorum*, *B. hypnorum*, *B. lapidarius*, *B. lucorum*, *B. pascuorum*, *B. pratorum* and *B. terrestris*). Sample sizes (given in brackets) vary between measurements as data for nests producing gynes or declining were not always reported.

Nests that produced gynes were detected a mean of 6.28 days earlier than non gyne producing nests (mean Day 165.93 ± 32.7 (mean ± SD) and Day 157.65 (std dev 31.41) for gyne producing (n = 200) and gyneless (n = 112) nests respectively but this difference was not significant ($T_{\text{D.F. } 222}$ =1.67, $P = 0.100$), (sample did not including *B. hypnorum* due to probable disproportionate recording, due to above ground locations and swarming behaviour of males, etc).

Nests situated above or on the surface of the ground, for example in bird boxes, outbuildings and compost heaps are likely to be noticed sooner than subterranean nests (typically in small mammal burrows or under buildings ($T_{\text{D.F. } 614}$ = 5.90, $P < 0.001$; Figures 5.4). Data analysis discounted *B. hypnorum* which are known to nest earlier, predominantly above ground and congregations of males is likely to have disclosed *B. hypnorum* nest location more readily than other species.

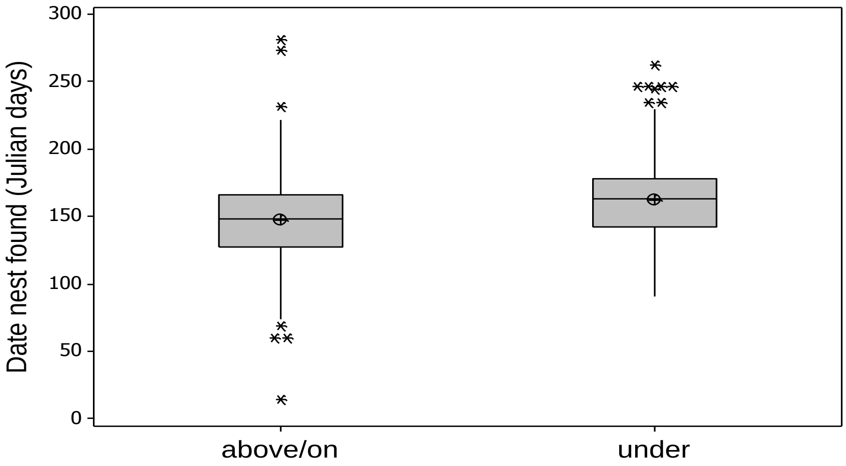

Figure 5.4. Date when nests were first noticed and the position of nest; above ground/on the surface and underground with means, interquartile ranges and outliers. (Data included both unknown

species and unverified accounts which had reported nest location, but excluded *B. hypnorum* due to their propensity to nest above ground (n=647)).

5.4. 1 Causes of failure

Some evidence of nest decline was noted for 100 nests (excluding wax moths which are considered separately; Table 5.1). Large animals were responsible for the greatest number of nest failures (50%). Human disturbance (for example, gardening and construction projects) resulted in 26% nest failures, but also resulted in other (disturbed but not destroyed) nests being reported.

Table 5.1. Possible causes and available evidence for mortality of 100 nests.

Nests (*n*)	**Cause**	**Evidence for cause. Number (*n*) given where relevant.**
50	Large animal	Nests excavated by animal larger than rabbit. Soil or vegetation removed, tooth and claw marks in soil, tree roots, etc. (9 nests were known prior to predation)
26	People	Nests disturbed through gardening or building (21) Long grass around entrance trampled by people or dogs, or mown, resulting in workers failing to relocate entrance. (5)
7	Flooded	Nest in flood water from heavy rain.
4	Ants	Many ants found in nest post death.
3	*Psithyrus*	*B. sylvestris* filmed entering nest. (1) *Psithyrus* photographed in nest or leaving. (2)
2	Mice	Filmed covering/blocking entrance. (2) Droppings/mice found within nest remains. (2)
3	Wasps	Nest contained wasps during decline. (2) Observed wasp attack and kill a worker at nest entrance. (1)
2	True bumblebee	*B. terrestris* queen filmed repeatedly entering *B. pratorum* nest which failed shortly afterwards. (1) *B. terrestris* workers filmed repeatedly entering *B. lapidarius* nest which ceased shortly afterwards. (1)
2	Birds	Great tit filmed ousting queen *B. hypnorum*. (1) Green woodpecker bill marks in destroyed *B. pascuorum* nest. (1)
1	Spider	Spider and queen filmed fighting repeatedly. Several days later, queen was dead.
100	Total	

5.4.1i Large mammals

Nests predated by large animals were found from May to September (Figure 5.5). Of approximately 780 nests which were observed more than once, nine were subsequently excavated by a large animal (i.e. 1.15%). The remainder (41) were only discovered after they had been excavated.

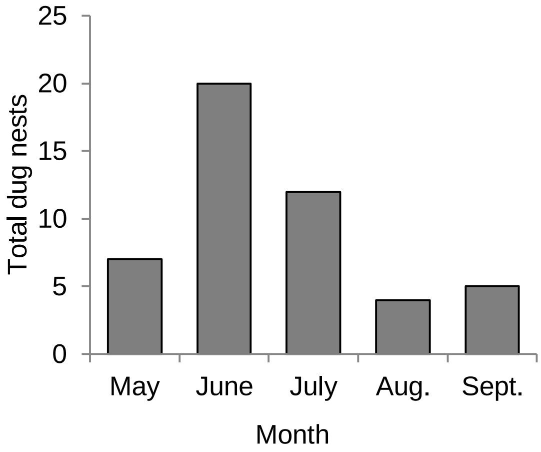

Figure 5.5. Month in which nests excavated by a large animal were discovered (n=48; no date was given for two reported dug nests).

5.4.1ii Birds

Bird boxes and nests provided nesting sites for at least 175 bumblebee colonies, although this figure is likely to be inflated due to the propensity for members of the public to check and watch bird nest boxes. There were 31 incidences where bumblebees interacted with nesting birds. In one case, a great tit was filmed used its bill to remove a queen *B. hortorum* which had entered the box three days previously (Table 5.2. Nest 27). In at least one case, birds were able to rear a brood in a nest which afterwards was used by bumblebees within the same season (Table 5.2; Nest 2: *B. hypnorum* arrived after fledglings of blue tits had left). Nests 16 and 24 were in vacated nests of the tit, *Parus* family, and it is not clear if these had successfully reared broods of chicks or if they were recently abandoned, suggesting possible ousting by bumblebees.

In the remaining 28 nests where some bird-bee interaction may have taken place, birds had at least inspected (n = 8), started to build (*n* = 17) or laid eggs (*n* = 1) in nests which they then abandoned and immediately or soon after were inhabited by bumblebees. It is impossible to know the proportion of bird nests which were usurped by bumblebees versus those abandoned for other reasons shortly before bumblebees took up residence (Table 5.2). Construction of bird nests had taken place in at least 18 cases and it is tentatively suggested that bumblebees ousted birds in these instances. Bird species succumbing to bumblebees using this estimate included 14 blue tits (*Parus caeruleus*); 2 house sparrows (*Passer domesticus*) and a single great tit (*Parus major*) and coal tit (*Parus ater*).

There was a single record of a possible green woodpecker (*Picus viridis*) predation of a nest of *B. pascuorum* in a meadow-garden in Norfolk. The long grass was mown at the beginning of October, partially revealing a *B. pascuorum* nest, which was re-covered with grass clippings in an attempt to camouflage it. By the following evening the nest had been destroyed and the reporter (V. Matthews of the British Trust for Ornithology) was confident that the nest was predated by green woodpecker, diagnosed by characteristic bill impressions left in the nest remains and soil. Great tits were filmed predating bumblebees at ten nest entrances in Scotland.

Table 5.2. Interactions between nesting birds and bumblebees throughout the UK; Bird species as follows: blue tit; house sparrows; great tit and coal tit. *Species identified by recorder, but not verified.

Nest Ref	**Year**	**Bumblebee species**	**Bird species**	**Summaries of quotes and evidence**	**Evidence for competition**
1	2010	*B. hypnorum*	*P. caeruleus*	*B. hypnorum* ousted *P. caeruleus* after a clutch of eggs were laid.	Yes
2	2010	*B. hypnorum*	*P. caeruleus*	Blue tits raised a clutch of eggs and fledged them before bees moved in.	No
3	2011	*B. hypnorum*	*P. caeruleus*	“Started nesting, then gave up, then got bees.”	Yes
4	2010	*B. hypnorum*	*P. caeruleus*	“Blue tits once thought about it but didn't stay!”	No
5	2010	*B. lapidarius*	*P. caeruleus*	“The nest was built by blue tits but the bees took over whilst the blue tits were starting to nest.”	Yes
6	2010	*B. lapidarius**	*P. caeruleus*	“visited by blue tits but they didn’t nest before bees took over.”	No
7	2010	Unknown*	*P. caeruleus*	“Ousted blue its who wanted to establish a nest here." (Queen bee only)	Yes
8	2012	*B. terrestris**	*P. caeruleus*	“Blue Tits investigated and then left.”	No
9	2012	*B. pratorum**	*P. caeruleus*	“...blue tits started to nest, but bees took over”	Yes
10	2010	*B. hypnorum**	*P. caeruleus*	Blue tits disappeared from nest.	No
11	2011	*B. hypnorum**	*P. caeruleus*	“Nesting material was put in by birds this year before bees took occupancy.”	Yes
12	2011	*B. terrestris**	*P. caeruleus*	The blue tits had started to build a nest before the bees came.	Yes
13	2011	*B. pratorum**	*P. caeruleus*	Tits started to build a nest but did not use.	Yes
14	2011	Unknown*	*P. caeruleus*	"Occupied by blue tits before the bees came."	Yes
15	2011	*B. lapidarius**	*P. caeruleus*	Blue tits made a nest between 28.2.11 and 6.4.11, birds vacated due to queen bee in box (filmed).	Yes
16	2010	*B. hypnorum**	*P. caeruleus*	“Vacated by tits earlier this year.”	No
17	2011	*B. hypnorum*	*P. caeruleus*	"Nest started by tits before adopted by bees."	Yes
18	2010	*B. lapidarius**	*P. caeruleus*	"Visited by blue tits but they didn’t nest before bees took over."	No
19	2010	*B. pratorum**	*P. caeruleus*	The blue tits had just started to build their nest in the box in early May. The tits abandoned the box when the bees came.	Yes

Nest Ref	Year	Bumblebee species	Bird species	Summaries of quotes and evidence	Evidence for competition
20	2011	*B. hypnorum**	*P. Major*	Sparrow terrace type nest box. Great tits nesting in end one but driven out when bees arrived in middle one.	Yes
21	2012	Unknown*	*P. caeruleus*	“The box was originally used by a pair of blue tits who have moved elsewhere.”	Yes
22	2010	*B. lapidarius**	*P. caeruleus*	Blue tits started taking nesting materials in, but didn’t finish.	Yes
23	2011	Unknown*	*P. caeruleus*	“They showed the usual interest in the box as a nest site but nested elsewhere."	No
24	2011	*B. hypnorum**	*P. caeruleus*	Recently vacated blue tit nest.	No
25	2010	*B. hortorum**	*P. ater*	“Coal tits had just finished building a nest and the bumblebee has now taken over.”	Yes
26	2011	*B. hypnorum**	*P. major*	"Great tits investigated earlier this year."	No
27	2011	*B. hortorum*	*P. major*	*P. major* and *B. hortorum* queen disturbed one another for three days before *P. Major* removed *B. hortorum* in bill.	Yes[1]
28	2010	*B. jonellus**	*Parus* spp.	Bees using old tit nest in October. Tits nested here earlier this year)	No
29	2012	*B. hypnorum**	*Parus* spp.	"Saw two tits 'checking it out' in early spring but they disappeared."	No
30	2010	Unknown*	*Passer* spp.	"..There were sparrows nesting in there a few weeks ago."	No
31	2011	*B. hypnorum**	*Passer* spp.	House sparrows ... began nesting this spring, but the bees had begun nesting at the same time.”	Yes

5.4.1iii True bumblebees

There were six events of true bumblebees entering other nests, although in most cases there is no reason to associate the visit with intruder with the demise of the nest.

One observer of a video recorded nest (species unknown) saw a second queen (also unidentified) enter the nest. No further information was given. In a separate instance, a *B. pratorum* nest also in a bird box and fitted with a camera was visited by a *B. lucorum* for 9 min, and again several days later by another *B. pratorum* queen. There is some evidence for negative impact of other species visits; a *B. pratorum* nest that had hatched the first brood was repeatedly visited by one or more *B. terrestris* queen(s) over several days before it failed. In another case, a gyne producing *B. lapidarius* nest was visited frequently by *B. terrestris* workers before all traffic ceased (see Chapter 4).

Two *B. terrestris* nests were visited by a *B. terrestris*/*B. lucorum* queen in July 2010 (see Chapter 4). A worn queen left one nest shortly afterwards, whether this was the foundress or the intruder is not known. Subsequent genetic analysis of nest mates from the other colony showed a batch of unrelated bees which may have resulted from the filmed queen (see Chapter 6).

5.4.1iv Psithyrus

A *Bombus sylvestris* was filmed at a *B. pratorum* nest in Scotland and photographs of another *B. pratorum* nest in Yorkshire appeared to contain males and females of both *B. pratorum* and *B. sylvestris* (Figure 5.6a). Another incident showed a *B. vestalis* at the entrance to a *B. terrestris* nest in a bird box near Cambridge (Figure 5.6b).

Figure 5.6a *Bombus sylvestris* in *B. pratorum* nest (photograph by S. Dyer). (b) *Bombus vestalis* at entrance to a *B. terrestris* nest, 27th May 2010 (photograph by R. Pridmore).

5.4.2 **Wax moth (*Aphomia sociella*)**

The wax moth infestation status of 133 nests was known. However, due to the nature of incomplete data sets other ecological information is only known for subsets of these data. For example, three were of unknown species and gyne production/nonproduction was known for only 90 of the nests. Therefore, sample size varies depending upon the aim of the statistical test or figure and are provided throughout.

Table 5.3. Summary of data for nests infected with wax moths by bumblebee species and gyne production.

					Nests without wax moths			**Nests with wax moths**		
Species	**Wax moth status known**	**Present**	**Absent**	**Gyne status known**	**No gynes**	**Gynes**	**Total**	**No gynes**	**Gynes**	**Total**
B. hortorum	4	3	1	1	1	0	1	0	0	0
B. hypnorum	48	7	41	36	0	4	4	4	28	32
B. lapidarius	16	5	11	6	1	1	2	2	2	4
B. lucorum	8	5	3	6	1	3	4	1	1	2
B. pascuorum	17	15	2	11	4	5	9	2	0	2
B. pratorum	10	7	3	7	1	4	5	0	2	2
B. terrestris	27	16	11	23	5	9	14	2	7	9
Unknown	3	2	1	2	1	0	1	1	0	1
Totals	**133**	**60**	**73**	**92**	**14**	**26**	**40**	**12**	**40**	**52**

Nests of *B. hypnorum* were disproportionately inspected for their wax moth status (48, *B. hypnorum* were inspected compared with 85 nests of other species; Table 5.3). A greater proportion of nests above ground had wax moth infestations ($\chi^2_{\text{D.F. } 2} = 13.82$, $P = 0.001$; Figure 5.7). *Bombus hypnorum* nests were more likely to contain *A. sociella* than other species pooled ($\chi^2_{\text{D.F. } 1} = 26.01$, $P < 0.001$; Figure 5.7b).

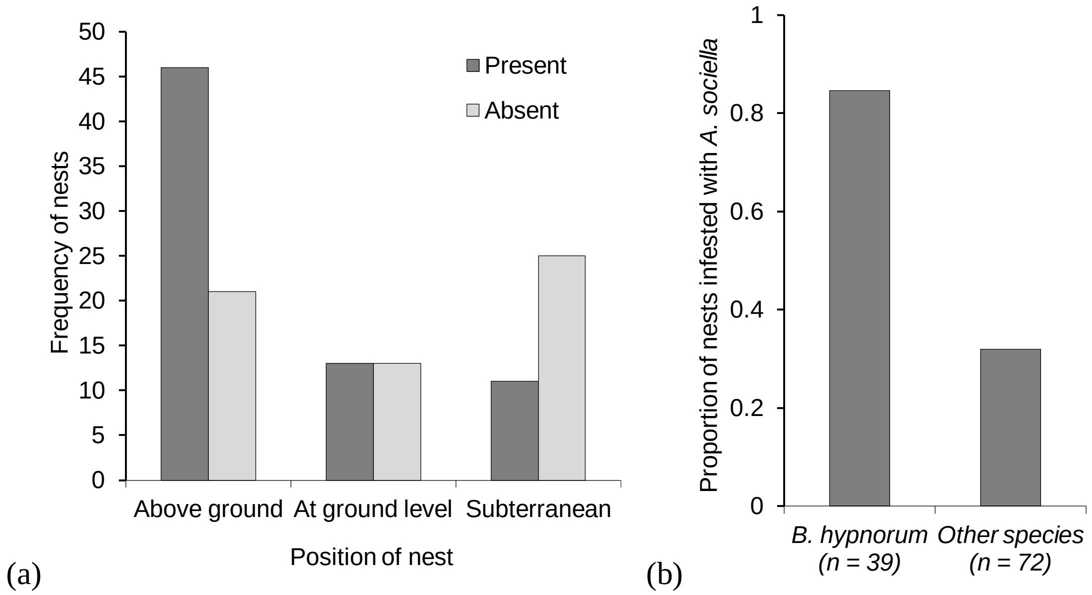

Figure 5.7. (a) Nests (all species pooled) above ground are more likely to be infested with wax moths than nests on the surface or below ground. (b) A larger proportion of *B. hypnorum* nests were infested with wax moths compared with all other species pooled together (verified species records only).

There were more reports of *B. hypnorum* nests which had wax moths and yet successfully reared gynes than were reported for other species. A comparison between the gyne/no gyne production of *B. hypnorum* nests compared to all other species pooled together revealed that this difference was not significant ($\chi^2_{\text{D.F.1}} = 2.86$, $P = 0.091$; Figure 5.8).

The earliest infestation of wax moth was recorded on 5th June and adult moths were recorded in nests on 15th June and 10th August. It should be noted that the majority of nests were inspected after the bumblebee nest had declined, i.e., there was a lag of several weeks between hatching of wax moth caterpillars and infestation being recorded.

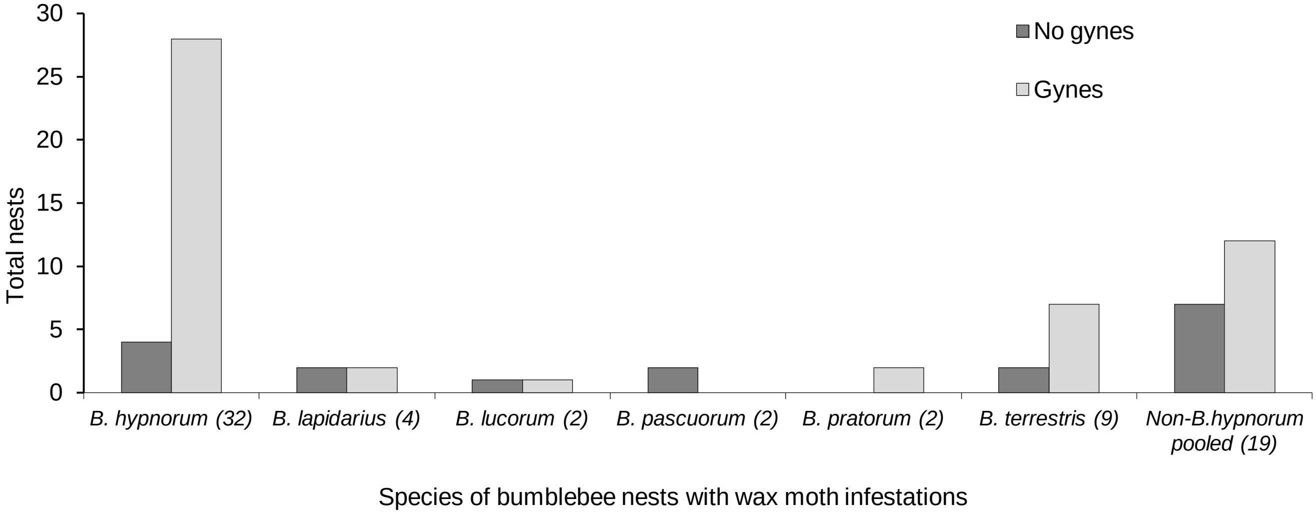

Figure 5.8. Bumblebee nests of different species infested with wax moths and gyne production using records from nests of both verified and unverified species (44 and 7 for species verified and unverified respectively). Sample sizes are given in brackets.

5.4.3 Predators of bumblebee nests

A summary of some possible bumblebee nest mammalian predators' diets is given from studies conducted within the British Isles (Table 5.4).

5.4.3i Small mammals

This study found no conclusive evidence for the predation of bumblebee nests by small mammals. During video analysis of the 33 nests, small mammals (wood mice, bank and field voles and shrews) frequented bumblebee nest entrances >1000 times. Mice were observed entering bumblebee nests during volunteer observations (n=3) and signs that small mammals had recently used the tunnels were evident (for example, chewed grass around entrance, droppings, disturbance of spider webs and dew, maintenance of hole >2cm diameter, etc.) at the majority of subterraneous nests examined. It appears that in the majority of cases, the small mammals coexist with the nests of bumblebees without harming them (as worker traffic continued regardless of small mammal visits. However, two filmed nests suffered a different experience and failed after wood mice blocked the entrance tunnels with leaves and debris (see Chapter 4).

Signs of wood mice at failed nests were present in two instances. Both were small in size and wood mice activity had been observed (video recorded at one) in the days before the nest failed. Nests contained mouse droppings and in one, a live wood mouse was found upon inspection. However, in neither instance was the remains of the brood ‘characteristically torn apart’ as described by Sladen (1912) and so it is perhaps more likely that nests failed due to other causes and mice simply took up residence. (In one the brood clump was abandoned and the compost heap containing it was damp (pers. obs.) in the second ten bees remained, but they were notably lethargic and all nectar and pollen stores had been depleted.)

Traces of chitin have been found amongst the stomach and gut contents of wood mice and bank voles throughout the year, indicating that insects are routinely eaten in small quantities (Watts, 1968; Flowerdew and Gardner 1978; Table 5.4). No hymenopteran remains have been reported, identifiable invertebrates which have been reported include: lepidopteran larvae, a beetle, a harvestman (*Phalangida*) and occasional earthworms (Watts, 1968). Bank voles stomach and gut contents (n =143) also revealed traces of chitin, but the only identifiable invertebrates were Lepidoptera larvae and an earth worm (Watts, 1968).

Table 5.4. Summary of the percentage composition of diets of mammalian predators from studies from within the British Isles, using frequency of occurrence data. (For further detail, see Appendix II; Table 5.5.). Insect occurence is provided as it may be inferred that if a species were frequently observed consuming insects, they may be more likely to occasionally eat bumblebees, whereas species which have not been found to eat insects are highly unlikely to be bumblebee predators. Sample sizes vary because studies reported prey items to different levels. Studies describing food items to Order were used to calculate rates of consumption of Hymenoptera, whereas only studies that identified prey to genus could be used to estimate frequency of occurrence of *Bombus*, studies that reported prey items in 'groups' (e.g. birds, plant matter, insects, etc) could only indicate frequency of insects in the diet [Number of studies used].

Species	**Total British Isles samples**	**Insects**	**Hymenoptera**	***Bombus***
Martes martes Pine marten	2836 [2]	21.5% (609 of 2826)	3.5% (98 of 2826)	1.9% (47 of 2449)
Vulpes vulpes Red fox	2617 [4]	16.5% (432 of 2617)	0% (0 of 1868)	0% (0 of 1868)
Meles meles Badger	3037 [3]	76.2% (2314 of 3037)	5.7% (161 of 2845)	6.1% (132 of 2159)
Mustela ermine Stoat	957 [2]	1.5% (14 of 957)	0% (0 of 168)	0% (0 of 168)
Mustela nivalis Weasel	1297 [3]	1.7% (22 of 1297)	0% (0 of 152)	0% (0 of 152)
Talpa europaea Mole	>135 [1]	Frequent	*Lasius flavus* cocoons (mentioned once)	0% (0 of 135)
Erinaceus europaeus European hedgehog	177 [1]	100% (177 of 177)	14.6% (20 of 177)	>2.7% (<14 of 177)
Apodemus sylvaticus Wood mouse	240 [2]	Frequent traces	0% (0 of 240)	0% (0 of 240)
Clethrionomys glareolus Bank vole	187 [2]	Frequent traces	0% (0 of 240)	0% (0 of 240)

Similarly, insect remains, but no hymenopterans were found in samples of crowned shrews (*Sorex coronatus*), pygmy shrews (*Sorex minitus*) or water shrews (*Neomys fodiens*) in studies examining the species in Northern Spain (Castién and Gosálbez, 1999; Appendix II).

5.4.3ii Vulpes vulpes (fox)

During video filming, four foxes were recorded passing directly over four bumblebee nest entrances and demonstrated no change in behaviour. Some dietry studies found insects infrequently (2-4% of diet) Leckie et al. (1998), but the majority recorded insects often (for example, in 25%; 9.6%; 21.0% in Lever (1959) Saunders et al. (1993) and Baker et al. (2006), respectively; Table 5.4). In particular, several studies note Coleopterans as a frequently occurring prey (Lever, 1959; Baker et al., 2006). However, of 1,868 samples where insect remains were identified as far as possible, no hymenopterans were found, suggesting that foxes do not regularly depredate bumblebee nests.

5.4.3ii Meles meles (badger)

A volunteer witnessed a badger attacking a known bumblebee nest in a domestic green house (pers. coms. 2010) and many of the 50 observations of destroyed nests were assumed by other volunteers to have been predated by badgers, either through prejudice and prior knowledge of badgers as bumblebee predators or due to observed signs, such as proximity to a sett, the scale of excavation, removal of house bricks or large stones, deep claw marks, chewed tree roots, etc.

Bumblebees have been found in stomachs and faeces of badgers (Table 5.4). For example, examination of the stomach contents of 686 badgers (Cleary et al., 2009) from March 2005 –

September 2006 in Ireland found that bees and wasps occurred in 3% of all samples and made up an estimated 1% of the total ingested bulk of badgers' diets. In June-August, bees and wasps remains occurred in 12% of samples, accounting for an estimated 6.5 % ingested bulk of the badgers' summer diets (n=77; Cleary et al., 2009).

A Scottish study of 2,159 badgers faeces, found bumblebee remains in 6.1% of samples, while wasp remains occurred in only 0.4% (Kruuk and Parish, 1981). It was estimated that 0.8% of the bulk Scottish badger's annual diet was bumblebees (Kruuk and Parish, 1981). If we consider that this food source is primarily available during the four months from mid May to mid September, then this would suggest that bumblebees constituted ~2.4% of the bulk of badgers' summer diet in Scotland.

Others did not detect any hymenopteran remains; for example Shepherdson et al. (1990) who examined 192 scats in Ireland and several studies from mainland Europe (Goszczynski et al., 2000; Sidorovich et al., 2011). Badgers diets vary widely across Europe (Marassi and Biancardi, 2002; Rosalino et al., 2005; Remonti et al., 2011).

5.4.3iv Martes martes (pine marten)

Pine martens predate bumblebee nests (Lynch et al., 2007; Caryl et al., 2011). Bumblebee remains appeared in 1.9% of over 2000 samples, (Table 5.4). Both pollen and wax were also recorded in the scats, confirming that nest predation had taken place in at least some cases (Caryl et al., 2011).

5.4.3v Mustela erminea and Mustela nivalis (stoats and weasels)

Insect remains occurred occasionally (<2%) in the stomachs of both stoats and weasels (Day, 1969; Tapper, 1979; McDonald et al., 2000; Table 5.4). However, the species were consistent with accidental ingestion, for example, being consumed with carrion or in the stomachs of prey animals (Day, 1969).

5.4.3vi Talpa europaea (mole)

The diet of moles predominantly consists of earth worms, (Mellanby, 1971; Funmilayo, 1979; Castien and Gosalbez, 1999) although larval and adult insects are increasingly consumed when abundant or in suboptimal habitat (for example coniferous woodlands in Germany; Mellanby, 1971). Examples of insects eaten include cock-chafer grubs, cerambycid larvae and cinnabar moth larvae. Although cocoons of the ant *Lasius flavus* have been mentioned, no bumblebee remains have been reported (Mellanby, 1971).

5.4.3vii Erinaceus europaeus (European hedgehog)

In total, 38 adult bumblebees were found in between 3 and 14 of 177 hedgehog stomachs. The author states that 2 stomachs each contained 13 adults and an additional 15 and 29 cocoons of immature bumblebees, which is strongly indicative of nest raiding having taken place (Yalden, 1976). The remains of a further 12 adult bumblebees were also found, but the author does not provide the number of hedgehog stomachs that they occurred in, (hence the vague figure; 3-14). Adult bumblebees accounted for up to 2.7% of hedgehog diets.

Both bumblebees and hedgehogs have been introduced to New Zealand and become established (Cumber, 1953; Alford, 1975; Edwards and Williams, 2004; Jones et al., 2005). A New Zealand study of 192 hedgehog guts revealed bumblebee remains in 42% of hedgehog stomachs (Jones et al., 2005). The author states that they often occurred in large numbers, suggesting nest raiding behaviour, for example, one gut contained 40 bumblebee legs, and it does seem unlikely that hedgehogs would catch such large numbers of foraging bumblebees.

During the filming of bumblebee nests, hedgehogs were recorded investigating nests, in a manner that one would expect if they were attempting to gain access to predate nests. They rooted in leaves and dug at the entrances, but were unable to gain access to the subterranean nests. Whilst actual predation of the bumblebee nests did not occur, it seems likely that given the occurence of bumblebee remains in the dietry studies, hedgehogs would readily predate bumblebee nests frequently if given the opportunity.

5.5 Discussion

Rates of gyne production from wild nests are largely unquantified. A large proportion of observed nests produced gynes (76.2%). This is a greater rate of gyne production than has been found in experiments using reared colonies, for example, 14 (56%) of 25 commercially reared *B. terrestris* colonies (which were fed ad. lib pollen and sugar water for two weeks before being allowed to forage outside produced gynes (Whitehorn et al., 2012); 5 (13.89%) of 36 and in another experiment, 7 (21.9%) of 32 laboratory reared nests which were placed outside after the initial stages of development, produced gynes (Müller and Schmid-Hempel, 1992; Imhoof and Schmid-Hempel, 1999). Lower rates of gyne production have been experienced by others, for example, none of 14 laboratory reared *B. terrestris* colonies placed in the field produced gynes (Otti and Schmid-Hempel, 2008). Data on the gyne production of wild nests in Britain is limited to a study by Cumber (1953) who found 23 (28.75%) of 80 *B. pascuorum* nests produced gynes.

The majority of nest failures are thought to occur in the early stages, although evidence for this is lacking (Darwin, 1906; Sladen, 1912; Alford, 1975). A solitary queen occasionally frequenting the nest is unlikely to be noticed; approximately 3.4% of the nests reported by volunteer observers were noticed at the 'queen-only' stage and some of these were only fully appreciated as nests after workers were seen (i.e. volunteers admitted they wouldn't have reported the queen only occupation as a nest without subsequent appearance of workers).

The figure quoted here is likely to be an overestimate due to the confidence required in declaring a nest not to have produced gynes. In this study, nests were either observed producing gynes or there was sufficient evidence from the nest remains (i.e. opened gyne sized pupae cells were found within the nest). Classifying a nest as gyneless is somewhat more difficult and subjective as many observers reported some information for a nest, but without regular observation of nests which would be expected to detect gyne production. A conservative approach was adopted, with the gyne status of many nests remaining unknown. Absence of gyne production was determined only where (1) observers had submitted regular, sensical reports of nests without recording new gynes, (2) dissected nest remains did not reveal any opened gyne-sized pupae cases, or (3) those where activity ceased while the nest was at a very early stage were classed as not having producing gynes. Therefore, the figures for gyne production which have been quoted here are highly unlikely to provide a realistic measure for all bumblebee nests in Britain.

In addition, it is likely that *B. hypnorum* nests may be easier to detect than other species for several reasons; firstly, as their common name suggests, the 'tree bumblebee' usually nests above ground, (often in bird boxes, attics and outbuildings,) and it appears that such nests are found more readily (and generally earlier) than subterranean nests. Secondly, male *B. hypnorum* congregate in a conspicuous 'swarm' outside nests producing gynes , which also seems to lead to their being discovered, and also makes it more likely that gyne production will be detected. Mating attempts were frequently observed at *B. hypnorum* nests whereas this was uncommon for any other species. Finally, *B. hypnorum* have been reported to select sites close to human habitation (Benton, 2006). Nests of *B. hypnorum* (and *B. pratorum*) were found earlier and produced gynes earlier than other species, a niche which until now has been occupied by *B. pratorum*. This assumes that the date of detection provides a useful reflection of the date nests were established. Benton (2006) states that *B. hypnorum* nests continue until mid August, but this was not typical for the nests reported here; the mean finishing date for 78 verified *B. hypnorum* nests was 5th July, and the latest continued until 1st August. Paxton et al. (2001) states that *B. hypnorum* nests grow through spring and early summer before switching to sexual offspring, which is in agreement with our findings. It is thought that bumblebee species specialising in early, small nests which reproduce quickly, *B. pratorum* is able to avoid interspecific competition with other species of bumblebees by utilising spring forage and also avoid high densities of protozoan parasites which have been found to peak later in the summer (Goulson, 2010; Whitehorn et al., 2011). Conopid parsitoids are most active in summer months rather than spring and this is thought to explain the observed reduction in parasitoid incidence in *B. pratorum* (Schmid-Hempel et al., 1991).

The impacts of *B. hypnorum*'s successful invasion of Britain could be far reaching, they may for example, reduce the effectiveness of the 'early' niche of *B. pratorum* by increasing the numbers of bees in spring and early summer, leading to competition for flowers or increased parasite transmission. The additional pollination service early in the year may be beneficial to earlier flowering wild plants and cultivated species such as orchards of fruit trees and oil seed rape.

In rural farmland, the majority of *B. terrestris*, *B. lapidarius* and *B. lucorum* nests are subterranean (Sladen, 1912; Alford, 1975; Osborne et al., 2008a; O'Connor et al., 2012). This preference was not clearly demonstrated here, with nests for all species reported above, at, or below ground level. This may suggest that bumblebees are more plastic in their habits than previously thought, or indicate biases in previous studies (i.e. efforts to search dense hedges or trees were not made), but is likely to be at least partially an artefact from subterranean nests being less readily detected than those that are at or above ground level and an increased availability of three dimensional above-ground structures in an urban setting (for example, houses, out-buildings, walls, compost heaps occur frequently in urban environments). Alternatively, with an increased bumblebee population in urban areas, competition for nesting sites may force some queens to select sub-optimal sites which they would otherwise not choose.

Studies of artificially reared nests have shown that those established early in the season are most likely to produce gynes, (although they also suffer higher rates of invasion from *Psithyrus*) whereas nests beginning later in the year are more likely to specialise in male only reproduction (Owen & Plowright, 1982; Muller and Schmid-Hempel, 1992; Carvell et al., 2008). Nests producing gynes were found a mean of six days prior to non-gyne producing nests, but this difference was not statistically significant.

Bumblebee species which establish colonies late in the season have suffered the greatest declines and range restrictions in Britain, for example, *B. distinguendus* and *B. sylvarum* (Benton, 2006; Williams and Osborne, 2009; Williams et al., 2009). Of the common six species, numbers of spring queens of *B. hortorum* and *B. pascuorum* peak later than the others (Lye et al., 2009; also see Chapter 3). *Bombus pascuorum* and *B. hortorum* and were the least frequently reported species here (6.3 % and 3.9 % of verified records respectively). This study may provide further evidence for a declining trend for later emerging or longer tongued species identified by Lye et al. (2012) given the frequency of nests of *B. pascuorum* in similar bumblebee nest surveys. Lye et al. (2012) found *B. pascuorum* and *B. hortorum* accounted for 11% and 9% of records respectively, Osborne et al. (2008a) reported 7% and 5% of records for *B. pascuorum* and *B. hortorum* respectively and the earlier study by Fussell and Corbet (1992b) 20% and 5% of nest records for *B. pascuorum* and *B. hortorum* respectively.

5.5.1 Bumblebees - Psithyrus and true bumblebees

The data collected by volunteers suggest that successful *Psithyrus* invasions of nests occur at a very low frequency compared to those reported by others (Carvell et al., 2008). However such data ought not to be relied upon as it may be likely that many volunteers would be unable to distinguish between true bumblebees and their similarly coloured *Psithyrus* species, and invasions are unlikely to occur at the moment of observation. In addition, this figure is reduced further as *B. vestalis* do not occur in Scotland, where the majority of filmed nests (in which invasions were more likely to be detected) were located and a social parasite of *B. hypnorum*, *Bombus norvegicus* (recorded in 5-10 *B. hypnorum* colonies; Løken, 1984) do not occur in Britain (Alford, 1975; Williams, 1986; Benton, 2006).

Theft of nectar from other bumblebee’s nests has been reported (Free and Butler, 1959 p64; Andrews, 1969). Queens may have been intending to usurp nests (Free and Butler, 1959; (Carvell et al., 2008) but in at least some cases, visiting queens did not remain in nests. Free and Butler (1959) note that different species may live peacefully alongside one another and Sladen (1912) recorded incidences of queens sometimes incubating initial brood jointly for a few days before fighting ensued. Competition for good nest sites has also been recorded before (Lye et al., 2011b; Alford, 1975) but our data do not provide further insight into this behaviour.

5.5.2 ***Aphomia sociella***

Wax moths have been reported to pupate in June and fly until August (Alford, 1975; Free and Butler, 1959). As the first infestations were well established in early to mid June, it seems likely that under certain circumstances, wax moths may pupate earlier than previously recorded and are able to infest nests at the end of May. Nests above ground have been thought to succumb to wax moths more often than their subterranean counterparts (Sladen, 1912; Alford, 1975). This trend appears to have been confirmed in this study.

A large proportion of *B. hypnorum* nests were infested with wax moths and the majority of these produced gynes prior to succumbing to the infestation. *Bombus pratorum* is commonly known as the ‘early bumblebee’ due to its reproductive timing which appears similar to *B. hypnorum*. Both species often nest above ground (Alford; 1975; Benton, 2006; Lye et al., 2012) and it may be that early reproduction is an adaption which allows avoidance of the ravages of wax moths whilst enabling use of raised nesting sites, out of the reach of mammalian predators, floodwater, etc.

Alford (1975) noted that he had never recorded wax moths in nests of *B. lapidarius*. Of sixteen nests of this species inspected in this study, wax moths were discovered in eight (although it should be noted that only ten were verified *B. lapidarius* with four of these infested with wax moths).

5.5.3 Large mammalian predators of bumblebee nests

From the literature, it appears that the only established larger mammalian predators of bumblebee nests in the UK are pine marten, badger and hedgehog. There was no evidence for the predation of bumblebee nests for several mammalian species, including, mole, stoat, weasel or fox. Confusion over the status of fox as bumblebee nest predators may have arisen from a statement in Southern and Watson (1941) in which they quote an earlier study as saying that bumblebees occasionally featured in the diet of foxes (Baranovskaya and Kolosov, 1935). However, the location of the quoted study is not provided, but the account is written in Russian and therefore the report is unlikely to be of British origin. Of 1,868 scat samples, where insect remains were identified as far as possible, no hymenopterans were found. It therefore seems unlikely that fox regularly depredate bumblebee nests, but given their propensity to consume insects of other types (mostly beetles) they perhaps shouldn’t be ruled out as bumblebee nest predators.

The scale of disturbance seen at attacked nests (i.e. large holes, claw marks, displacement of house bricks and large stones, tree roots bitten through, etc.,) was consistent with a large animal and one volunteer witnessed (and ended) an attempted badger predation. Considering the lack of evidence for fox consumption of bumblebees and the restricted range of pine martens in the UK, it seems likely that the majority of the 50 nests recorded as attacked by a large animal during surveys were predated by badgers.

The studies considered detected bumblebees from sections of adult exoskeletons. The number of fragments is used to estimate total number of bees and then total mass, and proportion of the bulk of diet, that each animal consumed. However, adult bumblebees consist of only a part of the total mass of a nest. Eggs, larvae, wax, pollen and nectar stores make up a considerably larger volume nests, yet constituent parts of nests were only reported in two of the examined studies (one of pine martens; Caryl et al., 2011) where ‘pollen and wax’ was given and in a study of hedgehogs (Yalden, 1976) where two stomachs were reported to contain a total of 44 bumblebee ‘cocoons’ yet this brood was not included in the calculations of total ingested bulk). It is therefore suggested that the figures in many of the reports of mammalian diet considerably underestimate the actual contribution of bumblebee nests to the diet.

Of the observed nests 1.2% of were excavated by a large animal and it appears that badgers were responsible for the majority of these. This figure is likely to be an underestimation as many of the nests surveyed were inaccessible to mammalian predators (for example, those in bird-boxes and inside or below buildings, within secured gardens, etc,) and it may be that nests away from human habitation are more vulnerable to attack.

We can make some simple calculations to estimate the impact of badgers on bumblebees at a national scale. Thriving *B. terrestris*, *B. lapidarius* and *B. lucorum* nests may rear 300-400 workers (Free and Butler 1959). The largest *B. terrestris* nests were found to weigh between approximately 500 - 700g at their maximum (Goulson et al., 2002b). Nests before maturity will weigh considerably less and those of other species of bumblebees remain smaller, rarely raising more than 100 workers (Sladen, 1912; Free and Butler 1959). Therefore, mean bumblebee nests could be estimated to weigh 250g.

Badgers typically consume approximately 900g of food per day (Kruuk, 1989 pp.57). If bumblebees nests were taken to account for a conservative estimate of 1.5% of the ingested bulk and excavated bumblebee nests were found from mid May until mid September (~4 months), an average badger may predate (1.5% of 0.9 kg) 13.5g of bumblebees per day.

Scaled up for the four month summer season (124 days) is ~1.674 kg of bumblebee nest, or using the crude estimate of 250g /nest above, approximately 6.7 nests per summer. In 2005, the UK badger population was estimated at 288,000 (Battersby, 2005). Assuming equal rates of bumblebee predation for the entire badger population, this would equate to 482,112 kg of bumblebee nest consumed annually, or approximately 1,928,448, bumblebee nests. There is no current estimate for the total number of bumblebee nests in Britain. Estimates of nest densities vary between habitat and method. Osborne et al. (2008a) estimated that there were approximately 7 nests per hectare in an area of typical British arable farmland (Hertfordshire, England). The UK landmass is approximately 24,361,000 Ha, if the above estimate was applied to all habitats, this would equate to 170, 527,000 bumblebee nests in Britain. This would mean that badger predation cause the failure of approximately 1.13% of the bumblebee nests in Britain. Whilst this very approximate figure relies upon several estimations and generalises unrealistically between all habitats, regions, etc, it is encouraging that it is within the same order of magnitude as the proportion of the 780 observed bumblebee nests which were excavated by a large animal (1.15%).

Hedgehogs consume up to approximately 60g of food per day (actual estimates vary 57-71g; Kruuk, 1989; Morris, 1967) and 2.7% of the diet could be estimated to be adult bumblebees (Yalden, 1976). From this it can be estimated that an average hedgehog would consume approximately 200g of bumblebee nest each summer (4 months). There are approximately 1,555,000 hedgehogs in Britain (Harris et al., 1995; Battersby, 2005) and assuming equal consumption, this would be approximately 311,000 kg or 1,244,000 nests (at 250g each). This equates to around 0.73 % of the estimated total 170,527,000 bumblebee nests in Britain.

Pine martens are uncommon in much of Britain, their total population is estimated to be only 3560 (Harris et al., 1995). It is therefore unlikely that most bumblebee nests will encounter attack from this predator.

5.5.4 Predation by small mammals

Mice, shrews and voles frequent runs also containing bumblebee nests (from filmed nests, observations from volunteers, etc,). All have been shown to consume insects when available (Watts, 1968; Flowerdew and Gardner 1978; Castien and Gosalbez, 1999). No bumblebee remains were reported in any of the stomach samples, but considering that they are supposed to only predate nests prior to the emergence of the first workers and in the absence of the queen, (Sladen, 1912) it is entirely unsurprising that identifiable remains of adult bumblebees were not detected. It seems likely that bumblebee brood, pollen or wax would not have been identified. This non-detection issue and the comparative lack of literature on small mammal diets do not facilitate any conclusion or estimations of rates of predation to be drawn. Lepidopteran larvae are consumed by wood mice, bank voles and shrews. It is therefore perhaps likely that bumblebee larvae would also represent a suitable food source for such small mammals.

Communal use of runs and burrows by small mammals and rabbits and bumblebees occurs frequently as detected by observations, signs of disturbance and video recording of nest entrances. In the majority of cases there was no apparent interaction with bumblebees with the exception of two cases where wood mice blocked tunnels that bees were using causing the demise of both nests. Further work is required to elucidate the relationships and potential rates of predation between small mammals and bumblebee nests, and efforts should be targeted at incipient nests, prior to the hatching of the first brood of workers.

5.5.5 Birds

Great tits predate bees at the entrances to subterranean nest sites. Attacks are not sustained enough to cause the destruction of the large nests that they target although they have been seen to occasionally attack gynes, with possible population consequences. Green wood peckers may predate surface nesting bees, (which are typically carder bumblebees) although this was reported only on a single occasion and not directly witnessed.

Birds also provide nest sites for bumblebees, in particular *B. hypnorum* (Free and butler, 1959; Alford, 1975; Benton, 2006; Lye, 2012). *Bombus pratorum* and *B. hortorum* have been shown to have variable nesting habits and may also use bird nests, and there are occasional records of other bumblebee species using bird nests. Competition over nest sites seems to exist between birds and bees in Britain and elsewhere. *Bombus niveatus vorticosus* oust common redstart (*Phoenicurus phoenicurus*) from bird boxes at all stages of nesting, even after brood have hatched, however, nests of great tits using nest boxes were never invaded (Rasmont et al., 2008) and *Bombus polaris* queens may utilise the nests of snow buntings (*Plectrophenax nivalis, nivalis*) in the Arctic (Heinrich, 1993), sometimes causing the birds to abandon their clutch of eggs (Kukal and Pattie, 1988). It seems encounters with nesting *P. caeruleus* typically result in bumblebees ousting birds, whereas in at least one instance, great tits was seen to remove a queen *B. hortorum*. It was also demonstrated that bumblebees and birds could use nests sequentially during the same breeding season.

5.6 Conclusions

Members of the public can enable data to be collected on a large scale which would not be possible otherwise. The observations of nests carried out by both volunteers and researchers have gathered useful data on aspects of bumblebee nest ecology. Wax moths were more likely to attack nests above than below ground level, as has long been suspected. It appears that some bird species have a complicated relationship with bumblebees, in particular, great tits which both provided nest sites for bumblebees and predate the insects.

Three British mammals are confirmed as predators of bumblebee nests, but further studies will be needed to establish the relationships between small mammals and bumblebees. Such studies should be targeted at early nests, prior to eclosion of the first brood of workers.

5.7 Acknowledgements

We would like to thank all of the people who volunteered their time to observe bumblebee nests, as well as research technicians and field assistants who assisted with finding bumblebee nests. Thanks also to land owners who allowed some studies to be carried out on their ground the Bumblebee Conservation Trust who forwarded nest records, in particular, Leanne and Natasha.

Chapter 6

Worker drift and egg-dumping by queens in wild *Bombus terrestris* colonies.

This Chapter has been published as:

O'Connor, S., Park, K.J. and Goulson, D. (2013) Worker drift and egg-dumping by queens in wild *Bombus terrestris* colonies. *Behavioral Ecology and Sociobiology* **67**, 621-627.

6.1 Abstract

Wild bumblebee colonies are hard to find and often inaccessible, so there have been few studies of the genetic structure of bumblebees within natural colonies, and hence it is not clear how frequently events such as worker reproduction, worker drift, and queen usurpation take place. This study aimed to quantify the occurrence of natal worker reproduction, worker drift and drifter reproduction, within 14 wild colonies of *Bombus terrestris* in Central Scotland. Four microsatellites unlinked were used to identify patterns of relatedness of the colonies' adults and broods. In colonies with queens (queenright colonies), worker reproduction accounted for just 0.83% of males, increasing to 12.11% in queenless colonies. Four colonies contained a total of six workers which were not daughters of the queen, and are assumed to be drifters, and four male offspring of drifters. Drifting is clearly not common and results in few drifter offspring overall, although drifters produced approximately seven times more offspring per capita than workers which remained in their natal colony. Unexpectedly, two colonies contained clusters of sister workers and juvenile offspring that were not sisters to the rest of the adults or brood found in the colonies, demonstrating probable egg dumping by queens. A third colony contained a queen which was not a sister or daughter to the other bees in the colony. Although usurping of bumblebee colonies by queens in early season is well documented, this appears to be the first record of egg dumping, and it remains unclear whether it is being carried out by old queens or newly-mated young queens.

6.2 Introduction

In colonies of social hymenopterans, the majority of individuals do not reproduce; instead they assist with rearing their siblings, some of which will go on to reproduce. It is also possible for workers to reproduce directly by laying unfertilised eggs which develop into males (Sladen, 1912; Van Honk et al., 1981b; Duchateau, 1989; Bourke and Ratnieks, 2001).

Worker bees entering colonies other than their own are known as drifters. Drifting is a well described phenomenon in *Apis mellifera* and can be extensive, with drifters sometimes comprising more than half of the work force (Free, 1958; Jay, 1966; Neumann et al., 2000; Chapman et al., 2010a). Drifting is thought to be largely the result of orientation errors and occasionally due to nectar robbing (Pfeiffer and Crailsheim, 1998; Neumann et al., 2000). The layout and external appearance of hives, as well as environmental factors such as the prevailing wind and sun direction can also affect the likelihood of drifting (Free, 1958; Jay, 1965; Jay, 1966; Jay, 1968; Jay, 1971). Worker drift and reproduction has been documented in other social hymenopterans, where drifters may act as social parasites, entering the colonies and laying eggs. For example, *Halictus ligatus* females drift between nest areas to lay eggs and do not carry pollen or assist with burrowing duties (Packer, 1986). Workers of other bees also drift into, and lay eggs in, non-natal colonies, including *Apis cerana* (Nanork et al., 2007), *Melipona scutellaris* (Alves et al., 2009) and *Apis florea* (Nanork et al., 2005; Chapman et al., 2010b).

Drifter bumblebee workers were found in non-natal colonies in an experiment using laboratory reared colonies of *B. terrestris* which were allowed to forage freely (Lopez-

Vaamonde et al., 2004). Colony inspections of colour marked workers confirmed that workers were entering non-natal colonies, with a mean of nine drifters observed per colony. Microsatellite analysis of callow males revealed that of the 1,501 males examined, 28 were produced by resident workers and 53 by drifters. Furthermore, nine of the drifter males were not attributable to any other colonies in the experiment and so must have resulted from drifting from wild bees.

Drifting has been found in glasshouses and agricultural systems where commercially reared bumblebee colonies are used to pollinate crops. In *B. terrestris*, depending on colony orientation, up to 50% of the workforce can be comprised of drifters (Lefebvre and Pierre, 2007). For *Bombus occidentalis* and *Bombus impatiens* an average of 28% of workers were discovered to be drifters (Birmingham and Winston, 2004). The ovaries of drifters are more likely to be developed than those of resident workers (Birmingham et al., 2004). Whilst some experiments that detected drifting workers were carried out at high densities, others were at lower densities, not dissimilar from those expected in the field. For example, Birmingham and Winston (2004) studied *B. impatiens* colonies that were stocked at a maximum of 7 colonies per Ha, which is comparable with estimates of wild colony densities (Knight et al., 2005, 2009; Osborne et al., 2008a).

Most observations of drifting in bumblebees have been in artificial systems where the bees were housed in domiciles with similar appearance and entrances. Wild colonies are typically in the burrows and colonies of small mammals (Fussell and Corbet, 1992b) and the entrances are often hidden amongst vegetation. The entrances of artificial colonies tend to be comparatively short compared to the several meters of tunnels typical of some species such as *B. terrestris* (Sladen, 1912; Carvell et al., 2008). It is therefore likely that drifting may not occur in the wild to the same extent as in artificial settings. Drifting has been confirmed at a low level in wild colonies of *Bombus deuteronymus* in Japan, and this remains the only evidence for drifting in natural colonies (Takahashi et al., 2010). Of 11 colonies examined, three contained four drifters in total, and 11 of their male offspring. Intraspecific parasitism has also been demonstrated within other hymenopterans. Queenless colonies of *M. scutellaris* may be usurped by foreign queens who take over the colony and lay eggs (Alves et al., 2009). Colony usurpation has been documented in bumblebees and is thought mainly to occur early in the season, before the second brood of workers have emerged (Sladen, 1912; Hobbs, 1965; Alford, 1975). The presence of one or more dead queens in the colony is often the only evidence for usurpation or attempted usurpation by queens (e.g., Carvell et al., 2008). Molecular techniques now allow the identification of foreign queens and reveal the extent to which such queens are successful at reproducing. Usurping has been detected using genetic techniques where it might otherwise have gone unnoticed. For example in one colony of *Bombus hypnorum* (Paxton et al., 2001), workers in this colony were not all of the same lineage, suggesting usurpation had taken place although drifting could not be ruled out in this case.

Here we examine patterns of relatedness among the adults and brood of 14 wild, mature colonies of *B. terrestris,* the largest sample of wild bumblebee colonies yet to be examined. We present evidence for drifting, drifter reproduction and egg dumping by queens.

6.3 Method

In July, August and September 2009 and 2010, fourteen colonies of *B. terrestris* were located by searching, aided by the use of a dog trained to detect bumblebee colonies (Waters et al., 2010 O'Connor et al., 2012). All were from the campus of the University of Stirling (Lat:Long 56°8.874852"N, 3° 54.928386"W) and nearby farmland. The colonies were excavated and the entire comb removed and frozen at -18°C. As far as possible, all adult bees were caught using nets. Colonies were later dissected, removing adult bees, eggs, pupae and larvae, including degraded specimens. A colony was considered queenless if no live queen was collected, or if the only queens present appeared to be new gynes (without signs of wear).

DNA was extracted from thoracic muscle for adults and pupae, whole eggs, or a similar sized portion of material from larvae, using HotShot protocol (75 µL of HotShot lysis solution, incubated at 95°C for 1 hour followed by addition of 75 µL neutraliser solution, Truett et al. 2000) and stored at -18°C until needed. Where colonies had more than 315 specimens, all adults were genotyped and a non-random sample of eggs and brood to a total of 315. The non-random sample avoided those that were obviously degraded, and later stage brood which appeared to be developing gynes as these were not likely to be natal worker or drifter worker offspring. DNA was amplified using Multiplex PCR Kits (Sigma) at four, labelled, unlinked microsatellite loci: B126, B10, BT09 and BT26 (Estoup et al., 1993; Estoup et al., 1995; Estoup et al., 1996; Funk et al., 2006; Wilfert et al., 2006). PCR was carried out according to the following protocol: 0.02 µL each of forward and reverse of the four primers (primer concentration, 0.2µM), 1 µL Q-Solution, 5 µL Multiplex Mix, 2 µL water and 1 µL DNA. Samples were firstly denatured at 95°C for 15 minutes then subjected to 35 cycles of annealing temperature of 48°C for 30 seconds and denaturing of 94°C for 90 seconds, then extension at 72°C for 90 seconds and a final extension step at 72°C for 10 min.

PCR product was sent to The DNA Sequencing Service, Dundee, UK where fragment analysis was carried out at 1:50 dilution on an ABI 3730 capillary DNA sequencer, and LIZ500 dye (Applied Biosystems) as the size standard. Fragment analysis output was examined using STRand software (Veterinary Genetics Laboratory, University of California at Davis, http://www.vgl.ucdavis.edu/informatics/strand.php).

A negative and positive control was included in each 96 well plate of samples prior to PCR to check for contamination of reagents and consistency of PCR and electrophoresis output. Where electrophoresis output was weak for one or all loci, the samples were amplified a second time and genotyped. Samples with one or more alleles foreign to the colony consensus group (i.e. possible drifters) were rechecked with fresh DNA extractions. The error rate was estimated by amplifying DNA and blindly scoring electrophoresis output from 94 samples including individuals from most colonies and all life stages (eggs, brood and adults). Identical results were obtained at the four loci in all cases. Therefore genotyping error rates were estimated to be acceptably low.

All European *B. terrestris* queens that have been examined to date have been monandrous (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000). One exception has been found; the spermatheca in one of nine *B. terrestris* in feral populations in Japan appeared to contain sperm from two males (Inoue et al., 2012). This means that for any locus, there are a maximum of three possible alleles that a bee can inherit: two maternally (assuming heterozygosity) and one paternally (assuming the male's allele differs from those of the queen). The parent alleles are easily deduced from examining colony mates and a drifter is taken to be any diploid individual carrying at least one allele that is not possessed by the queen or her mate. Data were inspected visually for individuals that did not conform to the parentage of colony mates. Expected heterozygosity for each of the four microsatellites was calculated using GENEPOP (Raymond and Rousset, 1995; Rousset, 2008) using data for genotypes of the inferred maternal bees of all nests.

A drifter can be misclassified by chance if she shares alleles with the rest of the bees in the colony at all four microsatellite loci. The probability of missing a drifter can be estimated by calculating the frequency of the paternal and maternal alleles in the population (the population estimate was calculated from the deduced parental alleles) to assess the likelihood of their occurring in a non-sister individual by chance. The probability of misclassification of drifters has been calculated by multiplying the frequency of the paternal allele with the mean frequency of the maternal alleles (estimated from the deduced colony parents). This estimation is carried out for each locus and the results multiplied to give the probability of a non-natal individual being misclassified by chance in a method similar to that used by Nanork et al. (2007) and Takahashi et al. (2010). The probability is:

$$\prod_i P_{i(1)}\,(P_{i(2)}+P_{i(3)})$$

Where $P_{i(1)}$ is the frequency of the inferred father's allele at the ith locus and $P_{i(2)}$ and $P_{i(3)}$ are the frequency of the inferred queen's first and second alleles respectively at the ith locus.

Males were identified either through direct morphological examination of adults and late stage pupae or because they were homozygous at all loci. For all but one colony (colony 10; Table 6.1) where only morphologically identifiable individuals were used, the inferred male parent had a different allele to those of the queen for at least one locus, so that diploid offspring could not be homozygous at all loci. We classified a male as a worker's son if he carried a paternal allele at one or more loci. It is possible that a worker-laid male only inherits maternal alleles, in which case he would not be detected as a worker's son. The probability of non-detection is 0.5^x where x is the number of informative loci (e.g. loci at which the male and queen carry different alleles).The total number of worker sons (detected and non-detected) was estimated by $n/(1-0.5^x)$, where n is the number of worker sons that were detected and $1-0.5^x$ is the probability of detection. Drifter worker sons were so classified if they possessed an allele not corresponding to those of the natal bees. A drifter worker was considered a possible mother of a non-natal male found in the same colony if the male possessed an allele corresponding to those of the drifter worker at each locus.

6.5 Results

In total 1,456 individuals from the 14 colonies were genotyped at the four loci (Table 6.2). Of these, 603 were identified as males. The majority of males were immature (eggs, larvae and pupae), 77 were adults and nine had been dead for some time previous to collection. The proportion of worker reproduction varied considerably between colonies (Table 6.1) and accounted for 78 of the 603 males detected (12.94%). Worker reproduction was greater in colonies that were queenless (n=73 males in 8 colonies), accounting for 12.11% of overall males detected, while we found just five worker-produced males (0.83%) amongst the six queenright colonies.

Table 6.2. Allelic richness of four microsatellites in *B. terrestris*, calculated using inferred genotypes of colony queens.

Locus	N	Alleles	HExpected	HObserved
B126	14	10	0.88	0.93
B10	14	12	0.92	0.93
BT09	14	6	0.74	0.50
BT26	14	14	0.93	0.86
Mean	14	10.5	0.87	0.80

Ten non-sister (drifter) workers were found in five colonies (Table 6.1). They differed from their host bees at a mean of 4.7 alleles across the four loci. Four of these in colony 9 appeared to be a group of sisters and notably, were also sisters to a female pupa within the same colony, apparently indicating that a foreign queen had entered the colony relatively recently and laid eggs. Similarly, a non-natal immature female was found in colony 10 as well as a male egg. The immature female and male may have been siblings but the male was not an offspring of the drifter workers found in this colony (see supplementary material available online). Interestingly, the genotype of one of the drifter workers found in colony 10 was consistent with bees from colony 11. It should be noted that the entrances to these two colonies were close (approximately 2.5 meters apart). A further colony (3) contained a foreign, worn queen.

A total of four male offspring of drifters were detected in three colonies (Table 6.1). Mean natal worker reproduction across 13 colonies (n=501 natal workers) was 0.164 males per natal worker whereas drifters (n=6) produced an average of 0.667 sons each. Colony 9 was removed from the calculation as the non-natal bees appeared not to be drifters. If we consider only the four colonies where drifting had taken place (following Takahashi et al. (2010)), then natal worker reproduction amounts to just 0.093 sons per bee.

Table 6.1. Summary of non-natal bees, brood and worker reproduction in 14 colonies of *B. terrestris*.

				Genotyped				Foreign individuals					Natal-worker reproduction	
Ref. no.	Date collected dd/mm/yy	Total adults, eggs and brood	Queen	Adults	Eggs	Brood items	Total	Probability of misclassifying a non-natal	Adults (n)	Description	Offspring (n)	Description	Sons (n)	Description
1	8.8.09	24	Present	13	0	0	13	$1.49x\ 10^{-5}$	0	-	0	-	0	-
2	10.8.09	352	Present	180	9	124	313	$1.04x\ 10^{-6}$	0	-	0	-	0	-
3	13.8.09	145	Absent	57	29	19	105	$2.10x\ 10^{-5}$	1	Queen	0	-	5	eggs
4	14.8.09	313	Absent	37	83	101	221	$1.96x\ 10^{-6}$	1	Worker	0	-	0	-
5	19.8.09	38	Absent	7	0	8	15	$4.23x\ 10^{-9}$	0	-	0	-	0	-
6	19.8.09	159	Absent	31	21	18	70	$6.30x\ 10^{-4}$	0	-	0	-	34	4 eggs; 1 larvae; 6 pupae; 1 adult
7	15.9.09	98	Present	14	37	35	86	$4.01x\ 10^{-6}$	0	-	0	-	0	-
8	16.7.10	15	Absent	12	0	0	12	$3.21x\ 10^{-7}$	0	-	0	-	0	-
9	29.7.10	44	Present	8	21	7	36	$5.61x\ 10^{-7}$	4[a]	Workers	2	1 pupa (female); 1 egg (male)	0	-
10	17.8.10	65	Absent	14	0	8	22	$3.92x\ 10^{-4}$	3[b]	Workers	2	1 egg (male); 1 pupa (female)	0	0
11	17.8.10	224	Absent	19	93	2	114	$2.35x\ 10^{-6}$	1[c]	Worker	2	2 pupae (male)	3	eggs
12	2.9.10	676	Present	130	72	93	295	$1.34x\ 10^{-4}$	0	-	0	-	1	adult
13	3.9.10	96	Absent	14	39	3	56	$1.25x\ 10^{-5}$	0	-	0	-	31	eggs
14	7.9.10	103	Present	43	6	49	98	$9.06x\ 10^{-6}$	1[d]	Worker	1	1 pupa ♂	4	eggs
Totals		2352	50%	579	410	467	1456	$8.59x\ 10^{-5}$	11	-	7	-	78	-

[a] Workers and female pupa appeared to be sisters. The egg was either a brother or son of these workers.

[b] One of the drifter workers genotype was consistent with workers from colony 11. The ‘foreign offspring’ may be siblings of each other, but were not related to the drifter adults.

[c] Pupae may be brothers, but the drifter worker was discounted as their mother.

[d] Foreign worker did not lay the pupa.

6.5 Discussion

Our data add to a growing body of literature which demonstrates that the reproductive strategies of bumblebees are more flexible than was once thought. In 14 wild bumblebee colonies, we find evidence for reproduction by workers, drifting of workers between colonies, reproduction of drifters within colonies, and possible egg-dumping by queens from foreign colonies.

Natal worker reproduction in bumblebees varies considerably between species. For example, in a sample of five *Bombus wilmattae* colonies, workers produced the majority of males (Huth-Schwarz et al., 2011). However, in 32 *B. terrestris* colonies, natal workers produced just 2.2% of male offspring (Lopez-Vaamonde et al., 2004). The presence or absence of the queen may have a profound effect on worker reproduction in many social hymenopterans (Beekman and Oldroyd, 2008) as has been shown in *A. cerana* (Nanork et al., 2007) as well as several *Bombus* species. Within eleven *B. deuteronymus* colonies, natal workers had produced males in six colonies (Takahashi et al., 2010). On average 27% of males in six queenless colonies were worker-males versus 16% of males in the five queenright colonies. This pattern was also found in *Bombus ignites* where workers produced 5% and 57% of males in queenright and queenless colonies, respectively (Takahashi et al., 2008), and in *Bombus melanopygus* colonies where workers produced 19% and 39% of males before and after queen death respectively. In ten *B. terrestris* colonies Alaux et al. (2004) found a greater number of worker derived males in colonies in which the queen had recently died (within 20 days, taken to be less than male development time). In our queenright colonies only 0.83% of males appeared to have been workers' sons, whereas this percentage was 12.11% in queenless colonies.

Lopez-Vaamonde et al. (2004) showed that drifting could occur between workers of laboratory reared colonies which were allowed to forage outside, and it is clear that drifting may occur frequently when many bee colonies are artificially contained in close proximity to one another (Birmingham et al., 2004; Birmingham and Winston, 2004; Lefebvre and Pierre, 2007). Lower rates of drifting have also been found, comparable to those described here, in wild colonies of *B. deuteronymus* (Takahashi et al., 2010). Four *B. deuteronymus* drifters were reported in three of the eleven colonies that were examined. Discounting colony 9 (as it appears a queen was responsible for the non-natal bees here) 4 of 13 colonies contained drifters and drifter offspring. Takahashi et al. (2010) suggest that *B. deuteronymus* may be vulnerable to social parasitism because it is a surface nesting species and therefore colonies may be easy to locate. However, *B. terrestris* nest in subterranean tunnels which may extend for several meters (up to 6m long, S.O. pers. obs.), but appears to suffer from drifting at a similar frequency. What determines whether workers become drifters remains unclear; it seems likely that some or perhaps all drifters are workers from failed colonies, but it would require exhaustive monitoring of a large number of colonies in a particular area to test whether this is so.

In studies using laboratory reared colonies, drifter reproduction was greater than resident worker reproduction. For example, in queenless *B. terrestris* colonies, drifter workers produced 28.1% of males whereas resident workers were responsible for 19.0% of males (Lopez-Vaamonde et al., 2004). The same study also showed that in queenright colonies sampled before the competition point (the date the first worker egg is laid (Duchateau and Velthuis, 1989)) drifter reproduction accounted for significantly more males than resident workers (17 and 1 respectively). In *B. deuteronymus* colonies Takahashi et al.

(2010) found drifter workers produced 20 times more offspring per individual than resident workers in colonies where drifting occurred. In line with these findings, we found drifter reproduction was approximately 7 times greater than that of natal worker bees. Not all of the eggs laid by natal or non-natal workers will develop into adults due to worker and queen policing of eggs (Duchateau, 1989; Beekman and Oldroyd, 2008). Extensive worker policing has been found in colonies of *A. cerana* (Oldroyd et al., 2001) and in *B. terrestris* colonies, the majority of worker laid eggs are destroyed within two hours of being laid (Zanette et al., 2012). In this study nine of the worker-produced males detected had developed beyond the egg (and therefore policing) stage.

Perhaps the most unexpected of our results is the finding in two colonies of female pupae which were not laid by the resident queen, but which appears to be sisters to adult workers within the colony. A foreign queen was found in a third colony. Usurpation of colonies by queens has been documented in bumblebees (Sladen, 1912; Alford, 1975; Paxton et al., 2001; Carvell et al., 2008). For example, Paxton et al. (2001) found several bees of similar lineage within a single colony of *B. hypnorum*, and Carvell et al. (2008) found that 30 *B. terrestris* queens had invaded 18 of 48 experimental colonies housed in boxes. However, colony usurpation is typically thought to occur primarily in spring, before the emergence of the second brood of workers, and carried out by late-emerging queens (Alford, 1975; Goulson, 2010). Given that our colonies were sampled in July, August and September, this appears to be more akin to egg-dumping, whereby queens enter a colony, lay a batch of eggs, and then leave. This behaviour has been recorded in invertebrates such as lace bugs (*Gargaphia solani*) (Tallamy, 2005), lady bird beetles (*Adalia* species) (Tallamy, 2005; Ferrer et al., 2011) and in the ant *Leptothorax tuberum* (Roberts et al., 1999), but was not previously known from bumblebees. Given the lateness of these events, it is unclear whether the queens engaging in egg-dumping are old queens from the previous year, or young queens produced in the current year. At least in the colonies we studied, their effort would seem to be ineffective as a reproductive strategy since they resulted only in worker offspring, but it seems likely that this may sometimes result in new queens being reared. It is also possible that non-natal male offspring may have been laid by queens rather than drifting worker bees.

Movement of bees between colonies has potentially important consequences for disease transmission. It has been assumed that the primary route of horizontal parasite transmission between bumblebee colonies is through infected bees visiting flowers which are later visited by uninfected individuals (Imhoof and Schmid-Hempel, 1999). Infections of the protozoa gut parasite *Crithidia bombi* have been shown to spread rapidly between individuals within colonies which is thought to be due to the close proximity of individuals and use of shared nectar pots and lack of destructive UV light (Rutrecht et al., 2007). Thus if drifters were carriers of a transmittable parasite, colony mates might be infected rapidly. *Nosema bombi* has been found to severely reduce or entirely prevent the production of new reproductives by colonies (Otti and Schmid-Hempel, 2008) and so it must rely upon horizontal transmission between colonies in order to infect future generations. Seasonal patterns of disease prevalence in sympatric bumblebee species can be markedly different even when they share many of the same floral resources (Goulson et al., 2012), suggesting that mechanisms such as worker drift which result in intra-specific transmission may be more important.

Drifting and egg-dumping also have implications for bumblebee conservation for they have the potential to increase the effective population size. Monogamous social insects such as *B. terrestris* are at enhanced risk of inbreeding, since the large majority of the population produce no offspring (Goulson et al., 2008; Whitehorn et al., 2009). Successful drifter reproduction, or reproduction by multiple queens in a single colony, would increase the effective population size and so reduce rates of genetic drift in small populations.

Genetic techniques provide a fascinating window on the colony structure of social insects. Further insights, for example into the origins of drifters and egg-dumping queens, could be obtained by intensive sampling of colonies within a defined area and over multiple years.

6.6 Acknowledgements

We thank the Leverhulme Trust for funding this research and field assistants James Mackenzie, Iain McNicol, and Lilly Herridge. We would also like to thank Andrew Bourke and one anonymous reviewer for their useful comments on the manuscript.

Chapter 7

Neonicotinoid pesticide reduces bumblebee colony growth and queen production

This chapter has been published as:

Whitehorn, P.R., O’Connor, S., Wackers, F.L. and Goulson, D. (2012) Neonicotinoid pesticide reduces bumblebee colony growth and queen production. *Science* **336**, 351-352.

The experiment presented in this chapter was a collaborative work by the above authors. Planning and experimental design was carried out by P.W., S.O. and D.G

Execution of the experiment was carried out equally by P.W. and S.O. Preparation of the initial manuscript was by P.W. All authors commented on drafts of the manuscript and responded to comments and questions posed by reviewers prior to publication.

7.1 Summary

The global decline of pollinators is of great concern due to the valuable ecosystem services they provide. Neonicotinoid insecticides have been implicated in these declines as they occur at trace levels in the nectar and pollen of crop plants. We exposed colonies of the bumblebee *Bombus terrestris* to field-realistic levels of the neonicotinoid imidacloprid, then allowed them to develop naturally under field conditions. Treated colonies had a significantly reduced growth rate and suffered an 85% reduction in reproductive success compared to control colonies. Given the scale of use of neonicotinoids, we suggest that they are likely to be having a considerable negative impact on wild bumblebee populations across the developed world, and may be in part responsible for the global pollination crisis.

7.2 Introduction

Pesticides play an important part in global food production and stability (Oerke and Dehne, 2004). Imidacloprid was first produced in 1991, one of a new generation of pesticides known as neonicotinoids, which are selectively toxic towards insects, acting as agonists of nicotinic acetylcholine receptors and disrupting neural pathways (Matsuda, 2001). Imidacloprid is now the most widely used systemic insecticide in the world and is routinely used on all major crops including cereals, oilseed rape, cotton and sugar beets (Elbert, 2008). Due to its systemic properties it is often used as a seed dressing, from where it migrates in the sap to all parts of the plant and it has been detected in the pollen and nectar of a number of flowering crops, for example maize, sunflower and oilseed rape, typically at concentrations ranging from 0.7-10μg L^{-1} (Bonmatin et al., 2003;

Brittain and Potts, 2011). As a consequence, beneficial pollinators may be exposed to trace levels of this insecticide as they forage on the crop plants (Schmuck et al., 2011). The majority of research investigating the effects of imidacloprid on beneficial insects has focused on honey bees. A recent meta-analysis based on 13 studies found that field-realistic doses of imidacloprid consumed by honey bees, under laboratory and semi-field conditions, had no lethal effects but reduced their expected performance by 6 to 20% (Cresswell, 2011). Fewer studies have been carried out on bumblebees and results are conflicting (Tasei et al., 2001; Gels et al., 2002; Morandin and Winston 2003; Tasei et al., 2011). There is some evidence that low doses of neonicotinoids may reduce foraging ability (Mommaerts et al., 2010), which is likely to have significant impacts under natural conditions when colonies rely on their workers to locate forage across the landscape, but is likely to have little effect in cage studies. In the only field study to date (Tasei et al., 2001), colonies were placed in the field for 8 days during which time 54% more foragers from the ten colonies exposed to neonicotinoids failed to return compared to the ten control colonies, although this difference was not statistically significant. However, the colonies were then returned to the lab and fed *ad libitum*, so any long term effects of impaired foraging ability on colony development would not be detected. No previous studies have examined impacts of exposure to neonicotinoids on colony development while foraging naturally in the field.

In order to address this gap in the research we conducted an experiment using 75 *B. terrestris* colonies divided evenly between one control and two treatment groups. Our experiment was designed to simulate the likely effect of exposure of a wild bumblebee colony to flowering of a nearby crop such as oilseed rape. Pollen and nectar were provided *ad lib* over a period of 14 days in the laboratory. The level of imidacloprid present in the pollen and nectar received by the ‘low’ treatment group was chosen to represent the level that has been found in seed-treated rape (Bonmatin et al., 2005). The ‘high’ treatment received double this concentration, still close to the field-realistic range. The colonies were then placed in the field where they were left to forage independently for a period of six weeks.

7.3 Method

Commercial *Bombus terrestris audax* colonies (Biobest N.V., Belgium) were randomly assigned to three treatments (control, low and high), with 25 colonies in each treatment. The number of worker bees present within the colony at the start of the experiment was recorded to control for variations in initial size (mean: 15.44, range: 5 to 34). Pure imidacloprid (Sigma-Aldrich, UK) was dissolved in a known volume of distilled water and used to dose pollen and sugar water. The low treatment pollen and sugar water contained 6μg kg^{-1} and 0.7μg kg^{-1} imidacloprid respectively. The high treatment pollen and sugar water contained twice these concentrations (12μg kg^{-1} and 1.4μg kg^{-1} imidacloprid respectively). Equivalent volumes of distilled water were added to the control pollen and sugar water. All colonies remained in the laboratory for 14 days, where they were provided with the treated food *ad libitum*, before being transferred to the field where the workers could forage under natural conditions for a further six weeks. The experiment was timed to correspond to wild colony development, with colonies being placed out in the field on 11th July. The field site was situated on the edge of Stirling University campus and close to ornamental gardens, deciduous woodland and mixed farmland. There were no flowering crops within 2 km. The doors on the nest-boxes were designed to ensure any new queens produced were not able to leave the colony. The fresh weight of all colonies was recorded at the start of the experiment and weekly thereafter. After field placement the colonies were weighed after dark to minimise disturbance. Any colonies that died during the course of the experiment were collected and stored at -20^{o}C. At the end of the experiment all colonies were freeze-killed and then dissected and the number of new queens present was recorded.

7.3.1 Data analysis

Data were analysed in R, version 2.12.0 (2010 The R Foundation for Statistical Computing). A linear mixed effect model was used to analyse determinants of colony weight. Treatment, week, week^2 (to account for the curved relationship of weight over time) and the number of workers present at week = 0 were entered as fixed effects. The interactions between week and both treatment and the number of workers were also fixed effects and the individual colonies were entered as a random effect. The difference between the numbers of new queens produced by the colonies in each treatment was analysed with a Kruskal-Wallis test.

7.4 Results

All colonies experienced initial growth followed by a decline as they matured. Colonies in both the low and high treatments gained less weight over the course of the experiment compared to the control colonies [(Figure 7.1); linear mixed effect model; t (568) = -4.03, $P < 0.001$ & t (568) = -5.39, $P < 0.001$ respectively]. By the end of the experiment the low and high treatment colonies were on average 8% and 12% smaller respectively than the control colonies. The weight change in the high treatment colonies was not significantly different to the low treatment colonies [(Figure 7.1); linear mixed effect model; t (568) = -1.44, P = 0.151].

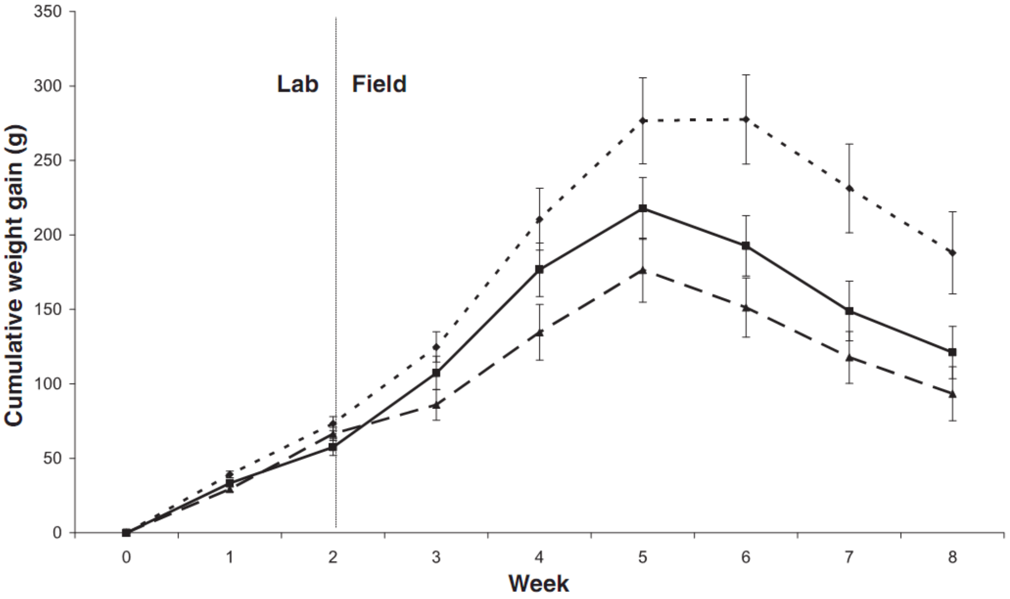

Figure 7.1. Mean observed colony weight for control (short-dashed line), low (solid line) and high (long-dashed line) treatments at weekly intervals. The change in weight over time was significantly smaller (P<0.001) in low- and high-treatments compared with control colonies. The number of colonies per treatment was 25 in weeks 0 to 3. In the following weeks, the numbers of colonies in the control, low and high treatments, respectively, were as follows: week 4 (25, 24 and 25), week 5 (25, 24 and 25), week 6 (23, 23 and 25), week 7 (22, 23 and 25), and week 8 (20, 18, 21). Points represent cumulative weight increase since week 0 (and their standard errors); weight includes all accumulated biological matterial (wax, brood, food stores and adult bees).

The rate of colony growth was also dependant on the number of workers present at week 0 [(Table 7.1); linear mixed effect model; t (568) = 2.61, P = 0.009], reflecting the importance of a large workforce for optimal development. The negative effect of imidacloprid on colony growth was reflected in a much reduced reproductive success.

The mean number of queens produced by colonies in the control treatment was 13.72 (± 5.70), whilst in the low and high treatments it was only 2.00 (± 1.13) and 1.4 (± 0.53) respectively [(Figure 7.2); Kruskal-Wallis test: H (2) = 9.57, P = 0.008]. The drop in queen production is disproportionately large compared to the impact of imidacloprid on colony growth. However, there is evidence that only the very largest bumblebee colonies succeed in producing queens: for example in field studies of reproduction of 36 colonies of the closely related *Bombus lucorum*, all queen production came from the largest 6 nests (Müller and Schmid-Hempel, 1992). Thus even a small drop in colony size may bring it below the threshold for queen production. Given that queens are the only stage to survive the winter to start new colonies in the following spring, reductions in queen production of 85-90% are likely to have significant impacts at the population level.

Table 7.1 Linear mixed effect model for colony weight. Parameter estimates are with reference to the control treatment. Degrees of freedom are given in parentheses.

Fixed effect	Value	SE	t - value	P
(Intercept)	564.21	39.59	14.24 (568)	<0.001
Treatment (High)	13.62	27.80	0.490 (71)	0.626
Treatment (Low)	13.62	27.11	0.502 (71)	0.617
Week	89.21	5.50	16.22 (568)	<0.001
Week^2	-6.68	0.430	-15.51 (568)	<0.001
No. workers at week = 0	0.759	1.92	0.396 (71)	0.694
Treatment (High)*Week	-13.42	2.49	-5.39 (568)	<0.001
Treatment (Low)*Week	-9.95	2.47	-4.03 (568)	<0.001
Week*No. workers at week = 0	0.448	0.172	2.61 (568)	0.009

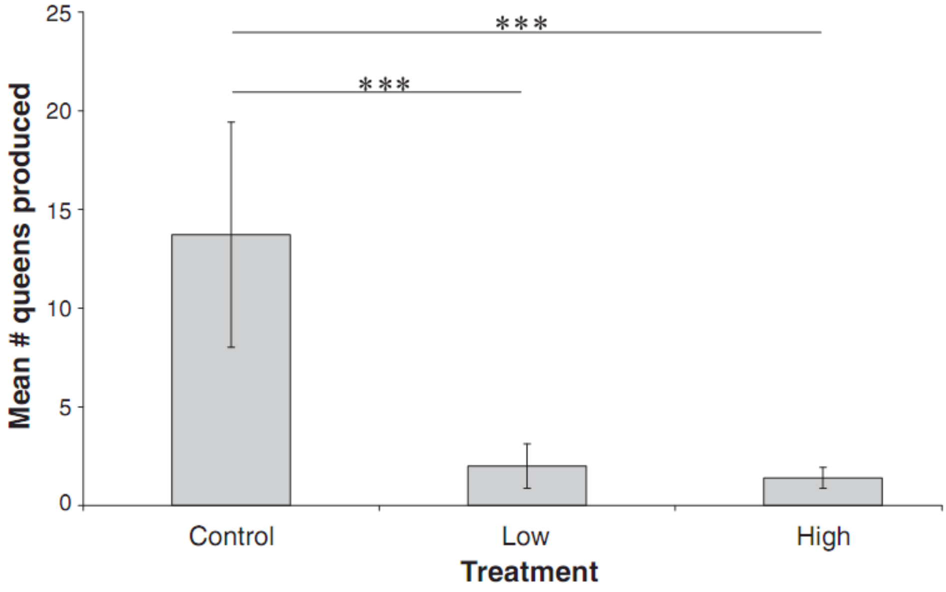

Figure 7.2. The number of new queens produced by the control colonies was greater than the number produced in both low- and high-treatment colonies. Bars represent the mean number of queens and their standard errors. Asterisks indicate significant differences.

7.5 Discussion

Our results are direct evidence that trace levels of neonicotinoid pesticides can have a severe negative consequence on the fitness of bumblebee colonies under realistic field conditions. Although we cannot infer the precise mechanism by which this loss of fitness occurs, it is probable that workers have a reduced foraging efficiency (Ramirez-Romero et al., 2005; Mommaerts et al., 2010) and therefore bring insufficient resources back to the colony, stunting its growth and ability to rear new queens.

Our experiment simulates exposure of a young colony to a flowering oilseed rape crop, which typically flowers for 3-4 weeks in the spring and attracts numerous foraging bumblebees of a range of species (Hayter and Cresswell, 2006). Bumblebee workers travel a kilometre or more to collect food (Knight et al., 2005; Osborne et al., 2008b) and in a recent study of a 10 x 20 km rectangle of lowland England, 100% of the land area in a 2007 snapshot was within 1 km of an oilseed rape crop, with rape providing the large majority of all floral resources in the landscape when flowering (Goulson et al., 2010). Thus we predict that impacts of imidacloprid on reproduction of wild bumblebee colonies are likely to be widespread and significant, particularly as this chemical is registered for use on over 140 crops in over 120 countries (Elbert, 2008). It should be noted that commercial colonies were used for this experiment and such colonies have been bred in captivity for many generations (Velthuis and Van Doorn, 2006). It is likely that wild British bumblebees have been increasingly exposed to imidacloprid and other neonicotinoids since the chemicals were first used in the mid 1990's (Goulson, 2013). The observed reduction in gyne production is likely to act as a strong selective pressure which may have led to wild populations having adapted to better cope with sublethal effects compared to captive bred commercial lines of bumblebees. Future experiments using colonies reared from wild queens (possibly collected in areas of high and low neonicotinoid use, such as intensive arable or extensive pastoral farming respectively) could be conducted to investigate adaption to neonicotinoid exposure. In addition, species may not be equally susceptible to toxicity from neonicotinoids and most experimentation has been carried out using very few species, for example, *B. terrestris* and *B. impatiens*. Future researchers may wish to investigate sublethal effects of neonicotinoids in other bumblebee species. As bumblebees are valuable pollinators of crops and wild flowers and vital components of ecosystems, we suggest that there is an urgent need to develop alternatives to the widespread use of neonicotinoid pesticides on flowering crops wherever possible.

7.6 Acknowledgements

We thank Biobest N.V. (Belgium) for providing the bumblebee colonies, Calum Brown for assisting with field work and Jeroen Minderman for statistical advice.

Chapter 8

General Discussion

The majority of species that have ever existed on the planet are now extinct (Yalden, 1999; Pough et al., 2002; Begon et al., 2006; Barnosky et al., 2011). Five mass extinctions have taken place during the last 500 million years and the probable causes ranged from volcanic activity, meteorite impact, to dramatic climate change (Barnosky et al., 2011; Sodhi and Ehrlich, 2011). The current wave of extinction is different, it driven by human action. The last few centuries have witnessed a rapidly increasing human population, industrialisation and ever greater consumption per capita which have ultimately resulted in the decline of animal populations and increasing numbers of species extinctions (Sodhi and Ehrlich, 2011). There are many facets to the current trend of declines, for example, direct harvesting or hunting of species (Wilkie et al., 2011), habitat loss through urbanisation, agriculture, or pollution (Sodhi and Ehrlich, 2011). Species have been introduced to new regions by humans, upsetting ecosystems, for example, Pyke (2008) attributes the decline and extinction of several native fish species in Australia to the introduction and establishment of the predatory mosquito fish (*Gambusia affinis*) which was introduced from Mexico. Alternatively, the effects may be more subtle, such as hybridisation, competition or introduction of pathogens for which native species have not developed any immunity. For example, American grey squirrels (*Sciurus carolinensis*) have spread throughout much of Britain (Yalden, 1999) and have been closely followed by declines of native red squirrels (*Sciurus vulgaris*) due to competition and spread of a pox virus (parapoxvirus) which red squirrels are more susceptible to (Rushton et al., 2000; Bertolino et al., 2008). These problems are not restrained to the Western world or developing countries and have been experienced by all taxa, in regions throughout the globe. Invertebrates have perhaps received less attention than larger more notable “flagship” species, which are typically large charismatic species (Sodhi and Ehrlich, 2011; Smith et al., 2012; Waldron et al., 2013). Insects, including bumblebees have not escaped population declines and extinctions, which are thought to be driven by the anthropogenic factors described. Bumblebee species, for example, have been introduced into new regions, such as *B. terrestris* into Japan and south America with the potential to hybridise with or outcompete native pollinators (Matsumara et al., 2004; Torretta et al., 2006; Kanbe et al., 2008) or assist the spread of invasive plant species such as *Lupin* spp. in New Zealand and Tasmania, (Stout et al., 2002; Goulson 2003) (Stout et al., 2002). The commercialisation of bumblebee colonies for agricultural crop pollination has been blamed for the introduction of bumblebee pathogens to new regions and enabling pathogens to spill-over into wild communities due to artificially increasing the bumblebee population in a concentrated area (Colla et al., 2006; Goka et al., 2001; Potts et al., 2010; Arbetman et al., 2012; Liu et al., 2013; Murray et al, 2013).

Bumblebee populations in Britain have declined over the last century, in particular since the introduction of the 1945 Agriculture Act (Robinson and Sutherland, 2002). The steps taken to improve efficiency of food production proved detrimental to the wildlife that had adapted to living on farmland under a lower intensity (Fuller et al., 2002; Hart-Davis, 2002; Robinson and Sutherland, 2002; Stoate et al., 2002). The primary cause is habitat loss, through actions such as removal of hedgerows, draining of wetlands, application of artificial fertiliser and herbicides to pasture and arable land and a movement from hay to haylage and silage production. Wild flowers which were once common in pastures and grew as weeds in hay meadows and arable fields have become increasingly rare and this has impacted upon those species that rely on them as a food source, such as bumblebees and butterflies (Southerton, 1998; Asher et al., 2001).

The lack of an appropriate, long term monitoring scheme for bumblebees means it is impossible to accurately quantify the declines precisely. Distribution maps were compiled from records collected by volunteers and museum specimens and published as The Atlas of the Distribution of the Bumblebees of the British Isles, (Alford, 1980). Data was split between pre and post 1960, and range retractions for some species, such as *B. humilis* and *B. ruderarius*, are apparent. These maps have underpinned bumblebee distribution research and form a baseline which is severely lacking in other countries (Winfree, 2010). Academic works published in the earlier half of the 20th century document and discuss species, which by the 1980's, were absent from large parts of the UK (e.g. Sladen, 1912; Cumber, 1953; Free and Butler, 1959; Alford; 1975). Since this time, attempts to document species ranges over time have continued using monitoring of both bumblebee nests (Fussell and Corbet, 1992b; Osborne et al., 2008a; Lye et al., 2012) and workers, for example to document spread of non-native *B. terrestris* in Japan (Kadoya, and Washitani, 2010) and to continue documentation of species populations (Dickinson et al., 2010; discussed in Goulson et al., 2011). Monitoring schemes are useful not only for informing on population declines and trends, but are also needed in order to target conservation efforts. For example, *B. sylvarum* was common in southern Britain before 1950's but faced declines thereafter (Free and Butler, 1959; Alford, 1980). By the 1980's, its range appeared to have retracted to only occasional reports at a few sites (Alford, 1980). In 1995, *Bombus sylvarum* was listed as a Biodiversity Action Plan species (BAP) but in order to establish the extent of *B. sylvarum*'s range retractions and enable conservation measures to be planned, knowledge of where the species persisted was required. A concerted effort was made to search the sites where it had been recorded since 1970 (Alford, 1980; Edwards and Williams, 2004). Six weeks worth of man-hours of searching in these sites produced just two *B. sylvarum* workers (Edwards and Williams, 2004).

Bumblebees are considered to be a keystone group due to the ecosystem service they provide in the pollination of both wild flowers and agricultural crops (Chapman and Bourke, 2001; Goulson, 2010; Thakur, 2012; Pearce et al., 2012). The reported bumblebee species declines (Alford, 1980; Williams, 1986; Goulson et al., 2008; Colla and Packer, 2008; Williams et al., 2008) are therefore concerning, as inadequate pollination causes a reduction in the yields from some crops, affecting the stability of agriculture (Ariza et al., 2012; Thakur, 2012) and wild flowering plants may become increasingly fragmented and go extinct (Corbet et al., 1991; Lennartsson, 2002; Potts et al., 2010; Pearce et al., 2012).

In addition to the pollination service provided by bumblebees, both adult bumblebees and nests are part of a complex network of ecological interactions. Many invertebrate species occur in bumblebee nests, for example the wax moth *Aphomia sociella* and dipterans such as *Brachicoma devia* and *Volucella bombylans* (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Benton, 2006; Goulson, 2010). Other species specialise in parasitism of adult bees, such as conopid flies and *Syntretus splendidus* (Alford, 1975; Müller and Schmid-Hempel, 1992; Benton, 2006). Parasitic nematodes, (e.g. *Sphaerularia bombi*), mites (e.g. *Parasitellus* spp.) and internal, bumblebee specific, parasites such as *Nosema bombi* and *Crithidia bombi* are entirely reliant on bumblebees (Pouvreau, 1973; Alford, 1975; Benton, 2006). The majority of *Psithyrus* are specific to a single true bumblebee species (Van-Honk et al., 1981a; Vergara et al., 2003; Benton, 2006; Martin et al., 2010). This means that the loss of a true bumblebee species from an area is likely to be matched by the loss of a corresponding *Psithyrus* bumblebee species (if previously present). Declines to date and any further losses of bumblebees will inevitably result in a reduction of all these dependent species (Benton, 2006; Goulson et al., 2008). This is likely to have further reaching implications for other species, for example bats or birds, which may feed upon some of the invertebrates which colonise bumblebee nests (Alford, 1975).

A third of filmed bumblebee nests were attacked by great tits. It is conceivable that bumblebees provide an important resource for great tits. Bumblebees are a recognised food source for *Muscicapa striata*, spotted flycatcher (Davies, 1977) and *Lanius collurio*, red-backed shrike (Pedersen et al., 2012). Nests are also consumed by British mammals, such as badgers, (*Meles meles*) (Sladen, 1912, Free and Butler, 1959; Kruuk and Parish, 1981; Kruuk, 1989; Cleary et al., 2009; Goulson, 2010), pine martens (*Martes martes*) (Caryl et al., 2012) and hedgehogs (*Erinaceus europaeus*) (Alford, 1975; Yalden, 1976). It appears these species are not bumblebee nest specialists, as the contribution of bumblebees to the mammals' summer diet is relatively small (for example ~1-2% of ingested bulk). However, if bumblebees suffer further declines, a reduction in availability of this food source could have a negative impact on the mammalian predators. Predators may make use of a range of prey species and reduction in the availability of one often results in prey switching to another (Begon et al., 2006). For example, in the short term, pine martens will increase consumption of hares (*Lepus* spp.) when vole (*Microtus*) populations crash (Helldin, 1999). Longer term prey switching behaviour has also been recorded. For example, stoats (*Mustela nivalis*) consumed more lagomorphs in the 1990's compared with 1960's, as rabbit (*Oryctolagus cuniculus*) populations recovered from the introduced disease; Myxomatosis (McDonald et al., 2000). This may result in increased competition with other predators of the secondary prey species, (Begon et al., 2006). It is therefore probable that the declines in bumblebee populations to date (Alford, 1980; Goulson et al., 2008) and any future changes will have far reaching consequences, in addition to their pollination service.

While many bumblebee species have declined, others have spread to new regions, both through deliberate introduction and natural immigration. Examples include intentional introductions of *B. terrestris*, *Bombus subterraneous*, *Bombus ruderarius* and *Bombus hortorum* to New Zealand in order to achieve adequate pollination of clover leys (Cumber, 1953; Donavan and Wier, 1978; Edwards and Williams, 2004) and *B. terrestris* in Tasmania (Goulson, 2003; Schmid-Hempel et al., 2007) and Japan (Matsumara et al., 2004; Kanbe et al., 2008). *Bombus hypnorum* recently arrived naturally in Britain and has since become established (Goulson and Williams; 2001; Benton, 2006). New arrivals may be considered a positive improvement to local pollination services (Cumber, 1953; Donavan and Wier, 1978), however, ecologically immigrations pose a risk to native species for example through displacement by competition (Matsumara et al., 2004) or introduction of pathogens, etc. (Potts et al., 2010; Liu et al., 2013). It is unclear how other species that interact with bumblebees will adapt to new arrivals. For example, we have found that wax moths readily target nests of *B. hypnorum* and *B. hypnorum* ousted birds from their nests, including great tits, which have been observed predating bumblebees of other species (Benton, 2006). As the majority of *B. hypnorum* nests are above ground level, they are likely to be out of reach of attack from mammalian predators (e.g. hedgehogs and badgers) and so will not contribute to the bumblebee proportion of the mammal's diets. There were no records in this study of *B. hypnorum* nests suffering from any *Psithyrus* parasitism, which is not surprising given that *Bombus norvegicus* (a social parasite of *B. hypnorum* (Løken, 1984)) does not occur in Britain

(Alford,1980). However, Benton, (2006) noted a *B. hypnorum* nest containing a *B. barbutellus*, so this recent arrival may provide host colonies for *B. barbutellus*.

8. 1 Methods for investigating bumblebee nests

8.1.1 Obtaining nests for study

The location of sufficient bumblebee nests for replicated study remains challenging (Carvell et al., 2008; Osborne et al., 2008a; Suzuki et al., 2009). Trained bumblebee nest detection dogs do not significantly increase the rate at which nests can be found (Waters et al., 2011; O'Connor et al., 2012). Instead, people should be recruited to assist in nest locating, as even inexperienced assistants may readily find nests, although the rate at which they do so is low (O'Connor et al., 2012). The numbers of located nests may be increased by counting nest site searching queens in spring time and searching only favourable areas (i.e. those with greatest numbers of queens). Alternatively, counts of nest site searching queens alone may be sufficient to assess attractiveness of sites for nesting queens (e.g. Svensson et al., 2000; Kells and Goulson, 2003; Lye et al., 2009) although clearly this provides no further ecological data, such as reproductive success (i.e. a site may be desirable for nesting sites, but fail to provide a succession of flowers to maintain colonies; Holm 1966). Whilst not empirically tested here the approach of a summer storm was a particularly good time to search for nests as foraging workers rushed back to their nest in an obvious stream of traffic (Cumber, 1953; pers. obs.).

Citizen science is proving increasingly popular as a method for collecting ecological data for a range of species. Perhaps the best known schemes are those conducted by the British Trust for Ornithology, founded in 1932, as well as butterfly recording schemes run by the charity 'Butterfly Conservation' (Asher et al., 2001; reviewed in Dickinson et al., 2010). With the development of the National Biodiversity Network, where species data can be uploaded for all to access and use, citizen science is becoming an important part of British wildlife monitoring and conservation. Willing members of the public are able to increase sample sizes and study areas dramatically. Bumblebees frequently nest in gardens (Osborne et al., 2008a; Lye et al., 2012) and members of the public spending considerable amounts of time in their garden (Gaston et al., 2005) are liable to notice worker and sometimes queen traffic (Lye et al., 2012; see Chapter 5). It is not uncommon for such people to search the internet in order to learn more about bumblebee nests and in this way, they may be recruited into research with little advertising effort, unlike previous studies of bumblebee nests which have relied upon advertising in magazines, etc. (e.g. Osborne et al., 2008a). Questionnaire websites and emails allow people to provide known details and upload photographs quickly and researchers may request further information in a timely manner. This data flow allows some of the issues faced with previous postal based surveys (Fussell and Corbet, 1992b; Osborne et al., 2008a) to be alleviated, for example photographs and footage of bumblebees facilitate more confident identification of species and in some cases a better understanding of the location of the nest, reproductive status, etc.

Artificially reared bumblebee colonies have provided a useful model for natural colonies (for example, Duchateau and Velthuis, 1989; Müller and Schmid-Hempel, 1992; Schmid-Hempel and Schmid-Hempel, 1998; Carvell et al., 2008; Lopez-Vaamonde et al., 2009; Whitehorn et al., 2012; see chapter 7). Such colonies can be more standardized with regards to the stage at which they are entered into an experiment and the conditions that they have previously experienced compared to wild nests. The boxed colonies facilitate experiments requiring translocation from laboratory to field sites, manipulation, measurements and treatments which would be undesirable or impossible to carry out with wild colonies. Whilst such reared colonies are unlikely to provide an entirely realistic model for wild nests (for example, they are often fed *ad. lib*. whilst developing, removed from environmental conditions, etc,) so results must be interpreted with some caution, such studies continue to be invaluable to developing our understanding of bumblebee nest ecology.

8.1.2 Methods for observing and studying bumblebee nest ecology

Cameras have been employed as an ecological tool, for example, studies of badger behaviour (Stewart et al., 1997) determining presence of large, elusive mammalian species (Kelly, 2008) and identifying predators of bird nests (Bolton et al., 2007). The camera recorders were crucial for discovering predation by great tits and tunnel blocking behaviour of wood mice, identifying nests visited by wax moths, and collecting information on other species that interacted with the nest, for example crows and hedgehogs. In addition, volunteers were able to provide interesting accounts of the inside of some bumblebee nests, which were located in bird boxes and fitted with cameras. This system may provide a useful model for future studies, especially considering the propensity for *B. hypnorum* to frequent bird boxes (Benton, 2006; Lye et al., 2012; see Chapter 5).

Regular observations of bumblebee nests (for example weekly or bi-weekly) are suitable for collecting data for some ecological measures, for example, continued activity, cessation of traffic, gyne or male production or animal excavation. However, this method was found to be less suitable for determining occasional or nocturnal events (such as *Psithyrus* or wax moth invasions, great tit predation, etc.). Neither observations, nor recording of entrances provide a clear understanding of behaviour within the nest (for example, actual wax moth infestations or interaction between the nest and small mammals). Inspection of the remains of nests allows some measurements to be taken, for example, infestation by wax moths, apparent small mammal destruction (Sladen, 1912; Donavan and Wier, 1972) and, when the comb has not degraded, total numbers of bees and gyne production (Alford, 1975; Whitehorn et al., 2012) along with evidence of intrusions by true bumblebees or *Psithyrus* (Goulson et al., 2002b; Carvell et al., 2008).

Developments in genetic analysis (for example, microsatellite markers) have revolutionised conservation biology, and informed upon many aspects of behaviour and ecology in a multitude of species. New species have been identified (Frankham et al., 2009) and breeding behaviours have been established and confirmed, for example, the brood parasite behaviour of brown-headed cowbirds, *Molothrus ater* (Alderson et al., 1998). Genetic techniques also allow other methods to be employed with more confidence. For example, hair and scat samples from animals can now be reliably distinguished (e.g. mustelids (Lopez- Giraldez et al., 2005; Rozhnov et al., 2008). Our understanding of bumblebee behaviour and biology has also been informed through the innovations of molecular methods. They are useful for the estimation of foraging ranges, nest densities and survival (Knight, 2005; Knight et al., 2009; Goulson et al., 2010) and queen dispersal ranges (Lepais et al., 2011), as well as for the differentiation of sub species (e.g. Estoup et al., 1996) and the differentiation of the biology of cryptic species, such as those of the *B. lucorum* complex (Murray et al., 2008). Molecular techniques also permit studies of the population genetics of bumblebees, especially of rarer species or those with fragmented populations, such as populations of *B. sylvarum* (Ellis, 2006) and *B. muscorum* and *B. jonellus* (Darvill et al., 2010). Genetic analysis also continues to be a vital tool for revealing the complexities of relationships within bumblebee nests (Alaux et al., 2004; Lopez-Vaamonde et al., 2004; Huth-Schwarz, 2011; see Chapter 6).

Estimates of fecundity or survival rates are hampered by the unknown parameters surrounding bumblebee nesting ecology (Carvell et al., 2008; Goulson et al, 2010; Winfree et al., 2012). Developmental time from an egg to worker is known to be 3-5 weeks, (Alford, 1975; Duchateau and Velthuis, 1989; pers. obs.) but there are only vague estimates for the duration of pre-egg-laying nest initiation of wild nests, for example ‘several days’ (Alford, 1975). If worker traffic is present at the point when the nest is discovered, it is not possible to determine whether they belong to the first or a subsequent brood. Longevity only provides data on nest survival and whilst growth (weight gain) is a useful measure of colony health, with heavier colonies more likely to produce gynes (Müller and Schmid-Hempel, 1992; Whitehorn et al., 2012), longevity may not always correlate with gyne production (see chapter 4). It is therefore important to establish regular observations of nests, by someone who is able to distinguish between casts of bumblebees, if nest success is the focus of the study.

8. 2 Relationships with other species

8.2.1 Vertebrates

The disused burrows and nests of some vertebrate species provide nest sites for bumblebees, for example, small mammals, hedgehogs, rabbits, rats, squirrels and numerous bird species such as sparrows, *Parus* spp. (Alford, 1975; Fussell and Corbet, 1992b; Lye et al., 2012; see Chapter 5), common redstart (Rasmont et al., 2008) and snow buntings (Kukal and Pattie, 1988; Heinrich, 1993). It seems likely that the nests of other species may also be adopted by bumblebees, (pers. obs.) of nest site searching *B.*

terrestris entering a disused fox's earth, and on a separate occasion, a nest site searching *B. terrestris* queen entering an active badger sett). Some of these nest site providers also predate bumblebee colonies, namely; hedgehogs (Yalden, 1976), great tits (see Chapter 4.) and badgers (Kruuk and Parish, 1981; Cleary et al., 2009) and supposedly certain small mammals, (Darwin, 1906; Sladen, 1912; Pouvreau, 1973). This thesis was unable to clarify the relationship between any small mammal species and bumblebees. This is not surprising because small mammals are thought to predate nests before emergence of the workers (Sladen, 1912; Pouvreau, 1973; Alford, 1975) and no nests were under a suitable observation regime to detect such activity. Similarly, conclusions could not be drawn from the dietary literature due to the limited sample size and possible null detection of wax, pollen and bumblebee brood (Watts, 1968; Flowerdew and Gardner 1978). It seems likely that mice predate bumblebee nests as this has been reported repeatedly (Darwin, 1906; Sladen, 1912; Cumber, 1953; Pouvreau, 1973; Donavan and Weir, 1978). Wood mice have a propensity to consume larvae of insects (such as lepidopteran larvae) in preference to seeds or other vegetable matter (Watts, 1968; Flowerdew and Gardner 1978) and in captivity will chew pieces of undefended, previously frozen bumblebee comb (pers. obs.), so it seems likely that considering mice as predators of small bumblebee nests is accurate. In this study, wood mice caused two nests to fail by blocking entrance tunnels with leaf litter, an avenue of interaction which had not been previously described. In addition, wood mice, voles and shrews frequented tunnels containing bumblebee nests without any apparent depredation of colonies (i.e. worker traffic did not cease), although partial depredation of nests by these small animals could not be ruled out (see Chapter 4).

The impact of various predators upon bumblebee populations is difficult to estimate. Great tits predated bees and occasionally targeted gynes (see Chapter 4). However, these nests produced more than one gyne and the birds' visits did not cause the nests to 'fail' (in a manner dissimilar to nests excavated and consumed by a large animal for example). Some worker predation is likely to be tolerated by nests, for example, experimentally increased worker mortality (removal up to 40% of the workforce per week) did not decrease the likelihood of *B. lucorum* nests producing gynes (Schmid-Hempel and Heeb, 1991). It can perhaps be assumed that great tits are therefore not responsible for the demise of a large proportion of nests.

Two mammalian species that are widespread throughout Britain, badgers and hedgehogs, and also the range-restricted pine marten, predate bumblebee nests (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Yalden, 1976; Kruuk and Parish, 1981; Cleary et al., 2009; Caryl et al., 2012). Annual consumption of bumblebee nests by badgers and hedgehogs has been estimated at 482,112 kg (1,205,280 nests) and 311,000 kg (777,500 nests) respectively. *Bombus pascuorum* is the only common surface-nesting species in Britain (Benton, 2006; Goulson, 2010; Lye et al., 2012; pers. obs.). Other surface-nesting species, such as *Bombus humilis, Bombus sylvarum, Bombus muscorum* and *Bombus ruderarius,* have suffered range restrictions in recent decades for example (Alford, 1980; Williams, 1982; Benton, 2006; Goulson et al., 2008). It is therefore probable that *B. pascuorum* suffer a disproportionately large amount of damage from hedgehogs compared to subterranean nesting species as filming showed that hedgehogs were unable to excavate subterranean nests (see Chapter 4).

It appears that badgers and to a lesser extent hedgehogs inflict considerable damage to bumblebee nests where they occur in Britain. I estimate that badgers may destroy in the region of 0.6-1.5% of all nests across the country, (see Chapter 5) but this very crude estimate includes areas where they do not occur. Under some circumstances the predation rates may be considerably higher, for example areas which have a large badger population or where other foods, such as earthworms are limited through dry weather, etc. (Kruuk, 1989). It is impossible to know if this figure is a cause for concern. Badger predation of bumblebee nests may be of increased interest where rare bumblebee species are known to exist in fragmented meta-populations which are vulnerable to stochastic extinction (Goulson, 2010). Whilst rates of predation may be inaccurate, this increased knowledge of bumblebee predators may facilitate inclusion of predation in future studies (Winfree, 2012). For example, where previous studies have considered the impact of mass flowering crops and urban areas on the survival of wild nests (Lepais et al., 2011) such models may also include proximity of badger setts.

8.2.2 Invertebrates

Wax moths are more likely to infest nests above than below ground as expected (Sladen, 1912; Pouvreau, 1973, Alford, 1975, Benton, 2006). Provision of nest boxes has been studied as a method of avoiding nest site competition (especially among later emerging species), improving habitat and to provide nests for amateur and professional researchers to observe (Fussell and Corbet, 1992b; Intenthron and Gerrard, 1999; reviewed in Lye et al., 2011b). Whilst well intentioned, nests in artificial domiciles that are above ground are more likely to be infested with wax moths, possibly leading to reduced fecundity of nests (also suggested by, Free and Butler, 1959). Studies of rates of infestation by wax moths using artificial nests above ground are likely to have detected inflated infestation, although variation within the experiment would still be expected to reflect real trends. For example, Goulson et al. (2002b) found infestation rates were greater in urban than rural areas.

Other than the large crab spider *Misumenia vatia*, British arachnids are not considered to be predators of bumblebees as their webs are considered too weak to catch bumblebees (Alford, 1975), although others dispute this with photographed examples of captured bumblebee victims (Benton, 2006). In this study a 'large spider' (species unknown) was seen to battle with a *B. terrestris* queen via a bird box camera recorder, resulting in the death of the queen (personal communication with B. Dawson). Whilst this was an isolated record of such an interaction, very few natural bumblebee nests have ever been observed in this way (24 hour film recordings of an incipient nest) and it may be possible that large arachnids ought to be considered another enemy of nesting bumblebees (at least in the pre-worker stage).

On a few occasions, social wasps *Vespidae* spp. were recorded frequenting bumblebee nests which later declined. Social wasps are known to steal nectar from nests, (Free and Butler, 1959; Alford, 1975) and have been observed invading laboratory reared nests which were placed outside in artificial domiciles (pers. obs.). In these domiciles it was possible to view the interaction between social wasps and bumblebees and it became apparent that the wasps caused the bumblebee colonies great harm, by stealing nectar, fighting and killing workers that defended nests (pers. obs).

Competition for nest sites, (Sladen, 1912; Alford, 1975; Lye et al., 2011b) usurping queens (Alford 1975; Paxton et al. 2001; Carvell et al., 2008), drifting and drifter reproduction (Birmingham and Winston 2004; Lopez-Vaamonde et al. 2004; Takahashi et al. 2010) have all been described previously and some evidence for each was found in natural nests during this study. A small proportion of nests were usurped by *Psithyrus,* compared to experiments in England (for example, Carvell et al., 2008) but this study was poorly designed to collect data on *Psithyrus* as filmed nests of *B. terrestris* were unlikely to be invaded by *Bombus vestalis* as the species has not been recorded in central Scotland (Benton, 2006) and it is unlikely that *Psithyrus* would be noticed entering or leaving at the moment when observations of the entrance were carried out either by researchers or members of the public (although they were in a few cases). Evidence for egg dumping (reviewed in; Tallamy 2005) by foreign *B. terrestris* queens was also found. Alternative reproductive strategies of true bumblebees (i.e. usurpation, drifting and egg dumping) will result in a nest containing bumblebees of several lineages. Such nests have been reported to have increased vigour, (Sladen, 1912) grow more quickly (Fisher and Pomeroy, 1989) and colonies resulting from gynes artificially inseminated with several males have increased internal parasite resistance (Baer and Schmid-Hempel, 2007). They may also produce reproductives of different lineages and therefore increase the effective population size (Chapman and Bourke, 2001). Invasions from foreign bees including *Psithyrus* may facilitate horizontal pathogen transmission if intruders are carrying an infection (Erler et al., 2012). Internal parasites such as *C. bombi* and *N. bombi* spread rapidly through nest-mates due to close proximity, shared food stores and absence of U.V. light (Rutrecht et al. 2007; Otti and Schmid-Hempel 2008). Previous studies describe minimal effects of *Crithidia bombi* on bumblebees, especially under favourable conditions (Schmid-Hempel and Schmid-Hempel, 1998; Brown et al., 2000; Brown et al., 2003). We found that *B. terrestris* nests with a greater proportion of nest-mates presenting infections of *C. bombi* in their faeces were less likely to produce gynes.

This is the first time that wild bumblebee nests have been assessed in this way, although the sample-size is somewhat small (n=29). This finding may have implications for previous studies that have considered *C. bombi* as common and relatively harmless (Brown et al., 2003) and studies assessing spill-over of pathogens from commercial colonies (Colla et al., 2006; Goulson et al., 2012; Murray et al., 2013).

It has been suggested that British bumblebee species may be broadly split into two categories, those that are woodland edge/garden specialists and those that are open grassland species (Edwards and Williams, 2004). The woodland edge species (such as *B. terrestris*, *B. lucorum*, *B. lapidarius*, *B. pratorum*, etc) tend to emerge earlier, have shorter tongues and make use of a succession of flowers from woodland floors, foraging further afield during the summer months. Whereas species adapted for open grasslands such as emerge later in the summer to coincide with the appearance of summer flowers and have longer tongues to allow them to handle flower species (such as red clover, birds-foot-trefoil, etc,) typical of open, meadows, or down-land (Corbet et al, 1991; Goulson et al., 2004; Benton, 2006). It may not be coincidence that the majority of declines have been suffered in later emerging species, whilst earlier emerging species are still widespread throughout Britain (Alford, 1980; Edwards and Williams, 2004). Surface nesting bumblebee species are presumably more exposed to attack from badgers, hedgehogs and possibly wax moths. It is therefore somewhat curious that any bumblebees preferentially nest on the surface, but in such open meadow habitats field voles thrive (Hart-Davis, 2002) and these tend to create runs and nests among tussocks of grass rather than digging subterranean burrows. It appears the bumblebees make use of the resources available.

8.3. Other causes of nest failure

8.3.1 Rates of nest failure and success

Percentages of nests producing gynes and those failing from various causes have been given. Extrapolation from these data may result in unrealistic measures of gyne production, predation, etc, due to the small sample size of filmed nests (n=32), constrained study site and biases in observation of different phenomena. For example, 6% of the 32 filmed nests failed due to wood mice blocking entrance tunnels, but it seems unlikely that this figure applies to all nests in British woodlands as it has not been recorded previously and there were only two incidences. The variation in gyne production in 2010 and 2011 is thought to reflect a genuine trend and not be an artefact of experimental procedure as bumblebees were notably scarcer in 2011 and nests were found less frequently than in previous years (pers. obs.). Gyne production has been found to vary widely between experiments using artificially reared colonies. Some experiments resulted in no gynes (Carvell et al., 2008) which was sometimes attributed to poor weather (Otti and Schmid-Hempel, 2008) and others found high gyne production, for example, 58% of 25 *B. terrestris* colonies produced gynes (Whitehorn et al., 2012) and 28% of 80 wild *B. pascuorum* nests produced gynes (Cumber, 1953). Muller and Schmid-Hempel (1992) reported that colonies of *B. lucorum*, which were initiated early and had a strong workforce were most likely to produce gynes. Whereas others have found that colonies which have large first broods are more likely to switch to male production at an earlier stage, thus limiting the change of gynes being reared (Duchateau et al., 2004). An alternative explanation for differing gyne production between years (and experiments) may be due to the winter/spring weather conditions and in particular temperatures in early spring which signal the end of queens' diapause (Benton, 2006). In a well replicated laboratory study of *B terrestris* colonies, length of queen diapause positively affected male production and negatively effected gyne production (Duchateau et al., 2004). This is thought to be due to increased metabolic demand on the queens' body during diapause resulting in lowered life expectancy and therefore an investment in males rather than gynes as they are more costly to produce (in terms of their pollen requirement and developmental time (Alford, 1975; Duchateau et al., 2004; Velthuis and Van Doorn, 2006)).

A meta-analysis of survival and fecundity of reared colonies used as controls (and in some cases treatments) from published experiments may be useful.

8.3.2 Anthropogenic causes of failure

People caused some nests to fail through deliberate interaction, such as intentional extermination and attempted nest relocation, or through accidental damage, for example, when gardening. In a few cases, simply walking through long grass or vegetation was sufficient to cause nests to fail as returning foragers were unable to locate the nest entrance amongst the flattened stems. Whilst such losses accounted for a very small percentage, throughout Britain actions causing such damage or alteration to the appearance of nest entrances must occur frequently, for example, through cutting of hay fields and roadside verges, building work and gardening. Collisions with moving vehicles are another anthropogenic source of bumblebee mortality (Benton, 2006) but this was not examined here.

Concerns have been raised regarding possible effects of agrochemicals on pollinators, especially honey bees and bumblebees (Thompson and Hunt, 1999; Potts et al., 2010) and in particular, neonicotinoids such as imidacloprid. We found a stark reduction in numbers of gynes produced from *B. terrestris* nests dosed with imidacloprid. Colonies were fed with a typical sub-lethal dose of imidacloprid that they might encounter whilst foraging on treated crops (such as oilseed rape) or garden flowers (Schmuck et al., 2001; Bonmatin et al., 2003) for two weeks, before allowing them to forage freely. Neonicotinoids are used extensively in Britain and around the world on many crops including those that flower and require pollination (Cresswell, 2011). Imidacloprid is retained in soils and may be recruited into untreated plants subsequently grown in them (Bonmatin et al., 2003). If the 85% reduction in mean gyne production found in our experiment is representative of wild nests which are exposed to sub-lethal levels of neonicotinoids (Gill et al., 2012), this will have a year on year negative affect on bumblebee populations.

There is an ongoing debate to decide whether neonicotinoid insecticides should be banned from use on flowering crops either in Britain or across all European Union member states. Recent studies have found further evidence for sub-lethal levels of neonicotinoids causing harm to bumblebees (Gill et al., 2012: Laycock et al., 2012), and a study of *B. terrestris* colonies feeding on Clothianidin treated clover similarly resulted in no new queens being produced, compared to a mean of more than 30 new queens per colony (Larson et al., 2013.) Negative effects of neonicotinoids have also been found in honeybees, for example reduced foraging ability (Henry et al., 2012) and increased *N. bombi* prevalence (Alaux et al., 2010; Pettis et al., 2012). Nevertheless, there is still considered to be sufficient doubt surrounding any negative impact of neonicotinoid insecticides on honeybee and bumblebee colonies to warrant continued use in some cases (Cresswell et al., 2012).

8.4 Suggestions for future research

This thesis has highlighted areas that require further attention, for example the relationship between incipient bumblebee colonies and wood mice as well as other mouse species such as house mice (*Mus musculus*), yellow-necked mice (*Apodemus flavicollis*), harvest mice (*Micromys minutus*) and other small mammal species, including the fat-dormouse (*Glis glis*) which has been witnessed to predate a bumblebee nest (C. Müller in Benton, 2006). Incipient colonies and those in their early stages of development remain a relatively poorly understood area of bumblebee ecology, although locating nests at this stage is particularly difficult. Some researchers have increased rates of nest founding in artificial domiciles by provisioning them with bedding, pollen and nectar and shutting wild caught, nest-site searching queens in them for 24-48 hours before restoring their access to the environment (e.g. Intenthron and Gerrard, 1999). Whilst this method would not provide entirely natural colonies, domiciles could be improved upon in order that they be more natural, for example, positioned below the ground, and possibly utilising existing small mammal tunnels and nests. This would enable footage to be recorded of the nest itself and might yield useful data on rates and causes of failure, usurpations by true bumblebees and *Psithyrus*, small mammal predation, etc., which might be approximately representative of wild nests.

Additional filming should be carried out in other areas of the UK, to observe other vertebrate species and determine if the predatory behaviour observed by great tits is constrained to the study site. The filming of other species of bumblebees, such as those that are surface nesting or rare may prove interesting. The literature review could be extended to include other British vertebrates, including those which haven’t previously been considered possible bumblebee predators. Further work is needed to establish the effect of field-realistic doses of neonicotinoids upon the gyne production of colonies under conditions which are more representative of wild nests in the field.

8.5 Conclusions

Bumblebees are crucial to the stability of ecosystems and agriculture. This thesis has attempted to develop our understanding of some aspects of bumblebee nest ecology. The relationships between bumblebees and mammals and birds are complex because bumblebees are dependent upon mammal and some bird species to provide them with nesting sites and may be depredated by the same species. It appears that nests below ground are protected from some large mammal depredations, as well as wax moths and presumably inclement weather.

Reproductive success may be reduced by high prevalence of *C. bombi* among nest mates, predation by great tits or exposure to trace levels of neonicotinoids. I have identified alternative reproductive strategies of wild bumblebees and offered advice to future researchers wishing to locate and observe bumblebee nests. It is hoped that these findings will enable the progression of our understanding of bumblebee ecology further and allow the development of strategies to halt and reverse species declines.

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Appendixes

Appendix I. Distribution of the sixty habitat transects at each of 14 rural farm sites around Stirling, Central Scotland. OS Grid reference (NS) is given for the centre of each site.

Site	**Grid Reference**	**Short Grass**	**Long Grass**	**Woodland Edge**	**Fence-line**	**Hedgerow**	**Bank**	**Total**
1	766965	0	1	1	1	3	2	8
2	765975	0	1	1	0	0	0	2
3	795986	2	0	0	1	0	0	3
4	825969	2	0	1	0	0	0	3
5	835963	1	0	1	1	1	0	4
6	850962	1	2	0	1	1	1	6
7	870969	0	1	0	0	2	3	6
8	933949	0	0	3	1	0	0	4
9	978947	0	0	0	1	0	0	1
10	012962	1	1	1	2	1	2	8
11	905899	0	2	0	1	2	2	7
12	844972	1	0	0	0	0	0	1
13	851955	1	2	0	1	0	0	4
14	925910	1	0	2	0	0	0	3
	Total	10	10	10	10	10	10	60

Appendix II. Summary of diet studies in relation to insects and bumblebees. Methods for reporting diet vary between studies. Frequency of occurence is presented as a percentage (%), or the actual numbers of relevant samples may be given (n). In some cases, authors have calculated the biomass for the pre-digested material and estimated the percentage of ingested bulk that different element contribute to the animals' diets [%]. Some studies have split their data further, (for example by month/season, or method of data collection and in some cases these have been presented.

Study and country	**Sample size and method**	**Invertebrates/Insects (n or FO%) [% Ingested Bulk]**
***Martes martes* (pine marten)**		
Heldin (1999) *Sweden*	398 scats	Beetles (n = 51) and Wasp (n = 11)
	483 intestines	Ants (n = 4) Beetle (n = 1); Wasp (n = 1) *Bombus* were not found in this study.
Lynch et al., (2007) *Ireland SW*	387 scats	Bees & wasps (7) [0.73%] Beetles (203) [22.99%] Bees found occasionally, no figure given for *Bombus*.
Caryl et al., (2011) *UK*	2449 scats	Insects (16.3%) n=399.19 [3.9 %]; *Vespa sp. (1.8)* n=44.08 [3%] *Bombus* spp. (1.9%) n=46.53 [3%]; Pollen and wax (1.5%) [2.9%] *Bombus* spp. some pollen and wax were found in winter suggesting dead nests?
Baltrunaite (2002) *Lithuania*	335 scats	Insects warm season (38.3%) [1.0%] Insects cold season (3.5%) [0.1%] No further detail given.
Vulpes vulpes (fox)		
Lever (1959) *UK*	420 stomachs 123 scats	Insects in (26%) (n=141.18) "Beetles common (mainly *Carabidae* and *Scarabaeidae*; 17 species beetle), syrphid larvae. Lepidoptera larvae (2 spp.) *Calliphora erythrocephala* where wool was also found, and larval Syrphid *Myiatropa fiorea* found in water in hollows in trees. Earwig (*Forficula auricularia*), bibionid flies, ant and three weevils *Phytonomus arator*, *P. punctatus* and *Alophbus triguttatus*. Solitary *elaterid* larva, *Athous* sp., adult *A. vittatus*" It seems likely that *Bombus* would have been identified had they occurred.
Baker et al., (2006) *UK*	985 scats	Coleoptera (n = 203) Orthoptera (n = 1) Diptera (n = 2) Lepidoptera (n = 1) Unidentified trace (n = 1) It seems likely that *Bombus* would have been reported.

Study and country	Sample size and method	Invertebrates/Insects (n or FO%) [% Ingested Bulk]
Sidorovich (2005) *Belarus*	Scats	Insects occurred frequently during the summer months, in 9.9 - 17.1% of samples, depending on soil type (Clay and Sandy soils respectively. No further detail given.
Leckie et al., (1998) *Scotland, moorland*	340 scats	Coleoptera in 2-4% of scats depending on season (n=approximately~10) There were no other identifiable invertebrate remains present.
Baltrunaite 2002) *Lithuania*	767 scats	Insects made up [1.0% IB] and occurred in 38.3% of scats during summer months. No further detail given.
Saunders et al., (1993) *England (urban)*	749 stomachs	Insects occurred often; 9.6% samples (n = 72.63). Insects and larvae contributed [11-12% ID] during summer and autumn, [Annual mean = 9.7% IB]. No further detail given.
Badger *meles meles*		
Shepherdson et al., (1990) *Ireland*	192 scats	Insects occurred in 45% samples (n=86.4) accounted for [~20%IB]; (n=38.4) during May and June, [~15%]; (n=29) during July and August. (figures estimated from graph). Described as “...mainly dipteran larvae and dung beetles, (eg. *Geotrupes* spp.)” No mention of hymenopterans.
Sidorovich et al., (2011) *Belarus*	1188 scats	In May, insects (all imago beetles) accounted for [24.2% IB] (67.4% of samples). During summer and autumn, insects present in 50.3-70.1% of samples [23.1 to 31.3% IB]. States that “most of the insects found were beetles.” Unclear if *Bombus* would have been reported.
Cleary et al., (2009) Ireland	686 stomachs	Insects = 73% samples (n=500.78) Bees and wasps occurred in 3% (n=20.58) [1%IB]. June-August, bees and wasps remains occurred in 12% (n =9.24) of samples, accounting for an estimated [6.5 % IB] of badgers summer diets (n=77 summer badgers.) Data not separated by bees and wasps.

Study and country	Sample size and method	Invertebrates/Insects (n or FO%) [% Ingested Bulk]
Kruuk and Parish 1981) *Scotland*	2159 faeces	Insects occurred in >80% (n=1727.2). Bumblebees occurred in 6.1% of samples (n= 132) [0.8% IB] Wasp remains occurred in only 0.4. (n=8.64).
Goszczynski et al. 2000 *Poland*	488 scats	Insects occurred frequently, (83-96% samples) but in small quantities, 1-3% I.B. Beetles (Carabidae and Goetrupes made up by far the greatest proportion of insects. From May to November badger dug out and consumed nests of wasps: 21.2% scats and accounting for 0.5% of biomass. It seems likely that other hymenopterans would have been reported in this study if relevant.
***Mustela ermine* (stoat)**		
Day (1968) *UK*	168 stoat stomach and gut	Insects in 6 (3.5%): Insects included blow-fly larvae, blow-flies, earwigs, midges and beetles. Suggests that the blow-flies and larvae may have been ingested with carrion. Non dipteran remains found in stomachs with birds and insectivores, suggesting they may have been accidentally consumed. Carabid beetle remains found in winter. No hymenopterans.
McDonald et al., 2000) *UK*	789 stoat gut and stomach	Insect material occurred in 8 stoats (1.0%). Insects included blow-fly larvae, blow-flies, earwigs, midges and beetles. No hymenopterans.
***Mustela nivalis* (weasel)**		
Day (1968) *UK*	152 weasel stomach and guts	Insects in 6 (4.0%).
Tapper (1979) *UK*	687 weasel guts	Insects occurred in 9 of the 687 No further detail given.
McDonald et al., 2000) *UK*	458 weasel gut and stomach	Insects present in 7 weasels (1.5%)

Study and country	Sample size and method	Invertebrates/Insects (n or FO%) [% Ingested Bulk]
***Clethrionomys glareolus* (bank vole)**		
Watts (1968) *UK*	143 stomach and gut	Lepidopteran larvae (1) Earth worm (1) No bumblebee remains
Flowerdew and Gardner (1978) *UK*	44 stomach and guts	1% of diet in the month of July was insects No bumblebee remains
***Apodemus sylvaticus* (wood mouse)**		
Watts (1968) *UK*	234 stomach and gut	Traces of chiatin throughout year. Lepidopterous larvae, a beetle, a harvestman (Phalangida) and an earthworm were found. No bumblebee remains
Flowerdew and Gardner (1978) *UK*	6 stomach and guts	20% of wood mouse diet was insects No further information given ~ no identifiable remains.
***Talpa europaea* (mole)**		
Funmilayo 1979 *Scotland*	135 (March-August) and a further 46 in January	Invertebrates included insect larvae; Elaterid, Dolichopodid and Carabid larvae; earthworm cocoons, slugs and centipedes. Adult insects included, Empididae, Formicidae, Muscidae, Noctuidae, Rhaginonidae, Tipulidae, Earthworms, slugs and Centipedes (goephilidae). No bumblebee remains
Castien and Gosalbez (1999) *Spain,* *Western Pyrenees*	73 stomach and guts	Invertebrates occurred, mainly Diptera larvae, oligochaetes, (mostly medium or large sized Lumbricidae) and Coleoptera larvae. No bumblebee remains

Study and country	Sample size and method	Invertebrates/Insects (n or FO%) [% Ingested Bulk]
Erinaceus europaeus **(hedgehog)**		
Yalden, 1976 UK	177 stomach	Invertebrates common. *Bombus* found in >3, <14 stomachs and contributed [2.7% IB]. Two stomachs each contained 13 adult *Bombus* and an additional 15 and 29 cocoons.
Jones et al., (2005) New Zealand	193 stomach	Hymenopter an 42% (n = 192 samples). Suggestion of nest raiding as large numbers of bumblebees sometimes found in individuals, e.g 40 *Bombus* legs in one stomach.
Sorex minutes **(pygmy shrew)**		
Castien and gosalbez (1999) *Spain, Western Pyrenees*	31 stomach and gut	Diet contained Myriapoda, Araneae, Opiliones, adult Coleoptera, Hemiptera and Lepidoptera larvae No bumblebee remains
Neomysfodiens **(water shrew)**		
Castien and Gosalbez (1999) *Spain, Western Pyrenees*	51 stomach and gut	The following species were eaten in high proportions: Diplopoda, Araneae, Opiliones, adult Coleoptera, larvae of Diptera, Tricoptera and Plecoptera. No hymenopterans