The Tree Bumblebee, *Bombus hypnorum*: ecology and genetics of a naturally colonising pollinator

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Thesis Abstract

Bumblebees are essential pollinators but, worldwide, many species are declining. The Tree Bumblebee, *Bombus hypnorum*, is a notable exception in that, having been first recorded in the UK (in southern England) in 2001, it has since rapidly spread to become common in much of England, Wales and Scotland. In this thesis I therefore investigated the ecology and genetics of *B. hypnorum* in the UK to better understand the factors underlying ecological success in bumblebees as a whole. In **Chapter 2**, I used biological recorder data to model and estimate *B. hypnorum*'s dispersal kernel. I found evidence for leptokurtic dispersal, with most queens dispersing a relatively short distance (mean, 4.3 km) but a few dispersing much further (e.g. 1% dispersing up to 23.9 km). In **Chapter 3,** I used a panel of neutral genetic markers (microsatellites) to investigate the demographic history of a representative UK *B. hypnorum* population. I found no evidence for a recent population bottleneck, suggesting that, rather than being the product of a single, chance event, *B. hypnorum*'s colonisation of the UK may be better explained by continuous migration from continental Europe. In **Chapter 4**, I used the same marker set to reconstruct the colony membership of workers sampled from a landscape in two successive years and to estimate the mating frequency of queens. This revealed notably short colony-specific worker foraging distances (mean, 103.6 m), high, variable nesting densities and a mean frequency of 1.7 matings per queen. In **Chapter 5,** I investigated the foraging ecology of *B. hypnorum* in the field and found that an absolute advantage in efficient flower handling and not low flower constancy ('generalism') may be contributing to its ecological success. Overall, these results greatly increase our understanding of the mechanisms by which bumblebees achieve ecological success and hence should help inform their conservation.

Table of Contents

Authorial contributions

Chapter 2: Tree Bumblebee queens colonise new sites by leptokurtic dispersal: long range colonisations occur at high frequency

Andrew Bourke, Claire Carvell, Liam Crowther, Gary Powney and Nick Isaac designed the study.

LC constructed the models and analysed the data.

LC wrote the manuscript.

AB, CC and NI contributed comments and edits to manuscript drafts.

Chapter 3: Tree Bumblebees (*Bombus hypnorum*) have colonised the UK without a severe genetic bottleneck

Andrew Bourke, Claire Carvell, Liam Crowther and David Richardson designed the study.

LC conducted the field and laboratory work.

LC analysed the data.

LC wrote the manuscript.

AB, CC and DR contributed comments and edits to manuscript drafts.

Chapter 4: Mating system, worker foraging distance and nest density of the Tree Bumblebee (*Bombus hypnorum*) in its recently expanded UK range

Andrew Bourke, Claire Carvell, Liam Crowther and David Richardson designed the study.

LC conducted the field and laboratory work.

LC analysed the data.

LC wrote the manuscript.

AB, CC and DR contributed comments and edits to manuscript drafts.

Chapter 5: Efficient foraging contributes to the ecological success of the Tree Bumblebee, *Bombus hypnorum*, a naturally colonising insect pollinator

Andrew Bourke, Claire Carvell, Liam Crowther and Elliot Reynolds designed the study.

LC and ER conducted the field work.

ER captured the data from the digital films.

LC analysed the data.

LC wrote the manuscript.

AB, CC and ER contributed comments and edits to manuscript drafts.

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Chapter 1: General Introduction

1.1 Background

Bumblebees, *Bombus* spp., are key pollinators of many wild plants (Ollerton, Winfree & Tarrant 2011) and economically important crops (Garratt *et al.* 2014). Along with those of other insects, their pollination services support food security (Klein *et al.* 2007) and contribute to around 10% of global agricultural production (Gallai *et al.* 2009). However, across both Europe and North America, many bumblebee species are declining (Williams & Osborne 2009; Potts *et al.* 2010; Cameron *et al.* 2011; Gill *et al.* 2016). Because of limited long-term data on their populations, such declines are typically inferred when contractions of species' ranges have been observed (Vanbergen *et al.* 2013). More recently, in the UK, data generated from standardised transect counts as part of a volunteer-led bumblebee monitoring scheme ('BeeWalk'), are beginning to reveal population trends. These suggest a declining trend for total bumblebee abundance in the UK between 2010 and 2016, with more species showing localised decreases than increases in numbers (Comont & Dickinson 2017).

The Tree Bumblebee, *Bombus hypnorum*, is a notable exception with respect to other bumblebee species in that is it expanding its range and also in that there are good long-term data on the year-by-year extent of its range expansion (BWARS 2017; Comont & Dickinson 2017). In particular, in recent years, *B. hypnorum* has colonised the United Kingdom and has undergone a rapid expansion of its new British range since it was first recorded in southern England in 2001 (Goulson & Williams 2001). In fact, in just 16 years *B. hypnorum* has expanded its range by 900 km and now occurs throughout all of England and Wales and in much of Scotland (Bees Wasps and Ants Recording Society 2017). This represents an average rate of range expansion of 56 km yr^{-1}. Offshore islands have also been colonised, presumably in secondary colonisation events originating from the mainland UK landmass. For example, *B. hypnorum* has now been recorded in the Scilly Islands, the Isle of Man and the Western Isles of Scotland (Bees Wasps and Ants Recording Society 2016).

B. hypnorum has a very large Palaearctic distribution, which extends from Western Europe in the west to Japan in the east, and from the Kola Peninsula in Arctic Russia in the north to the Himalayan Mountains in Nepal in the south (Goulson & Williams 2001). Throughout its Palaearctic distribution, *B. hypnorum* occupies a wide range of biotopes. In pristine habitats it is associated with boreal forests in the north and montane forests in lower latitudes, and it is reportedly absent from steppe environments (Goulson & Williams 2001; Rasmont & Iserbyt 2013). Very little quantitative data on *B. hypnorum*'s use of pristine habitats are available but, consistent with these associations, the presence of *B. hypnorum* is predicted by length of boreal forest edges in Estonian populations (Sepp *et al.* 2004).

Limited data point to a previous expansion of *B. hypnorum* into westerly maritime parts of continental Europe, specifically increases in abundance relative to other *Bombus* species in Belgium and observations of *B. hypnorum* in sites in north-western France where it was confirmed as absent in the 1980s (P. Rasmont, pers. comm.; Rasmont 1989). In western continental Europe, *B. hypnorum* can be found as far north as the tree line in northern Norway and as far south as the Pyrenees and coastal sites in northern Spain (Rasmont & Iserbyt 2013). In fact the earliest records of *B. hypnorum* ever made, when the species was first described, were of specimens collected by Linnaeus in Uppsala, Sweden (Linnaeus 1758). Generally, the Western European range of *B. hypnorum* suggests

that its climatic tolerance, at least in terms of latitude, encompasses a range of biomes and includes sites that are both colder and hotter than almost all of the UK landmass. Therefore, the expansion of *B. hypnorum* into Western Europe has been primarily an expansion westwards and its expansion northwards having reached the UK is likely to represent mainly an artefact of the north-south orientation of the UK landmass.

Across a habitat gradient typical of southern lowland parts of its UK range, the observed abundance of *B. hypnorum* on transects, in contrast that of most other widespread *Bombus* species, has a strong, positive association with suburban and woodland landscapes (Crowther, Hein & Bourke 2014). Compared to the same set of widespread *Bombus* species, it also has an 'early' phenology, in that within each season it appears to found colonies and produce sexuals earlier than all widespread common social (non-parasitic) *Bombus* species found in the UK, with the exception of *B. pratorum* (Benton 2006; Crowther *et al.* 2014; Comont & Dickinson 2017).

In *Bombus* species worldwide, range contractions have been correlated with 'late' phenology, small climatic range and proximity to range edge (Williams 2005; Williams, Araújo & Rasmont 2007; Williams, Colla & Xie 2009; Williams & Osborne 2009). Additionally, phylogenetic analyses suggest that *Pyrobombus*, the subgenus to which *B. hypnorum* belongs, appears to be the subgenus least susceptible to declines when compared with other *Bombus* subgenera (Arbetman *et al.* 2017). Therefore, *B. hypnorum*'s recent ecological success appears to match a larger pattern within *Bombus* as a whole. Nonetheless, given our depth of knowledge of the year-by-year changes in the pattern of *B. hypnorum*'s UK range expansion (see following section), it remains of considerable interest to investigate what features have potentially contributed to this expansion.

Other bumblebee species have also exhibited range expansions, typically when they have been transported for use as pollinators of agricultural crops. *B. hortorum, B. ruderatus, B. terrestris* and *B. subterraneus* were all introduced to New Zealand in the 1880s for this purpose, but there are few historical data on how quickly they colonised the archipelago (Macfarlane & Gurr 1995). *B. terrestris* has similarly been introduced to South America and Japan for crop pollination (Torretta, Medan & Arahamovich 2006; Yokoyama & Inoue 2010; Schmid-Hempel *et al.* 2014). In South America, the invasion front of *B. terrestris* was reported to have moved at 200 km yr^{-1} (Schmid-Hempel *et al.* 2014), whereas in Hokkaido, Japan, the same species has taken 11 years to expand its range by approximately 200 km, which would represent a slower rate of expansion of 18 km yr^{-1} (Kadoya & Washitani 2010). Hence, at 56 km yr-1, *B. hypnorum*'s observed rate of range expansion in the UK falls within the spread of values of other such rates for which data exist. Again, however, *B. hypnorum* in the UK remains a striking example of rapid range expansion and so one deserving of further investigation.

1.2 Aim of the thesis

In light of this background, the central aim of this thesis is to use the rapidly-expanding UK population of *B. hypnorum* as a study system from which we can learn about aspects of the ecology and genetics of bumblebees that contribute to the ecological success of their populations and hence that will potentially be of use in conserving them and their ecosystem function as pollinators. At the same time, because of the status of *B. hypnorum* in the UK as a remarkable case of rapid natural colonisation, the thesis aims to shed light on some general issues in invasion ecology, such as the

genetic paradox of invasion (Allendorf & Lundquist 2003)ludquist. Accordingly, each of the four data chapters uses a different data modelling, genetic or field observational approach to investigate specific elements of the colonisation, population genetics and foraging ecology of *B. hypnorum* that may have contributed to its success in the UK. Here, we first describe the characteristics of the species and existing available datasets that make it suitable for these different approaches.

The data modelling approach taken in this thesis is feasible because the active and organised community of amateur natural history recorders in the UK has provided distributional data on the UK's Aculeate Hymenoptera, including *B. hypnorum* and other *Bombus* species, with a very high level of spatial and temporal precision (Bees Wasps and Ants Recording Society 2016). Similar data are not available for other cases of range expansion in bumblebees. Indeed, the BWARS data represent the finest-resolution, wide scale distributional dataset for a range-expanding bumblebee population ever collected. Therefore, among other aspects, the colonisation of the UK by *B. hypnorum* offers a special opportunity to study the dispersal distances over which bumblebee queens can colonise new sites. This is a particularly important aspect of bumblebee ecology for three reasons. First, as many *Bombus* species that are now range-restricted exist in fragmented populations, the maintenance of genetic diversity in these populations may depend on gene flow across the matrix of unsuitable surrounding habitat (Darvill *et al.* 2006; Ellis *et al.* 2006; Jha & Kremen 2013; Jha 2015). Knowledge of queen dispersal distance will therefore aid in understanding the spatial scale over which such gene flow can occur. Second, some *Bombus* species ranges have been shown to be climatically structured and, if so, localised climatic changes under future scenarios of widespread environmental change may causes ranges to shift substantially (Williams *et al.* 2007; Hoiss *et al.* 2012; Kerr *et al.* 2015). The colonisation of new ranges by *Bombus* populations is likely to be limited by dispersal, so robust estimates of the dispersal capabilities of wild populations would again represent information useful to conservationists. Third, understanding the dispersal capabilities of queens will help inform researchers' understanding of those populations of bumblebees that, in various parts of the world, have become invasive and undergone range expansion following transportation.

The UK *B. hypnorum* population is also of particular interest because it has the potential to inform our understanding of how genetics and ecology can interact during colonisation events and range expansions. Previous studies have suggested that the *B. hypnorum* population in the UK has very low genetic diversity and have concluded that this is the result of founder effects following an extreme demographic bottleneck in the course of the colonisation of the UK landmass (Jones & Brown 2014). This would mean that the ecological success of the UK population has occurred despite severe genetic load. A similar phenomenon has been confirmed in *B. terrestris* in its invasive range in Tasmania (Schmid-Hempel *et al.* 2007). In general, the success of an invader despite genetic load is known as the ‘genetic paradox of invasion’, with the UK population of *B. hypnorum* having been cited as a premier example (Schrieber & Lachmuth 2017). However, whether *B. hypnorum* really did experience a severe genetic bottleneck during its colonisation of the UK has not been firmly established.

In recent years, genetic approaches have allowed significant progress to be made towards understanding the previously cryptic spatial ecology of bumblebees (Woodard *et al.* 2015). An important finding of this research is that bumblebee worker foraging distance, the distance that workers fly from their nest to forage at plants for nectar and pollen, is plastic with respect to resource availability (Carvell *et al.* 2012; Jha & Kremen 2013; Redhead *et al.* 2016). In addition we

know that *Bombus* nesting density can vary both across species and across populations within species (Knight *et al.* 2009; Charman *et al.* 2010). Therefore both worker foraging distances and the nesting densities of *B. hypnorum* in its new UK range are of interest to the central goals of this thesis. Potentially the most informative technique for estimating these parameters involves censusing of colony numbers and reconstruction of colony memberships by inferring sisterhoods among sampled workers using neutral genetic markers. However, unlike the queens of most bumblebee species, *B. hypnorum* queens are facultatively polyandrous (Estoup *et al.* 1995), hence reconstructing their colonies requires the reconstruction of networks of half-sisters rather than full sisterhoods. Therefore estimating worker foraging ranges and the nesting density of *B. hypnorum* necessarily requires information on the mating frequency of *B. hypnorum* queens.

Understanding the foraging ecology of bumblebees is critical because changes in forage plant abundances are thought to be driving population declines in many species (Carvell *et al.* 2006; Knight *et al.* 2009) and because their ecosystem function as pollinators depends on interactions with their forage plants. For this reason understanding the role of foraging ecology in the ecological success of *B. hypnorum* is key to goals of this thesis.

Taken as a whole, this thesis makes novel contributions to our understanding of the ecology and genetics of bumblebee populations at local landscape scales and to our understanding of meta-populations at the scale of hundreds of kilometres.

1.3 Specific objectives

Given the central aim of the thesis, its specific objectives as follows:

In Chapter 2, we used the BWARS dataset for the *B. hypnorum* range expansion in the UK over the period 2001 – 2013 and fitted a dynamic occupancy model to these data, to quantify the dispersal capabilities of *B. hypnorum* queens and characterise the shape of the distribution of their dispersal distances.

In Chapter 3, we used a panel of microsatellite markers to test hypotheses relating to the population-genetic history of *B. hypnorum* in the UK. Specifically, we tested for evidence of a demographic and genetic bottleneck to investigate whether the colonisation of the UK by *B. hypnorum* occurred as an event involving a single, small founding population or whether it has occurred via continuous migration, as part of a general westwards expansion of the species across Europe.

In Chapter 4, we used the same microsatellite panel to reconstruct the colony memberships of *B. hypnorum* workers collected over 2 years in a suburban landscape assumed to be typical of the habitats which are acting as sources for the colonisation of the UK. We used the data to estimate colony-specific foraging distances of workers, nesting densities and mating frequencies of queens.

In Chapter 5, we conducted a comparative field study of the flower-handling times of workers of *B. hypnorum* and other *Bombus* species to investigate whether foraging efficiency contributes to the ecological success of *B. hypnorum* in the UK.

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Chapter 2: Tree Bumblebee queens colonise new sites by leptokurtic dispersal: long-range colonisations occur at high frequency

Abstract

Bumblebees are globally important pollinators both of wild plants and agricultural crops. However, some bumblebee species have been introduced outside their native ranges, others are subject to habitat fragmentation and many may be sensitive to climate-induced range shifts under likely scenarios of environmental change. Estimating the distances over which bumblebee queens can disperse and either join other populations as migrants, or found new populations, is therefore of great importance for their successful conservation. To date, some estimates of queen dispersal distances have been published based on individual life-history measurements. However, consistent with Reid's Paradox (that biogeographic data suggest organisms disperse further than they appear able to), these are likely to be underestimates of the actual distances travelled by the relatively small subset of individuals that colonise new sites or migrate between populations. Coupled with the results of dispersal models and empirical evidence from numerous other taxa, this means we should expect the colonisation probability to decay with increasing isolation from a source population with a leptokurtic ('fat-tailed') shape rather than with the Gaussian (half-normal) shape expected under diffusion dispersal. In the current study, for the first time, we estimate a year-to-year dispersal kernel for a bumblebee. We focus on *Bombus hypnorum*, the Tree Bumblebee, a recent natural colonist of the UK the spread of which has been recorded with high spatial and temporal precision. Using a Bayesian occupancy-detection framework, we found that *B. hypnorum* has spread within the UK via leptokurtic dispersal and that, although queens dispersed a mean distance of 4.31 km, 1 in 20 queens are likely to have dispersed 14.4 km or more and 1 in 100 queens are likely to have dispersed 23.9 km or more. The finding that *B. hypnorum* queens have a leptokurtic rather than Gaussian dispersal kernel partially explains the rapidity of the recent range expansion of the species in the UK. Our results also have implications for our understanding of both fragmented populations of other *Bombus* species and introduced *Bombus* populations. This is because they suggest that the population-level productivity of new queens may more strongly influence the distances over which populations can exchange migrants and colonise new sites than would be expected under diffusion dispersal.

2.1 Introduction

Dispersal, the spatial displacement of individuals or propagules between generations, is an important ecological process that is highly relevant to some of the key challenges facing conservationists today. Many species' ranges are shifting due to climatic changes (Parmesan & Yohe 2003) and dispersal is the process that allows newly suitable sites to be colonised. Some invasive species have well documented impacts (Pimentel, Zuniga & Morrison 2005) and their ability to spread following translocation over biogeographic barriers is dependent on dispersal (Sakai *et al.* 2001). Dispersal ability also mitigates potential negative effects of spatial isolation due to habitat fragmentation ('rescue effects') and is therefore widely recognised as a key determinant of meta-population success in fragmented populations (Hanski & Ovaskainen 2000).

Reconciling the apparent dispersal ability of individual propagules to the actual rate of range expansion in natural populations has been a long-standing ecological challenge. In 1899 the naturalist Clement Reid measured the displacement of seeds and concluded, in what was subsequently known as Reid's Paradox, that many of the plant species found in Britain ought to have taken millions, rather than thousands, of years to reach their current distributions from their Ice Age refugia in Southern Europe (Reid 1899). It is this fundamental mismatch between estimates of dispersal ability using the measured life-history traits of organisms and their propagules, and the actual rates of range expansion observed in biogeographic records, that dispersal theory has sought to address (Skellam 1951).

Part of this mismatch could conceivably stem from a sampling issue, as finite-area studies where the potential dispersal distances of some individuals take them beyond the sampled area, should be expected to yield underestimates by under-sampling extreme long-distance dispersers (Koenig, Van Vuren & Hooge 1996). Conceptually, a separate, biological reason we might observe such a mismatch is that the propagules which colonise new sites are those that disperse much greater distances than the average propagule. A quantitative solution to this problem is to model the dispersal of propagules using a dispersal kernel, a probability distribution that describes the displacement of propagules in space. The classical model, in which the colonisation of new sites is directly based upon the random diffusion of individuals, i.e. based on measurable population-level life-history traits, produces a dispersal kernel with a Gaussian, i.e. half-normal shape (Skellam 1951). This is because, if we assume that propagules beginning at a fixed origin move randomly in space, i.e. by diffusion or a random-walk process, then their final density in space, in any given direction from the origin, will take a Gaussian half-normal shape. But, in order to make the tails of this type of kernel reach the distances at which propagules must have regularly moved, given the rate of range expansion, one must assume that the average propagule must have moved distances much greater than those ever observed. However, if we further assume that a relatively small subset of propagules move much further, perhaps due to their own behaviour or external stochastic events, and it is predominantly this subset that colonises new sites, then a leptokurtic or 'fat-tailed' dispersal kernel might better describe the rate of range expansion (Clark *et al.* 1998). Treating dispersal in this manner has been shown to reconcile Reid's estimates of seed dispersal distances and the rate of post-glacial advance in Western European flora measured by fossil pollen deposits (Clark 1998). Furthermore, large-scale modern-day patterns of genetic structure provide evidence for this mode of dispersal across a broad suite of vertebrate, invertebrate and plant taxa, as they are best explained by leptokurtic dispersal following the last Ice Age (Hewitt 1996, 2000; Ibrahim, Nichols & Hewitt 1996).

Bumblebees, *Bombus* spp., are important pollinators of many wild plants (Ollerton, Winfree & Tarrant 2011) and economically important crops (Garratt *et al.* 2014). Along with those of other insects, their pollination services support food security (Klein *et al.* 2007) and account for around 10% of global agricultural production (Gallai *et al.* 2009). However, across both Europe and North America, many bumblebee species are declining (Williams & Osborne 2009; Potts *et al.* 2010; Cameron *et al.* 2011; Gill *et al.* 2016). The dispersal abilities of bumblebees are of particular interest for several reasons. Firstly, *Bombus* species distributions have been shown to be linked to climatic gradients and hence could be expected to change, including by colonisation of new sites, under likely scenarios of climatic change (Williams, Araújo & Rasmont 2007; Hoiss *et al.* 2012; Kerr *et al.* 2015).

Secondly, multiple species of bumblebee have been introduced outside their native ranges and subsequently colonised new ranges via dispersal and successful establishment (Schmid-Hempel *et al.* 2007; Madjidian, Morales & Smith 2008; Kadoya & Washitani 2010; Goulson *et al.* 2011), so the potential of bumblebees as invasive species needs to be understood. Lastly, many species of bumblebee are restricted to a narrow range of suitable habitats that may be fragmented by a matrix of unsuitable habitat, and for these species dispersal between isolated populations is necessary to prevent inbreeding and maintain levels of genetic diversity (Darvill *et al.* 2006; Ellis *et al.* 2006; Jha & Kremen 2013; Jha 2015).

Bumblebees have an annual colony cycle, the dispersal phase of which is predominantly via young queens either before or after hibernation. Dispersal distances of queen bumblebees are, however, extremely difficult to observe directly. The maximum observed dispersal distances of two species in the UK (*B. pascuorum* and *B. lapidarius*), obtained using genetic markers to assign sampled queens to colonies whose workers were sampled in the preceding year, were 3 and 5 km respectively (Lepais *et al.* 2010). These estimates are widely cited and have been valuable for hypothesis formation and informing study design (e.g. Goulson *et al.* 2011; Jha & Kremen 2013; Dreier *et al.* 2014; Jha 2015) and interpreting results (e.g. Hagen *et al.* 2011; Lozier *et al.* 2011; Francisco *et al.* 2016; Bartlett, Hale & Hale 2016). More recently, Carvell *et al.* (2017) estimated the mean minimum dispersal distances of queens of three species (*B. lapidarius, B. pascuorum* and *B. terrestris*) as 1.2 km.

Given that resampling related queens at greater distances necessitates sampling across much greater areas (likely beyond the scale of 10 km), the above-cited observed dispersal distances are likely to be underestimates (Koenig *et al.* 1996). Accordingly, much of the literature treats the estimates of queen dispersal, including the individual-level maxima of Lepais *et al*. (2010), as proxies for population-level minima (Lozier *et al.* 2011; Jha & Kremen 2013; Jha 2015; Francisco *et al.* 2016). Leaving aside such sampling considerations, from Reid's Paradox we would expect these values, since these are estimates of the dispersal distances of individual propagules (queens), to be underestimates of the actual distances over which bumblebee populations can colonise new sites or exchange migrants. There is substantial and independent empirical evidence to support this assessment.

Firstly, from a population-genetic perspective, Dreier *et al*. (2014) estimated the degree of fine-scale spatial genetic structure between colony queens of five bumblebee species (*B. hortorum, B. lapidarius, B. pascuorum, B. ruderatus* and *B. terrestris*) and found either no isolation by distance or a very weak relationship at scales up to 10 km. This extent of genetic mixing at fine scales suggests that related queens must be dispersing over larger scales to produce a random pattern at the scale at which the study sampled the queens.

Secondly, the expansion of introduced bumblebee populations indicates the potential of queens to disperse to and colonise new sites. *B. terrestris* was introduced to South America in 1999, and although there is some uncertainty over the exact locations of all the initial release sites, it appears to have colonised an extensive new range with the invasion front moving at an average rate of 200 km yr^{-1} (Schmid-Hempel *et al.* 2014). Since its introduction to New Zealand in 1885, *B. terrestris* has colonised 28 offshore islands, including some separated by straits of over 30 km wide (Macfarlane & Gurr 1995).

Thirdly, bumblebee populations on volcanic islands, which have never been connected to continental land masses, also give an indication of queen dispersal distances realised at the extreme tips of dispersal kernels. For example, genetic analyses suggest that *B. terrestris* colonised the volcanic Atlantic island of Madeira independently of other Atlantic islands that may have otherwise acted as stepping stones. Instead, the founding queens are thought have dispersed across 630 km of ocean from North Africa rather than in steps over a series of straits, which would still have involved travelling across up to 400 km of open sea (Widmer *et al.* 1998). Finally, casual observations of bumblebee queens flying over sea straits, e.g. *B. lucorum* queens crossing the 80 km wide Gulf of Finland (Mikkola 1984), again suggest that queens are capable of flight over large expanses of sea.

In summary, the current evidence base regarding bumblebee queen dispersal represents a situation directly analogous to Reid's Paradox: direct estimates of queen dispersal suggest rates of range expansion orders of magnitude lower than must have occurred according to biogeographic evidence. Resolving this paradoxical situation requires data on rates of bumblebee range expansion and hence dispersal distances of queens with sufficient spatial and temporal resolution to make robust quantitative estimates. Unfortunately, such data have previously been lacking. One example of a recent bumblebee range expansion, that of *B. hypnorum*, the Tree Bumblebee, in the UK, has been captured with relatively high spatial and temporal resolution. *B. hypnorum* was first recorded near the southern coast of England in 2001 (Goulson & Williams 2001). It has since rapidly expanded its UK range, and can now be found throughout England and Wales, large parts of mainland Scotland and the Western Isles, the Scilly Islands and the Isle of Man (BWARS 2017). This constitutes a range expansion of approximately 900 km in 16 years. There is some evidence that suburban and woodland habitats may be facilitating the colonisation, as there are strong positive correlates of these landscape elements with observed abundances of *B. hypnorum* on standardised transects (Crowther, Hein & Bourke 2014). Throughout this range expansion, observations of *B. hypnorum* have been made by amateur entomologists and other natural history enthusiasts, and subsequently validated and curated by the Bees, Wasps and Ants Recording Society (BWARS 2017). In coat colour and pattern, *B. hypnorum* does not even superficially resemble any of the *Bombus* spp. native to the UK (Edwards and Jenner, 2005) and hence can be unambiguously recorded.

As in many large-scale biological recording schemes, BWARS records are observational and do not follow a systematic sampling approach. They consist of a minimum of: what was observed (a confirmed species record); where (a grid reference of at least 1 km^2 resolution); and when (the date on which the observation was made). A difficulty in modelling site-level presence/absence with observational data where we cannot assume perfect detection lies in determining how to separate true absences from false absences, i.e. how to separate truly unoccupied sites from occupied sites at which the focal species was not recorded. Occupancy models address this problem by modelling detection/non-detection conditional upon occupancy (MacKenzie *et al.* 2002).

Unstructured observational data like the BWARS data also have substantial unevenness in the spatial and temporal pattern of recording effort, potentially introducing further biases (Isaac & Pocock 2015). In recent years, a powerful refinement of occupancy modelling has been developed to address these problems, in which more complex occupancy models are fitted in a hierarchical Bayesian framework. This approach has been shown to be able to reliably detect species trends in occupancy over time despite unevenness in the spatial and temporal pattern of recording effort (Isaac *et al.* 2014). Hence, Bayesian occupancy models allow unstructured recorder data to be used

to infer changes in the pattern of occupied sites through time (van Strien *et al.* 2010; van Strien, van Swaay & Termaat 2013a; van Strien *et al.* 2013b; Kery *et al.* 2010; Isaac & Pocock 2015). In this implementation, records of other species recorded at the same site on the same recording visit are used to infer non-detections, and use the number of species recorded as a covariate with which to model the detectability of the focal species. Bayesian occupancy models have been used to calculate trends in range size across many species for taxonomic groups with suitable biological recorder data and for this reason formed a substantial part of the evidence incorporated in the UK State of Nature 2016 report (Hayhow *et al.* 2016) and in the compilation of UK Biodiversity Indicators 2015 (Isaac *et al.* 2015). These applications essentially estimate patterns of change in species range size, but a further refinement, Bayesian dynamic occupancy modelling, henceforth 'dynamic occupancy modelling', allows biological records to be used to estimate site-level changes, namely colonisation and local extirpation. Hence, dynamic occupancy models are now widely recognised as being highly suited to the analysis of biological records to test hypotheses relating to the ecological processes that underpin changes in species distributions (van Strien, van Swaay & Kery 2011; Woodcock *et al.* 2016).

In the current study, we used the BWARS *Bombus* data in a dynamic occupancy modelling framework to parametrise a dispersal kernel for *B. hypnorum* in the UK. This provided the first quantitative estimates of bumblebee year-to-year dispersal distances using biogeographic evidence rather than life-history observations. This work also presents the first inclusion of implicit spatial information (i.e. isolation distances) in an occupancy model fitted to unstructured biological recorder data. The study addressed the following research questions raised by the rapid spread of *B. hypnorum* in the UK: 1) Does the pattern of colonisation support leptokurtic rather than diffusion or random walk dispersal? 2) Over what distances can queens disperse and hence colonise new sites? 3) How does the inclusion of spatial information, in the form of a dispersal kernel, change the spatial pattern of estimated site-level occupancy of *B. hypnorum*?

2.2 Methods

Data source

Biological records of bumblebee species (*Bombus* spp.) in the UK collected and collated by BWARS are publicly available via the National Biodiversity Network (NBN) Gateway (The National Biodiversity Network 2017). All UK, excluding Northern Ireland, bumblebee records were downloaded from the NBN Gateway on 2014-04-05, representing a total of 99,538 records. Some records are in the form of weekly or monthly species lists and/or may only be recorded at the scale of a large area such as a 10 x 10 km 'hectad'. Therefore the records were filtered to include only those with the temporal precision of at least a day and the spatial precision of at least a 1 x 1 km grid square, hereafter 'site'; which left 92,979 records. Of these, we selected records spanning the years with known occurrence of *B. hypnorum* in the UK, 2001 until 2013 inclusive, which left 47,917 records. This dataset contained a mean (standard deviation) of 3,685.9 (1,709.8) records per year.

These records covered 25 bumblebee species and were made in 9,454 of the potential total of 250,000 one-km squares (hereafter 'sites') in the UK. In order to separate non-detections of *B.*

hypnorum from its genuine absence at a site, the records were grouped into 21,557 unique combinations of site and day, hereafter 'visits'. The number of separate bumblebee species recorded on the same visit was calculated for each visit and is hereafter referred to as 'list length'. The mean list length was 1.989 species per visit and lists ranged from 1 to 15 species. The species most frequently recorded were *B. pascuorum* (in 9,277 lists), *B. lapidarius* (in 6,190 lists) and *B. terrestris* (in 6,049 lists) and the species most infrequently recorded was *B. ruderatus* (in 153 lists). For each site in each year, we also calculated the 'isolation distance', the straight line distance to the closest site at which *B. hypnorum* had been recorded in any previous year.

We further filtered sites to include only those that had been visited in two or more separate years. This was because the dispersal processes that are the focus of the study act upon between-year changes in site-level occupancy, and hence sites visited in only one year have minimal information content for estimating relevant parameters. In this manner, the data-filtering employed maximised the per-record information content while including as much data as possible (Kamp *et al.* 2016). Overall, these steps resulted in a final dataset of 12,444 visits to 2,080 sites across the 13 years. Note that isolation distances were calculated from all 9,454 sites, i.e. using all the available information.

Description of the models

Two different occupancy models were fitted to the data, one intended to answer questions 1 and 2 and the second as a reference model for comparison to answer question 3. Both models use the Bayesian occupancy model framework of Kery & Schaub (2012). In this framework, the detection or non-detection of a focal species on a visit is a function of a detection sub-model, conditional on the unknown occupancy status of the site at the time of the visit. Both occupancy models in the current study used an identical detection sub-model. This detection sub-model used the visit-level list length to estimate the detectability of *B. hypnorum* on that visit. Unlike the case in other studies using similar occupancy models with biological recorder data, e.g. van Strien, van Swaay & Termaat (2013), our detection sub-model excluded effects of Julian date. Including Julian date as a covariate is intended to model seasonal changes in detectability of the focal species, for example those arising from phenological patterns of abundance. We excluded this term because, with BWARS records, including Julian date as a variable greatly increases computational requirements without substantially affecting the estimates of occupancy rates across sites (G. Powney, unpublished data).

The two occupancy models in the current study used different state sub-models to parametrise the changes in site occupancy across years. The first model was a dynamic occupancy model *sensu* Royle & Dorazio (2008) in which the underlying colonisation of unoccupied sites depends on a dispersal kernel *sensu* Clark *et al.* (1998), and is hereafter referred to as the 'dynamic dispersal model'. The second model was a static occupancy model which uses the previous year's rate of occupancy across all sites as a prior for that of the current year, and is hereafter referred to as the 'static model'.

The static model specification is optimised to inferring occupancy trends of rare focal species that are recorded at a low rate (Outhwaite *et al*., under review). This specification was chosen for the static model as, at least in the earlier years included in the model, *B. hypnorum* was recorded infrequently on only a small number of sites. Alternative specifications such as that used by van

Strien *et al.* (2010) produced estimates of the proportion of occupied sites with very high uncertainty for the years 2001-2006 (L. P. Crowther, unpublished data), which is unsurprising as it is already known that low recording rates present a challenge to occupancy models (Isaac *et al.* 2014).

Detection sub-models

In both models y_{ijt}, the detection or non-detection of *B. hypnorum* on visit *j* to a particular site *i* in a particular year *t* was a function of that visit's detection probability p_{ijt}, conditional upon z_{it}, the unknown binary 'true' occupancy status of that site in that year (Equation 1).

$$y_{ijt}|z_{it} = bernoulli(p_{ijt} \times z_{it}) \quad (1)$$

Again in both models, a visit's detection probability, p_{ijt}, was a logistic function of α_t, the annual logit probability that a single species list is a record of *B. hypnorum*, and of the visit's list length, expressed as a categorical factor where a 'short list' included 2-3 species and a 'long list' included 4 or more species (van Strien *et al.* 2013a). The parameters δ_2 and δ_3 estimate the difference in logit detection probability on short and long lists, respectively, relative to the detection probability on a list of length 1 (Equation 2). The unknown 'true' occupancy status, z_{it}, of the site in that year, is a function of an estimable probability Ψ_{it} (Equation 3).

$$Logit(p_{ijt}) = \alpha_t + \delta_2(short\ list)_{ijt} + \delta_3(long\ list)_{ijt} \quad (2)$$

$$z_{it} = bernoulli(\Psi_{it}) \quad (3)$$

All parameters in the detection sub-models were given uninformative priors, intended to express minimal information as to the value of the given parameter (Appendix 2.1).

Dynamic dispersal state sub-model

In the dynamic dispersal model, the probability that site *i* is occupied in year *t* when $t > 1$, Ψ_{it}, depends on that site's occupancy status in the previous year. If it was previously occupied, i.e. $z_{it-1} = 1$, then it will remain occupied with persistence probability Φ, which is constant across all sites and years. If it was unoccupied, i.e. $z_{it-1} = 0$, then it will become occupied according to the colonisation probability γ_{it} (Equation 4). When t = 1, i.e. in 2001, the initial occupancy rate of sites $\Psi_{initial}$, was estimated as an additional parameter.

$$\Psi_{it} = \phi z_{it-1} + \gamma_{it}(1 - z_{it-1}) \quad (4)$$

The colonisation probability of site *i* in year *t* is related to d_{it}, the isolation distance of that site in that year, by a dispersal kernel as specified by Clark *et al.* (1998). The dispersal kernel uses two parameters α_γ and *c* that affect the kernel's scale and shape, respectively (Equation 5). The kurtosis of the resulting distribution depends on *c,* such that, when $c = 2$, the distribution approximates a normal distribution, when $c = 1$, the distribution approximates an exponential distribution, and when $c < 1$, the kernel is leptokurtic. The gamma function is represented by Γ() (Equation 5).

$$\gamma_{it} = \frac{c}{2\alpha_\gamma \Gamma(1/c)}\ exp\left[-\left|\frac{d_{it}}{\alpha_\gamma}\right|^c\right] \quad (5)$$

The distance over which colonisations are likely to occur depends on both the scale and shape of the kernel and is proportional to α_Υ/c (Clark *et al.* 1998). The joint posterior distribution of α_Υ and c was used to calculate the mean dispersal distance, μ_d, as a derived parameter using the equation derived by Clark *et al.* (1998) (Equation 6).

$$\mu_d = \frac{\alpha\Gamma(2/c)}{\Gamma(1/c)} \quad (6)$$

In the dynamic dispersal model, one state sub-model parameter was given an informative prior; specifically, the initial occupancy rate of sites $\Psi_{initial}$ was assumed to be small as at that time *B. hypnorum* had only been recorded at one site in an intensely recorded part of the country. Therefore the prior distribution was specified using a truncated half-normal distribution with a low variance (Expression 7).

$$\Psi_{initial} \sim normal(\mu = 0, \sigma = 1/1000)\mathrm{T}(1,0) \quad (7)$$

To further quantify the dispersal distances of *B. hypnorum*, we calculated the cumulative density function of the dispersal kernel. For comparison with the results expected given diffusion or random walk dispersal, we generated a Gaussian dispersal kernel, with the same mean dispersal distance μ_d, but with $c_{NEW} = 2$. Since μ_d is proportional to α_Υ/c, this new kernel had $\alpha_{\Upsilon NEW} = 2\alpha_\Upsilon/c$.

Static state sub-model

In the static model, the probability site *i* is occupied in year *t,* Ψ_{it} is a logistic function of a yearly random intercept β_t and a random effect of site η_i (Equation 8).

$$Logit(\Psi_{it}) = \beta_t + \eta_i \quad (8)$$

The yearly random intercept β_t, in all years but the first (2002-2013), was given an informative prior of the effect in the previous year, β_{t-1} (Expression 9) with a half-Cauchy hyperprior for the precision across years (Expression 10). This, in effect, shares information on the proportion of occupied sites across years as it requires information from the data to infer large changes in the proportion of occupied sites between consecutive years (Outhwaite *et al*., under review).

$$\beta_t \sim normal(\beta_{t-1}, \tau_\beta) \quad (9)$$

$$\tau_\beta \sim t(\mu = 0, \sigma = 1, df = 1)T(0, \infty) \quad (10)$$

The random effect of site μ_i and the random intercept for year one (2001) β_1 were given uninformative priors (Appendix 2.1). The proportion of occupied sites when t = 1 (2001), $\Psi_{initial}$, was calculated as a derived parameter for comparison with the dynamic dispersal model.

Computational methods

All data handling and preparation of figures was carried out using R (R Development Core Team 2011) with extensive use of functions from, or adapted from, R packages 'sparta' (August *et al.* 2015) and 'BRCmap' (Harrower 2015). Both models were fitted using MCMc sampling with three chains, implemented using the Gibbs sampling program, OpenBUGS version 3.2.3.

The dynamic dispersal model was run for 64,000 iterations with 48,000 iterations as burn-in and the static model was run for 25,000 iterations with 15,000 iterations as burn-in. Convergence of all

parameters was tested using Gelman 'Rhat' diagnostic statistics between chains and Geweke diagnostic plots within chains. Chains of all parameters were thinned to every third iteration to avoid autocorrelation. It was not computationally practical to save estimates of the 'true' occupancy state z_{it} in this manner, given the large latent variable (2080 sites x 13 years x 28,000/3 iterations x 3 chains = 7.6 x 10^8 estimates of site-level occupancy). Therefore, once the results of the other parameters had been returned to R, the models were updated for a further 10,000 iterations with monitors set on z_{it} and the estimates, thinned to every tenth iteration, were saved directly. Gelman 'Rhat' diagnostic statistics were used to confirm that the other parameters in the updated run were not significantly different from those in the retained iterations of the original model runs. The R package 'bigmemory' (Kane, Emerson & Weston 2013) was used to extract estimates from the saved values.

2.3 Results

Both the dynamic dispersal and static models converged and produced posterior estimates for all of their parameters (Appendices 2.2, 2.3).

Question 1: Dispersal kernel kurtosis

The dynamic dispersal model produced a dispersal kernel with a leptokurtic shape (Figure 2.1), showing that *B. hypnorum* has colonised the UK by leptokurtic dispersal. The estimated colonisation probability per site per year (Υ_{it}) fell from 0.62 (credible interval, 0.35 – 0.81) at 1 km isolation distance to 0.064 (credible interval, 0.032 – 0.082) at 10 km and 9.43 x 10^{-8} (credible interval, 1.27x10^{-10} – 3.09 x 10^{-6}) at 100 km (Figure 2.1). The shape parameter of the dispersal kernel, C_Υ, was much lower than one (confidence, >99.99%; median estimate, 0.772; 95% credible interval, 0.662 - 0.878). Hence the alternative hypothesis that *B. hypnorum* has colonised the UK by diffusion or random walk dispersal can be rejected, as this hypothesis would predict C_Υ to be two or higher (Figure 2.2a).

Question 2: Dispersal distances

The dispersal kernel generated by the dynamic dispersal model predicts that the average propagule, i.e. a queen dispersing from one year to the next, travels a mean 4.31 km (credible interval, 3.45 – 5.35 km) (Figure 2.2b). As expected given the leptokurtic shape of the dispersal kernel, this means that we would expect most colonisations (i.e. successful establishment at new sites) to have been made by queens dispersing much greater distances (Figure 2.1). The quantiles of the dispersal kernel predict that 1 in 20 (95th percentile) colonisations were at distances greater than 14.4 km, 1 in 100 (99th percentile) were at distances greater than 23.9 km and 1 in 1000 (99.9th percentile) were at distances greater than 39.0 km.

A Gaussian dispersal kernel (i.e. one suggesting diffusion or random walk dispersal) with the same mean displacement μ_d, but c = 2, has a proportionally smaller increase in distance over the same quantiles, such that 1 in 20 colonisations (95th percentile) would be at distances greater than 10.0 km and 1 in 100 (99th percentile) at distances greater than 13.1 km. Hence, the observed leptokurtic dispersal kernel has a 99th percentile at 1.66 times greater distance than its 95th percentile (i.e. 23.9/14.4 = 1.66), whereas the Gaussian equivalent has a 99th percentile at only 1.31 times greater distance than its 95th percentile (i.e. 13.1/10.0 = 1.31).

Question 3, Effect of dispersal on estimated pattern of occupancy

Relative to the static model, the dynamic dispersal model predicted a very different pattern of change in occupancy across all sites through time. Overall, the dynamic dispersal model predicted a much smaller change in occupancy over the 13 years modelled, going from 23% of sites in 2001 to 39% of sites in 2013, whereas the static model predicts less than 1% of sites were occupied in 2001 and 84% in 2013 (Figure 2.2c; Appendices 2.2, 2.3).

However, the sites that are predicted as occupied by the dynamic dispersal model are predicted to be so with much higher certainty than those predicted as occupied by the static model (Figure 2.3). Very few sites were predicted by the static model to have a less than 0.5 chance of being occupied, whereas the majority of sites were predicted to be in this range by the dynamic model (Figure 2.2c). The sites that were predicted to be occupied by the static model include many beyond the recorded range boundary, as, for example, in the relevant time period (2001-2013) *B. hypnorum* had not been recorded in Scotland. The sites that the dynamic dispersal model predicted as being occupied were also predicted as being occupied by the static model (Figure 2.3). This suggests that the dynamic dispersal model is highly conservative and rarely predicts occupancy at sites where *B. hypnorum* was not recorded.

2.4 Discussion

In order to elucidate the distances over which bumblebee queens may be able to disperse and hence colonise new sites, we parametrised a dispersal kernel using a dataset of unstructured biological records of *B. hypnorum,* a new colonist of the UK, and other co-recorded bumblebees. We found evidence that the recent colonisation of the UK by *B. hypnorum* was underpinned by leptokurtic dispersal rather than diffusion (question 1). The dispersal kernel predicted an average *B. hypnorum* queen dispersal distance of 4.31 km (credible interval, 3.45 – 5.35 km), while the shape of the dispersal kernel suggested that many successful colonisations happened over much greater distances (question 2). Specifically, 5% of colonisations happened at distances over 14.4 km from the nearest known population, 1% at distances greater than 23.9 km and 0.1% at distances greater than 39.0 km (question 2). For comparative purposes, we also fitted a static occupancy model with an identically structured detection sub-model to the data and found that the inclusion of a dispersal kernel made the model highly conservative with respect to both the number of occupied sites and the predicted range size (Question 3).

This represents, to our knowledge, the first quantitative estimate of year-to-year bumblebee dispersal distances using biogeographic data rather than life-history measurements. This is noteworthy because there is a strong empirical case that estimates of dispersal distances that use life-history measurements greatly underestimate the distances over which organisms actually disperse to colonise new sites (Reid 1899; Clark *et al.* 1998; Clark 1998).

There is considerable evidence that leptokurtic dispersal has shaped the large-scale genetic structure of a broad range of North Temperate plant and animal taxa (Hewitt 1996, 2000; Ibrahim *et al.* 1996). Therefore, it is not surprising that we have found that *B. hypnorum* also has this pattern of dispersal. Previous work has shown that leptokurtic dispersal is capable of reconciling the rate of recolonisation of boreal biomes by their current flora following the last Ice Age, measured

biogeographically using pollen core data, with life-history measurements of seed dispersal distance (Reid 1899; Clark *et al.* 1998; Clark 1998). In the current study, we have demonstrated that in a similar manner it is possible, in bumblebees, to reconcile biogeographic data characterised by a fine spatial and temporal resolution with the best available estimates of dispersal based on life-history measurements (Lepais *et al.* 2010; Carvell *et al.* 2017).

Our estimates of the average queen dispersal distance of 4.35 km (credible interval, 3.45 – 5.35 km) are remarkably similar to the queen dispersal distances measured 'directly' from life-history measurements. Lepais *et al.* (2010) used genetic techniques to infer that queens of *B. pascuorum* and *B. lapidarius* could disperse 3 and 5 km, respectively. While these distances represent the individual level maxima of queen dispersal measured by Lepais *et al.* (2010), they are often cited as proxies for population level minima (Lozier *et al.* 2011; Jha & Kremen 2013; Jha 2015; Francisco *et al.* 2016). Since we have demonstrated that *B. hypnorum* queen dispersal follows a leptokurtic distribution, and this implies that colonisation and gene flow occur largely over distances greater than the average dispersal distance, this study provides support for interpreting the findings of Lepais *et al.* (2010) as population-level minima.

However, some studies cite the queen dispersal distances estimated by Lepais *et al.* (2010) as maximum year-to-year distances over which we might expect gene flow to occur. In this interpretation the individual-level maxima of Lepais *et al.* (2010) are effectively taken as proxies for population-level maxima. For example, Bartlett *et al.* (2016) reported that *B. ruderatus* gene flow in New Zealand is limited by habitat quality and that, since queens have been observed to disperse 3 and 5 km, the spatial connectivity of habitat at that scale is likely to explain the spatial genetic structure that the authors observed; they then inferred that longer-range gene flow is likely to occur as a 'stepping stone' process. The finding of the current study, that *B. hypnorum* has a leptokurtic dispersal kernel, cautions against such an interpretation and suggests that gene flow or colonisation of new sites by a minority of long-range dispersers may be relatively common.

The estimates of queen dispersal distances of both Lepais *et al.* (2010) and Carvell *et al.* (2017) are subject to sampling biases. While Carvell *et al.* (2017) generated much larger sample sizes and sampled continuously across the study landscape, both studies used finite sampling areas that are much smaller than the areas over which some queens are likely to disperse. While the current study is not subject to the same biases, there are factors which are likely to bias its estimation of dispersal distances. Firstly, since the BWARS dataset contained *Bombus* records for a small fraction of the sites that make up the UK landmass, on the one hand our estimates of 'isolation distance' are likely to have been overestimates of the 'true' distance to the nearest potential source population. On the other hand, our estimates of queen dispersal distances could be underestimates. Since the colonisation probability depends only on the 'isolation distance' and not on site-level variables such as habitat or climate, it could be that very long-range dispersers are more likely to disperse to less suitable 'climate space' and therefore be less likely to establish a local population.

The extent to which these results are representative of dispersal capabilities across other *Bombus* species requires examination. The estimates based on life-history measurements of Lepais *et al.* (2010) were for two species across an agricultural landscape in England, *B. pascuorum* and *B. lapidarius,* and are the estimates that have commonly been used to inform hypotheses or interpret results relating to a whole suite of other *Bombus* species (Goulson *et al.* 2011; Hagen *et al.* 2011;

Lozier *et al.* 2011; Jha & Kremen 2013; Dreier *et al.* 2014; Jha 2015; Francisco *et al.* 2016; Bartlett *et al.* 2016; Carvell *et al.* 2017). To our knowledge, there are no biogeographic data for any range-expanding *Bombus* population with levels of spatial and temporal resolution equivalent to those used in the current study. Therefore, it follows that we have no choice but to extrapolate across congeneric species if we want to use evidence to inform assumptions relating to *Bombus* queen dispersal distances.

In defence of this extrapolation, there is arguably not any unequivocal evidence that *Bombus* species differ in their propensity to disperse. Several studies have hypothesised that differences across species in individual-level propensities to disperse are a key trait with which to explain differences in species responses to habitat fragmentation (Darvill *et al.* 2006, 2010; Ellis *et al.* 2006; Goulson *et al.* 2011). While this may be case, the evidence presented essentially consists of different *Bombus* species having different levels of genetic isolation by distance over the same geographic barriers (e.g. Darvill *et al.* 2010). However, the genetic isolation of populations is a function of the absolute number of migrants per generation (Wright 1943). Therefore, if the number of migrants from population (A) to another (B) depends on not just the propensity of individuals in population A to disperse but also the rate at which potential migrants are produced in population A, then differences in genetic isolation by distance across *Bombus* species may actually be attributable to differences in the population-level rate at which new queens are produced.

Currently there are very few data on population-level variation in numbers of queens produced across bumblebee species in nature. However, even within the single species *B. terrestris*, the number of queens produced per colony varies across populations(Goulson *et al.* 2002; Lopez-Vaamonde *et al.* 2009; Whitehorn *et al.* 2012), as does the density of colonies in nature (Charman *et al*. 2010). In addition, the rate of bumblebee lineage survival, which is presumably strongly correlated with colony-level queen productivity, has been shown to vary across gradients in habitat quality (Carvell *et al.* 2017). Therefore, it follows that any estimates of dispersal distances, including those made in the current study, are potentially confounded by population-level queen productivity. For this reason any comparisons across species, biomes and habitat gradients should be made with caution. These caveats apply equally to the estimates based on life-history measurements of Lepais *et al.* (2010). This is because the differences they detected across the two species they studied could have been similarly confounded by differences in population-level queen productivity between the species. The cumulative density function of the dispersal kernel gives us a way of conceptualising the relationship of population-level productivity and the actual realised dispersal distances observed. The higher quantiles of the disposal kernel are more likely to be realised given a larger number of dispersers, and the increase in distance between the 95th and 99th percentiles is proportionally much larger for the leptokurtic dispersal kernel (95th, 99th; 14.4 km, 23.9 km) than the equivalent Gaussian kernel (95th, 99th; 10.0 km, 13.1 km). It follows that leptokurtic dispersal potentially makes the role of population-level productivity that much more important to understanding bumblebee habitat fragmentation and gene flow.

Similarly, the rapid range expansion of *Bombus* populations where they have been introduced outside their native ranges may likewise be a function of enhanced queen productivity in their introduced ranges. Schmid-Hempel *et al.* (2014) reported that *B. terrestris* may have increased its introduced range in South America by 200 km yr^{-1}. Assuming leptokurtic dispersal as demonstrated in *B. hypnorum* in the current study, such a rapid range expansion could be facilitated by the

population-level production of a very large number of new queens. This would result in a high number of potential dispersers, meaning that, at the population level, there would be an increase in the number of individually unlikely events of very long-range dispersals of hundreds of kilometres.

We found that including dispersal limitation in the state model in the form of a dispersal kernel and site-level occupancy dynamics made the occupancy predictions highly conservative. While the dynamic dispersal model predicted that fewer sites were occupied, they were predicted as such with greater certainty. It seems likely that some of the sites that were predicted as occupied by the static model but not by the dynamic dispersal model were actually occupied. If this were the case, then it is possible that the dynamic dispersal model increased the number of false negative detections. However, by 2013 *B. hypnorum* had not been detected in Scotland, so it seems unlikely that actually a large number of sites in Scotland were occupied, as predicted by the static model. Dispersal limitation can be a large component of the spatial autocorrelation that affects the estimation of species ranges (Dormann *et al.* 2007). Therefore including spatial information, perhaps in the form of a dispersal kernel or some other numerically simpler form, may be an effective way of accounting for spatial autocorrelation due to dispersal limitation in occupancy models fitted to unstructured recorder data. However, specific recommendations as to how to optimally include spatial information, including whether it necessarily requires the dynamic formulation of occupancy dynamics as in Equation 4, are beyond the scope of this study.

A limitation of the present study is that the dynamic dispersal model used the distance to the nearest site at which *B. hypnorum* had been recorded in previous years as a proxy for isolation by distance from potential source populations. In theory it is possible to specify a model in which dispersal kernels are fitted between each combination of occupied site and unoccupied site, in order to reflect the fact that dispersers may arrive at a location from multiple source populations. In this respect, such a model would be 'spatially explicit'. However, this approach would greatly increase the computational power needed to fit the model. In addition, studies that have specified such spatially explicit models to model the spread of invasive species have frequently had to make other potentially restrictive assumptions in order to do so. For example, a study by Kadoya & Washitani (2010) presented a spatially explicit model of the spread of introduced *B. terrestris* populations that are invasive in Hokkaido, Japan. This study had access to systematic survey data (as opposed to unstructured biological recorder data). Nonetheless, to construct a spatially explicit model, this study modelled presence or absence at a relatively coarse scale (10 x 10 km), did not account for imperfect detection and combined multiple years into two 'time periods'. These assumptions would not have been suited to either our research questions or our unstructured dataset.

A further caveat which has implications for the extent to which these results can be extrapolated to other *Bombus* species is that *B. hypnorum* may have a facultatively bivoltine colony cycle in the UK (Edwards & Jenner 2015). There are currently no structured census data for *B. hypnorum* with which we might estimate the prevalence of bivoltinism, and conceivably workers observed later in the season could be from later-founded colonies. However, even if it occurs, bivoltinism is almost certainly a trait shown by only some populations in some years, suggesting that its effect on our estimate of the dispersal capabilities of *B. hypnorum* queens is small.

In conclusion, the present study found evidence that *B. hypnorum* has colonised the UK by leptokurtic dispersal, so potentially adding to our understanding of bumblebee populations that are

fragmented and range-restricted, colonising new ranges or range shifting in response to environmental change. The findings underline the importance of long-term biological records data and the effort and expertise that go into collecting and collating them, as they further increase the range of important biological questions that unstructured biological records have been used to answer. Further research could aim to incorporate site-level variables such as land use in similar dynamic occupancy models to test whether the habitats that have higher densities of *B. hypnorum* are more readily colonised (Crowther *et al.* 2014).

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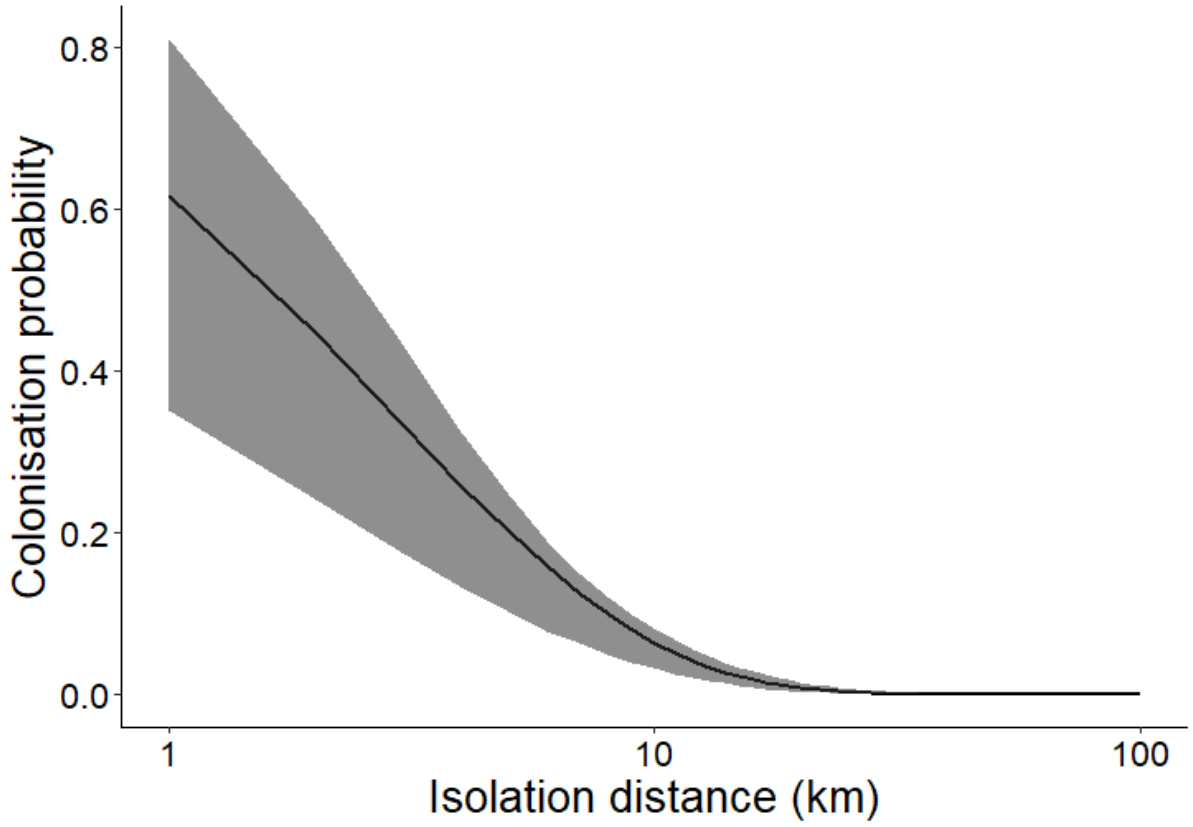

Figure 2.1. Posterior estimate of the dispersal kernel of *Bombus hypnorum* in the UK from the dynamic dispersal model, a Bayesian dynamic occupancy model fitted to biological records from 12,444 visits to 2,080 1 x 1 km sites over 13 years (2001 – 2013). Black line, median estimate; grey shading, 95% credible interval. Colonisation probability, annual probability that an unoccupied 1 km^2 site is colonised by *B. hypnorum*; Isolation distance, distance from nearest site with *B. hypnorum* recorded in a previous year.

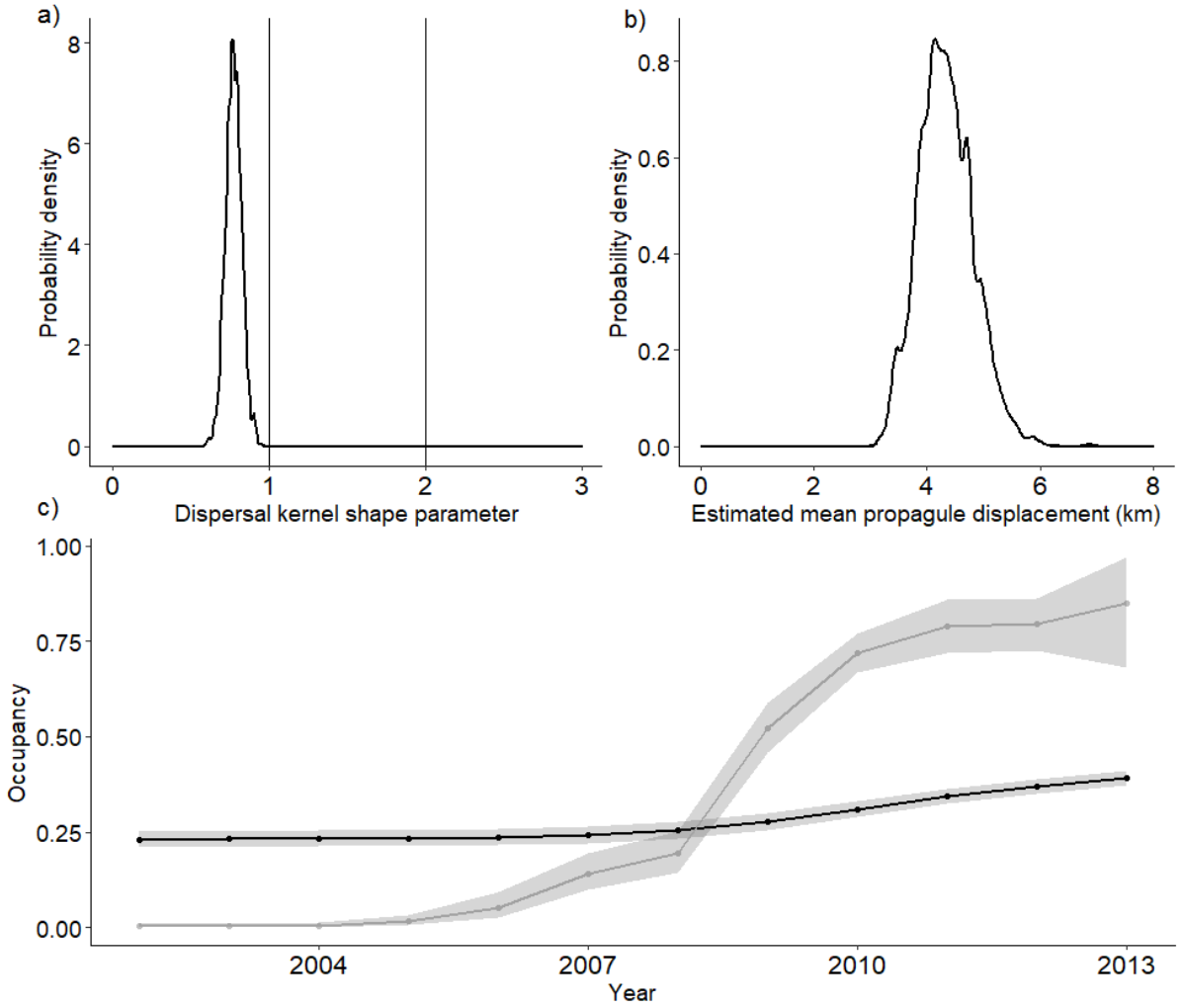

Figure 2.2. Posterior estimates of parameters and latent variables from Bayesian occupancy models predicting *Bombus hypnorum* occupancy, fitted to biological records from 12,444 visits to 2,080 1 x 1 km sites over 13 years (2001 - 2013). (a) Posterior density distribution of the shape parameter C_Y from the dynamic dispersal model; vertical lines indicate the expected value of C_Y if the dispersal kernel is an exponential distribution (x = 1) or a normal distribution (x = 2); values of C_Y below 1 indicate a leptokurtic distribution. (b) Posterior density distribution of μ_d, a derived parameter from the dynamic dispersal model giving the estimate mean distance over which *B. hypnorum* queens can colonise new sites from year to year. (c) Predicted proportions of occupied sites over time from the dynamic dispersal model (black line) and the static model (grey line); grey shading, 95% credible intervals.

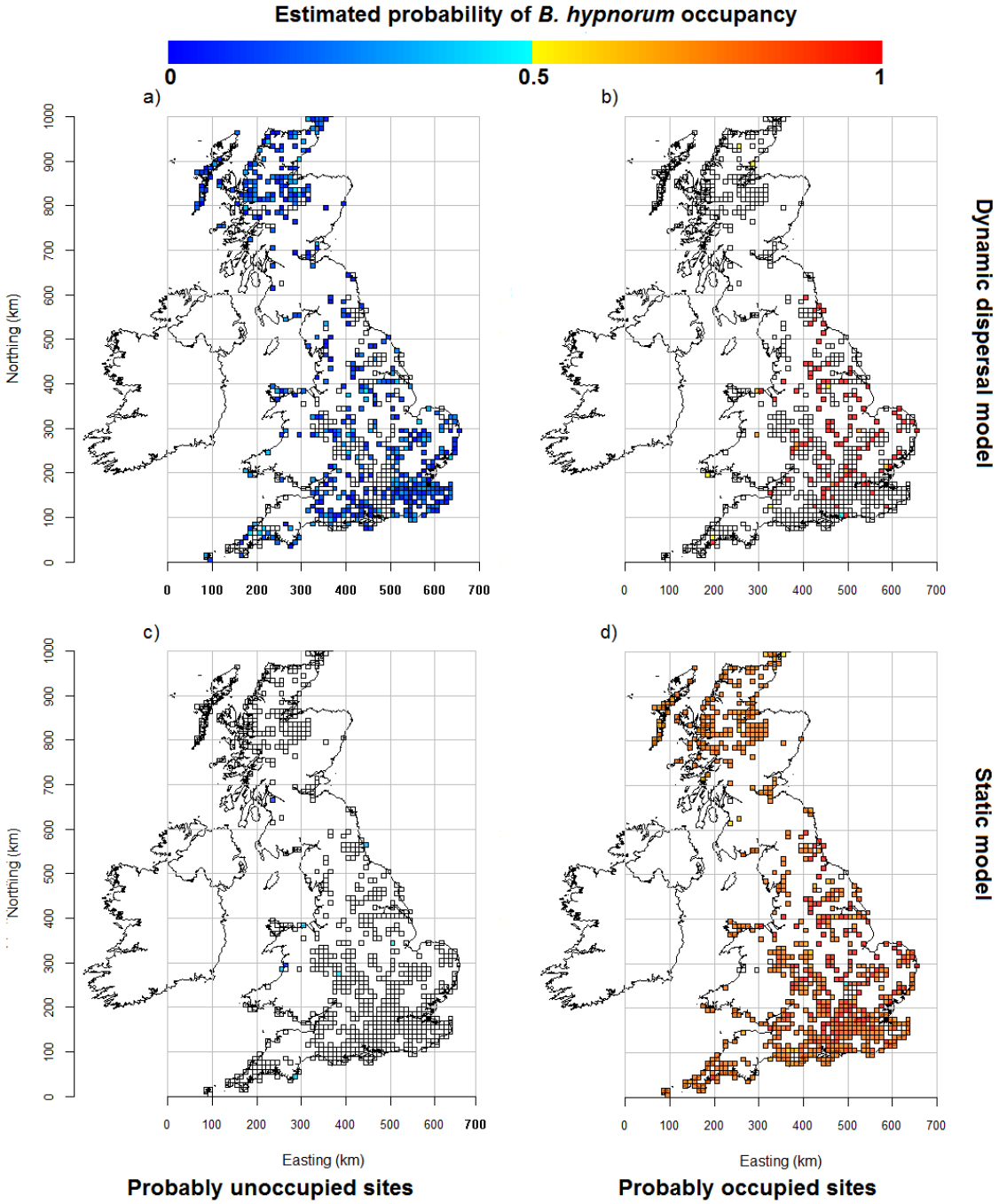

Figure 2.3. Spatial structuring of predicted *Bombus hypnorum* occupancy probability in the UK in the final year for which data were available, 2013. Occupancy probability is predicted by two Bayesian occupancy models fitted to biological records from 12,444 visits to 2,080 1 x 1 km sites over 13 years (2001 - 2013). Both models have identical detection sub-models but different state sub-models. (a), (b): dynamic dispersal model, site-level colonisation is dispersal limited according to the isolation distance from known populations. (c), (d): static model, occupancy probability is inferred only from detection histories with no spatial information. Sites are either probably occupied or unoccupied with occupancy probabilities of greater than ((a), (c)) or less than ((b), (d)) 0.5, respectively. For visualisation purposes the occupancy probability of 1 x 1 km sites is summarised at the 10 x 10 km scale. Such hectads (n = 762) containing the 2,080 constituent sites

are coloured according to the 'temperature bar' at the top of the figure by the median occupancy probability of their constituent sites. Open hectads have no constituent sites within the panel's range of occupancy probability, and in cases in which a hectad's sites fall within both ranges, its polygon is coloured in both panels.

Chapter 3: Tree Bumblebees (*Bombus hypnorum*) have colonised the UK without a severe genetic bottleneck

Abstract

Social insects are important providers of pollination services to agricultural crops, and for this reason many have been transported and, sometimes accidentally, released into new regions outside of their native ranges. A limiting factor in the spread of introduced invasives or natural colonists in their new ranges is genetic load due to the demographic 'bottlenecking' of a small founding population. However, paradoxically many successful invaders and colonists are thought to have succeeded despite this genetic load. Bumblebees (*Bombus* spp.), like other social Hymenoptera, are particularly vulnerable to genetic load following bottlenecking because homozygosity at their sex-determining locus is highly deleterious as it leads to the production of sterile, diploid males. Nevertheless, some introduced *Bombus* populations have successfully expanded despite severe bottlenecking and the consequent genetic load. *Bombus hypnorum*, which has rapidly colonised most of the UK landmass in the last 16 years, potentially represents a primary example of this phenomenon, as its success is hypothesised to have followed severe genetic bottlenecking, its UK population conceivably stemming from a founding population as small as one, doubly-mated queen. In the current study we test this hypothesis using a panel of microsatellite markers on a sample of individual *B. hypnorum* workers from a population within the core of the new UK range. We found no evidence for a severe genetic bottleneck. We also found that the study population exhibited a level of genetic diversity intermediate between those of widespread *Bombus* populations and those of range-restricted *Bombus* populations. Our analyses suggest that if a demographic bottleneck occurred at the likely time of an initial founding of the UK population, then it likely consisted of more than 60 diploid individuals. These findings support an alternative hypothesis under which the colonisation of the UK by *B. hypnorum* was not the result of a single, small, founding population, but instead may have been characterised by continuous migration from mainland Europe.

3.1 Introduction

Social insects are well represented among the most highly invasive animal species (Lowe S. Boudjelas S., & IUCN 2000). Furthermore, sociality itself often facilitates invasiveness because the social organisation of reproduction allows social insects to adapt to new environments and biomes (Chapman & Bourke 2001). However, social insects are also important contributors to ecosystem function and, where this benefits humans, to ecosystem services. Insect pollination is an important ecosystem service that underpins 9.5 % of global agricultural output (Gallai *et al.* 2009). On a global scale, dependence on insect-pollinated crops for sources of dietary micronutrients is positively correlated with levels of malnutrition (Chaplin-Kramer *et al.* 2014), so, in addition to its direct economic benefits, insect pollination also supports public health. Social bees (Apidae) are some of the most important insect pollinators of food crops (Klein *et al.* 2007) and globally, many bee species are in decline (Brown & Paxton 2009). However, at smaller regional scales, some bee species are expanding their ranges. Some of these are known to have been deliberately transported and introduced, while others may have naturally colonised new areas. In some cases these range-expanding species can have detrimental effects on taxa or ecosystems native to their new ranges (Hingston 2006; Madjidian, Morales & Smith 2008). Alternatively they may have little effect on native competitors and offer new pollination services to crops and wild flowers (Goulson & Hanley

2004). Whether colonists are introduced or naturally colonising, or beneficial or detrimental, it is important we understand the underlying mechanics and dynamics of range expansions by social insects. Here we investigate genetic factors underlying the range expansion of a recent bumblebee colonist of the UK, testing for evidence of a genetic bottleneck and its potential role in the colonisation success of the species.

Bumblebee species (*Bombus*) have been deliberately transported to areas outside their native ranges for commercial use and then either accidentally or deliberately released in the new area. This way, non-native bumblebees have entered and then colonised large parts of South America and Japan (Torretta, Medan & Arahamovich 2006; Yokoyama & Inoue 2010; Schmid-Hempel *et al.* 2014). The consequences of these invasions can be mixed but they are rarely without impact. For example, *B. terrestris* has been demonstrated to be a vector for transporting parasites to naïve hosts among South American native *Bombus* spp. (Schmid-Hempel *et al.* 2014). While *B. ruderatus* has been shown to compete for floral resources with native South American congeners, it is also an active pollinator of native South American plants (Madjidian *et al.* 2008). In Japan, where *B. terrestris* has colonised large areas after escaping from commercially-reared colonies in greenhouses (Kadoya & Washitani 2010), it disrupts plant-pollinator interactions by nectar robbing (Kenta *et al.* 2007) and depresses the population-level productivity of native *Bombus* species by interspecific mating (Kondo *et al.* 2009). *Bombus* species have also been introduced to Australasia, where there are no native *Bombus* species. For example, *B. terrestris* was introduced to New Zealand in 1885 (Hopkins 1914) and subsequently went on to colonise Tasmania (Schmid-Hempel *et al.* 2007). There is evidence that, due to the lack of native *Bombus* and the prevalence of introduced plants adapted to *Bombus* pollination, the pollination syndrome of *B. terrestris* alters the dynamics of Tasmanian plant communities in favour of invasive plants (Hingston 2006).

Generally, propagule pressure, a composite measure of the number of individuals introduced, which stems from either a large founding population or continued migration from the source population, can be a strong predictor of invasion success (Simberloff 2009). However, founding populations of organisms that subsequently colonise expanded ranges are often small. A small founding population creates a sampling effect on alleles that leads to reduced genetic diversity, known as genetic bottlenecking. This lack of genetic diversity can in turn, due to limited adaptive potential or inbreeding depression, be a barrier to successful further colonisation (Dlugosch & Parker 2008). Eusocial Hymenoptera, following bottlenecks, are potentially subject to an additional genetic load, relative to diploid organisms, due to their single-locus complementary sex determination mechanism (Chapman & Bourke 2001; Zayed & Packer 2005). Under complementary sex determination, individuals heterozygous at the sex-determining locus develop as females and those homozygous or hemizygous (with one allele) at the locus develop as males. Hence, under outbreeding (e.g. mating type AB x C), diploid offspring are heterozygous at the sex-determining locus (AC, BC) and develop as females and haploid offspring are hemizygous at this locus (A, B) and develop as males, generating the haplodiploidy characteristic of the Hymenoptera. But under conditions of reduced genetic diversity in eusocial Hymenoptera, a genetic load arises because a queen is far more likely to mate with a male that shares one of her alleles at the sex-determining locus (e.g. AB x A). Half of her diploid offspring will then be heterozygous and develop as females (AB) as under outbreeding, but the remaining half will be homozygous and so develop as males (AA). Such diploid males impose productivity and reproductive costs on the colony because males in eusocial Hymenoptera perform

no work and because diploid males, unlike haploid ones, are sterile (Beye *et al.* 2003). However, despite this phenomenon, among social bees and other eusocial Hymenoptera there are examples of dramatic and rapid invasions following severe genetic bottlenecks. For example, *B. terrestris* successfully colonised the island of Tasmania, where no native *Bombus* species are found, following introduction of an estimated two or three individuals (Schmid-Hempel *et al.* 1998). Among invasive organisms in general, that many introduced populations have successfully invaded despite the genetic load imposed by bottlenecks, is termed the genetic paradox of invasions (Allendorf & Lundquist 2003). Equally, many introduced populations are more genetically diverse than has been previously thought (Estoup *et al.* 2016).

Bombus hypnorum, the Tree Bumblebee, has recently dramatically expanded its range in the United Kingdom. It was first recorded near the southern coast of England in 2001 (Goulson and Williams, 2001), and can now be found throughout England and Wales, large parts of Scotland and the Western Isles, the Scilly Islands and the Isle of Man (BWARS 2017). This constitutes a range expansion of approximately 700 km in 15 years. In contrast to other *Bombus* species found in the UK, *B. hypnorum* has a marked preference for urban and woodland habitats, which are thought to be facilitating its range expansion, and prefers an overlapping but distinctive set of widespread flowering plants as forage (Crowther *et al*., 2014). There are some limited data pointing to a previous expansion into westerly maritime parts of continental Europe, specifically increases in abundance relative to other *Bombus* species in Belgium and records of *B. hypnorum* in sites within north-western France from which it was absent in the 1980s (Rasmont, 1989; P. Rasmont, personal communication). *B. hypnorum* presumably reached southern England by dispersal across the English Channel from the closest neighbouring area of the pre-2001 range, northern France (Rasmont & Iserbyt 2013). Although the possibility of accidental or deliberate introduction cannot be excluded, *B. hypnorum* is most likely to be a natural colonist of the UK, since it is not used or traded as a commercial pollinator. The spatio-temporal pattern of colonisation of the UK suggests that *B. hypnorum* has colonised the UK via leptokurtic dispersal, suggesting that it is capable of colonising new sites over distances much greater than those over which individuals typically disperse (Chapter 2).

There is some circumstantial evidence that *B. hypnorum* may have undergone a severe bottleneck on its arrival in the UK. Jones and Brown (2014) used the inferred rate of diploid male production to estimate that, in the UK *B. hypnorum* population, the sex-determining locus is most likely to have just four alleles. They thereby inferred that the most likely size of the founding population was as few as one or two doubly mated queens. Consequently, *B. hypnorum*'s success despite such an apparently severe bottleneck has been cited as a premier example of the genetic paradox of invasions (Schrieber & Lachmuth 2017). However, Jones and Brown (2014) studied a relatively small sample size of colonies and inferred male diploidy from the timing of male production within the colony cycle, without confirming it genetically. Therefore the conclusion that the UK *B. hypnorum* population has undergone a severe genetic bottleneck is open to question. Nonetheless, if the UK *B. hypnorum* population could be confirmed to have been subject to such a severe bottleneck, it would represent important evidence of a very rapid colonisation of a new area by a eusocial insect despite a high genetic load.

In summary, the colonisation of the UK by *B. hypnorum*, could have occurred according to one of two opposing hypotheses. Under Hypothesis 1, in a chance, single event, a very small number of

individuals, perhaps as few as one or two multiply-mated queens, founded the UK population. The preliminary support for this hypothesis is the circumstantial evidence of Jones and Brown (2014). It predicts that *B. hypnorum* in the UK has very low genetic diversity and will show evidence of a recent severe bottleneck. Under Hypothesis 2, the initial founding of the UK population was not a chance, single event and but instead was part of a wider westwards expansion of *B. hypnorum*'s range and therefore comprised many individuals and involved subsequent continued immigration from mainland European populations. The preliminary support for this hypothesis is the subsequent colonisation of offshore islands (BWARS 2017), the westwards expansion in mainland Western Europe (Rasmont 1989) and the leptokurtic dispersal implied by the rate of range expansion within the UK (Chapter 2). This hypothesis predicts that *B. hypnorum* in the UK does not have very low genetic diversity and will not show evidence of a recent severe bottleneck. Therefore, in the current study, using a panel of polymorphic microsatellite loci, we sought to discriminate between these hypotheses and specifically: 1) to determine the level of genetic diversity in a representative population of *B. hypnorum* in the UK, i.e. one within the core of its UK range; and 2) to test whether this population of *B. hypnorum* has undergone a severe bottleneck.

3.2 Methods

Bumblebee sample collection

Bombus hypnorum workers were collected from a 2 x 2 km sampling area on the western edge of Norwich, UK. The position of the sampling area's southwestern corner was: 52°36'56.12"N, 001°14'00.39"E (Appendix 3.1). The sampling area comprised a mix of suburban residential areas, parks, woodland, semi-natural areas and university campus and was taken to typify non-agricultural lowland areas of the UK. Workers were sampled in two successive summers during May and June, i.e. 15 May – 16 June 2014 and 28 May – 1 July 2015. This time period straddles the seasonal peak of observed worker abundance for *B. hypnorum* for the locality (Crowther, Hein & Bourke 2014). *B. hypnorum* was first recorded in the area, i.e. within the same 10 x 10 km grid square, in 2008 (BWARS 2017). In order to distribute evenly both (a) sampling effort in time and space and (b) the locations of sampled workers in space, the sampling area was split into 16 equally-sized (25 ha) divisions of 500 m x 500 m each, hereafter 'sampling squares'. In each year, *B. hypnorum* workers were sampled by free-searching of all the publicly accessible suitable habitat of every sampling square. A net was used to capture all encountered workers (from flowers or while free-flying) until either 40 workers had been caught from a given sampling square or three 2-hour searches of the square had been completed on separate days. Sampling took place during dry weather when air temperature was 15^{o}C or higher, during the hours 1000 - 1700. Tissue for DNA extraction was non-lethally sampled by temporarily restraining the worker and clipping the tarsal tip of a mid-leg (Holehouse, Hammond & Bourke 2003). Each tarsal tip was stored in 100% ethanol in a 1.5 ml tube at room temperature until later extraction. Additionally, for each worker sampled, the exact location of capture was recorded using a Garmin eTrex handheld GPS receiver, with an accuracy of approximately 4 m. A summary of the number of workers sampled by sampling square and year is given in Appendix 3.1

DNA extraction and genotyping

DNA was salt-extracted from the sampled tarsal tips using a modified ammonium-acetate ethanol precipitation (following Richardson, 2001). Each tarsal tip was individually frozen using liquid N for 2 minutes and then crushed to powder before the ligase treatment. In order to increase the reliability of DNA yield, the ethanol precipitation step included incubation at -20^{o}C for 3 hours. Extracted DNA was suspended in weak TE buffer (10 mM Tris.HCL, 0.1 mM EDTA) and kept at -20^{o}C until further use.

Twenty-four microsatellite markers, previously characterised from other *Bombus* species (Estoup *et al.* 1995; Reber Funk, Schmid-Hempel & Schmid-Hempel 2006; Stolle *et al.* 2009) were tested on a small number of individuals (10 - 20) to ascertain whether the associated primers could amplify polymorphic loci in *B. hypnorum*. This resulted in a panel of twenty polymorphic microsatellite markers (Table 3.1). The four loci excluded are detailed in Appendix 3.2. For PCR amplification, the 20 polymorphic loci to be screened were divided into three multiplexes. The multiplexes were designed using Multiplex Manager v1.2 (Hollely & Geerts 2008), with the minimum distance between same-dye markers being set at 14 base pairs and the complementarity threshold being set at 7. Multiplex characteristics and fluorescent dyes used are summarised in Table 3.1. PCR was carried out in a 2 µl reaction volume in 96-well plates. Where extraction yields permitted, up to 15 ng of sample DNA were added to each reaction well. In order to maintain consistent concentrations of all reagents in the small reaction volume, all liquid buffer was evaporated from the DNA solution at 50^{o}C, leaving a dry pellet of DNA before the addition of aqueous reagents. Each reaction contained 1 µl of Qiagen Multiplex Master Mix and 1 µl of primer mix with primer pairs at 0.08 – 0.50 M concentrations. Each reaction was then covered with a droplet (ca. 10 µl) of mineral oil to prevent evaporation. Each plate included (a) a negative control for the reaction, consisting of all the reagents and primers but no template DNA, and (b) two positive controls using DNA from *B. hypnorum* queens (from samples detailed in Chapter 4) whose multi-locus genotypes had been ascertained using multiple single-locus PCRs.

Amplification conditions comprised: an activation step for 15 min at 95°C; 30 cycles of denaturing for 30 s at 94°C, annealing for 90 s at 50°C and extension for 1 min at 72°C; with a final extension of 5 min at 72°C. PCR products were visualised using a 48-well capillary ABI 3730 DNA analyser and a ROX-500 internal size standard (Applied Biosystems), and fragments were sized using GeneMapper 4.0 software (Applied Biosystems, Paisley, UK). Alleles were only accepted when confirmed across two or more individuals. To quantify genotyping error, extracted DNA from 80 - 120 workers (i.e. 12 % - 19 % of samples) were re-run in each of the three multiplexes so as to repeat the PCR and analysis steps for 1,880 locus-level genotypes, covering all loci. These repeated genotypes were compared to the original genotypes to calculate locus-specific allelic mistyping rates. The per-locus mean (range) allelic mistyping rate was found to be 2.26% (0.91 – 3.17 %). No negative controls contained peaks that corresponded to any amplified alleles. Four workers were excluded from further analyses because they had peaks corresponding to three alleles at one or more loci, and because in these cases it was not possible to determine whether the workers were truly triploid or whether the original samples were contaminated. In total, 645 workers (375 from 2014 and 270 from 2015) were sampled and genotyped.

Hardy-Weinberg equilibrium, null allele frequencies and linkage disequilibrium

All data handling and statistical analysis was executed in R version 3.1.2 (R Development Core Team 2016) unless otherwise stated. The genotypes of the workers were tested for deviations from Hardy-Weinberg equilibrium (HWE) within both years, corrected for multiple comparisons, using the R package 'adegenet' (Jombart 2008). Years were treated as separate subpopulations because, due to the recent colonisation of the study site and surrounding area, it is possible that the local population was structured temporally. This could arise by between-year genetic mixing within a population structured at larger spatial scales creating temporal structuring of the population at the study site. The tests for HWE used all workers, some of which would have been full or half-sisters. This should not have introduced bias, as offspring genotypes can be taken to be a random sample of parental genotypes. Rather, this potentially made the tests more conservative (by inflating degrees of freedom). The frequencies of null alleles for all loci were estimated using the program Cervus 3.0 (Kalinowski, Taper & Marshall 2007). Pairwise tests for linkage disequilibrium across all combinations of the twenty loci were implemented using functions from the R package 'pegas' (Paradis 2008). To meet the assumptions of all subsequent analyses, loci were excluded that exhibited one or more of: (a) significant deviation from HWE in both years after correction for multiple comparisons; (b) null allele frequencies in excess of 0.1 (Dakin & Avise 2004); or (c) significant linkage disequilibrium with another, more informative locus, after correction for multiple comparisons.

Genetic diversity and bottlenecking

To test whether the study population of *B. hypnorum* had undergone a severe bottleneck, we looked for evidence of a recent reduction in effective population size. In Chapter 4 (Section 4.3), workers are assigned to lineages, defined as the matrilineal descendants of a single unsampled 2014 colony queen, such that within a lineage workers are related as full sisters, half-sisters or aunt and nieces. To avoid the confounding effects of sampling multiple related workers from the same lineage, the following analyses used only the set of genotypes obtained by sampling one worker randomly from each of the lineages inferred in Chapter 4. Therefore, workers sampled in 2014 and 2015 were pooled for these analyses, but all workers used were less related than half-sisters within years or aunts and nieces between years. Henceforth, these workers are referred to as 'unrelated' workers.

Firstly, the program Bottleneck 1.2.02 (Piry, Luikart & Cornuet 1999) was used to implement a sign test to determine whether there had been a recent reduction in effective population size (by assaying the extent to which allelic diversity is in excess of equilibrium). This analysis assumed a two-phase model of allelic mutation and a 9:1 ratio of one-step to multi-step mutations, as these assumptions have been shown to be most applicable to the mutation of microsatellite loci (Di Rienzo *et al.* 1994).

Secondly, the 'M-ratio' *sensu* Garza & Williamson (2001) was calculated for each of the loci. The M-ratio is the ratio between *k*, the number of alleles at a locus, and *r*, the size range of those alleles in base pairs. Reductions in population size cause alleles to be lost at random, due to the sampling effect, so reducing *k*. But since losing only the largest or smallest alleles will reduce *r*, it follows that usually *k* will be reduced more than *r* and hence the ratio of *k* to *r* will fall. A value of $M < 0.7$ (given the number of loci included and assumptions identical to those used in the Bottleneck sign test) was taken to indicate evidence of historic reductions in population size (Garza & Williamson 2001).

A reduction of the M-ratio could stem from either (a) colonisation from a small founding population or (b) historic population reduction, not connected to the founding of the UK population. Therefore,

we sought to use similar data on other UK bumblebee species which have not undergone such a rapid range expansion as a 'null model' against which to compare the results from *B. hypnorum*. Microsatellite genotype data (Dreier *et al.* 2014a, b) from workers of single populations sampled in 2011 in southern UK in each of five *Bombus* species were selected for this purpose. We calculated M-ratios from these reference populations to compare the M-ratio from *B. hypnorum* with those of single populations of bumblebees that should exhibit the level of bottlenecking associated with population fluctuations normal for long-established UK native *Bombus* species. The dataset of Dreier *et al.* (2014a, b) was selected for comparison with the *B. hypnorum* data due to its taxonomic breadth (the species comprising the dataset were *B. hortorum, B. lapidarius, B. pascuorum, B. ruderatus* and *B. terrestris*), relative geographic proximity to the study site in the current study, similar numbers of loci typed and a worker sampling protocol similar to that used in the current study (Dreier *et al.* 2014a, b).

3.3 Results

Hardy-Weinberg equilibrium, null allele frequencies and linkage disequilibrium

Three of the twenty microsatellite loci significantly deviated from HWE in both the 2014 and 2015 worker samples and a further four loci significantly deviated from HWE in the 2015 worker samples alone (Table 3.2). Six of the twenty loci had estimated null allele frequencies greater than 0.1, and, of these, three were the same loci that deviated from HWE in both years. Therefore six of the twenty loci were excluded from further analyses (Table 3.2). At the 14 retained loci, the 645 workers were successfully genotyped at a median (interquartile range) of 11 (10 - 14) loci. No pairwise combination of loci showed significant evidence for linkage disequilibrium after correction for multiple comparisons (400 pairwise comparisons, corrected alpha value = 0.00125, minimum p value = 0.0073).

Genetic diversity and bottlenecking

Across the 645 workers and 14 loci, the median number (range) of alleles per locus (k) was 5 (3 – 11) (Table 3.2); mean allelic richness was 5.9 alleles per locus; and mean observed and expected heterozygosities were, respectively, 0.48 and 0.51.

Sampling a single worker from each of the separate lineages yielded a sample of 89 unrelated workers. The sign test found no evidence of recent bottlenecking, as the observed and expected heterozygosity excesses were not significantly different across loci (expected heterozygosity excess, 7.51 of 14 loci; observed heterozygosity excess, 7 of 14 loci; $p = 0.493$).

The mean (standard error) M-ratio, calculated using allelic richness and size ranges at 14 loci from the 89 unrelated workers, was 0.381 (0.053). This was lower than the threshold of 0.7, indicating support for a historic population reduction. However, the mean M-ratio for *B. hypnorum* fell within the range of the mean M-ratios for the five reference *Bombus* populations (Figure 3.1), and overall there was no significant difference in mean M-ratio across all six populations, i.e. those of *B. hypnorum* plus the five other species (one-way ANOVA, not assuming equal variances, $F_{5, 31.90} = 1.947$, $p = 0.1139$). All of the reference populations had M-ratios under 0.7 (Figure 3.1), which also indicated support for a historic population reduction in all these populations.

3.4 Discussion

We used fourteen polymorphic microsatellite loci to estimate genetic diversity in a focal population within the core of *B. hypnorum*'s UK range and to test for evidence of a genetic bottleneck. The microsatellite loci had between 3 and 11 alleles, a mean allelic richness of 5.9 and an average heterozygosity of 0.51. We also used microsatellite data for five *Bombus* species native to the UK (Dreier *et al.* 2014a, b), to determine whether the extent of bottlenecking exhibited by the UK population of *B. hypnorum* was greater than that associated with population fluctuations in long-established species. We performed two analyses, neither of which found evidence for a genetic bottleneck. Firstly, a two-phase model of mutation-drift equilibrium sign test did not support a recent bottleneck in *B. hypnorum*. Secondly, while the mean M-ratio across loci for *B. hypnorum* was below the threshold value indicative of a historic population reduction, it was not significantly different from the mean M-ratios of populations of five *Bombus* species native to the UK, at least four of which (all except *B. ruderatus*) are widespread and abundant.

Overall, our results support Hypothesis 2, as they indicate that the rapid colonisation of the UK by *B. hypnorum* has not involved a severe genetic bottleneck and has not been associated with a lack of genetic diversity. Assuming that any bottleneck associated with the initial colonisation of the UK would have occurred in the year before *B. hypnorum* was first detected in the country (i.e. 2000), we can infer that this was at least 14-15 generations before the population in the current study was sampled. Power analyses suggest that a putative bottleneck occurring at this relative timepoint would have been detectable by each of the tests employed (Cornuet & Luikart 1996; Garza & Williamson 2001; Williamson-Natesan 2005). Therefore Hypothesis 1, that there was a single founding event involving a very small number of individuals, is not supported. We can also reject with some certainty the suggestion that the founder population consisted of as few as one or two multiply-mated queens (Jones & Brown 2014). This is because we found a maximum number of alleles of 11 (at the BTMS0125 locus), and, excluding rare mutation events, two queens mated with a mean 1.7 males each (Chapter 4) would yield an expected maximum of 7.4 alleles per locus ([2 + 1.7] x 2).

Power analyses allow us to estimate the minimum size of founding population for which a bottleneck would have been detected. Specifically, these analyses indicate that bottlenecks should be detected reliably 0.25 X N to 2.5 x N generations after the bottleneck occurring, where N is the effective population size, in diploid individuals, immediately after the bottleneck (Cornuet & Luikart 1996). Therefore, on the previous assumption that any bottleneck occurred 15 generations ago, the sign test should reliably have detected a bottleneck of at least 60 diploid individuals (i.e. 15/0.25 = 60). Hence, since no bottleneck was detected, it is unlikely that the initial founding UK population of *B. hypnorum* numbered fewer than 60 diploid individuals, which corresponds to either 45 singly-mated queens or 30 doubly-mated queens. In turn, supporting Hypothesis 2, these results suggest that the arrival of *B. hypnorum* in the UK involved either multiple colonisation events or continued migration after an initial colonisation event. This is because if we hypothesise that the initial founding population was relatively large, then it follows that the individual probability of a *B. hypnorum* queen dispersing from the putative source location was relatively high. If this is the

case, then, assuming that dispersers are independent, we should also expect either multiple colonisations or continued migration with a relatively high probability.

One caveat to these arguments is that some circumstantial evidence suggests that *B. hypnorum* may exhibit facultative bivoltinism, i.e. two colony cycles per year (Edwards & Jenner 2005). Specifically, observations suggest that in some years and localities a second, smaller peak in observed abundance of workers occurs in late summer (Edwards and Jenner 2005). If this phenomenon is a result of bivoltinism then this introduces some uncertainty as to the average generation time of the study population of *B. hypnorum.* There are currently no structured census data for *B. hypnorum* with which we might estimate the prevalence of bivoltinism, and conceivably workers observed later in the season are from later-founded colonies. However, even if it occurs, bivoltinism is almost certainly a trait shown by only some populations in some years, suggesting that its effect on our estimate of the number of generations since *B. hypnorum*'s arrival in the UK (15) is small.

The estimated M-ratios suggest that the sampled UK populations of both *B. hypnorum* and the other *Bombus* species have undergone historic population reductions. Because new alleles that replace the alleles lost to drift in a bottlenecked population arise only from mutation, M-ratios should take several hundred generations to return to equilibrium levels after a bottleneck (Garza & Williamson 2001). Any interpretation of the M-ratios across *Bombus s*pecies with respect to their known population history is highly speculative as data on the distributions and abundances of *Bombus* species in the UK are mostly limited to recent decades. Historical recorder data for the period 1921 – 1950 have been compared to contemporary data to infer changes in the pollinator community of the UK over an 80 year period (Senapathi *et al.* 2015). However, the geographical coverage of these historical records is limited to just 14 sites, so that it is difficult to extrapolate clear indications of long-term changes. Another indication of long-term population trends comes from comparisons of population genetic measures between hundred year-old museum specimens and modern samples from corresponding sites on continental Europe. These have found that some declining *Bombus* species were already genetically depauperate one hundred years ago (Maebe *et al.* 2016). Assuming that historical population reductions in widespread *Bombus* species might have been contemporaneous with the historical loss of genetic diversity in declining species, it is possible that population reductions in widespread species may have occurred more than one hundred years ago. Therefore, it is possible that the historic population size reductions that caused the M-ratios to fall below the equilibrium threshold level may also have occurred more than one hundred years ago, which is earlier than any period for which data exist on *Bombus* populations in the UK or elsewhere. Alternatively, lowered M-ratios when measured from *Bombus* populations sampled at this scale may be a result of population fluctuations and local extinctions resulting in a relatively high chance of losing alleles to drift. Either way, our analysis shows that the extent of genetic bottlenecking resulting from population size reductions in *B. hypnorum* is not particularly exceptional when compared to populations of other UK *Bombus* species.

Compared to other Western European *Bombus* populations for which there are population-genetic data, we found that the study population of *B. hypnorum* had an intermediate level of genetic diversity. For example, our study population of *B. hypnorum* had lower expected heterozygosity (0.52) than the four widespread species (*B. hortorum, B. lapidarius, B. pascuorum* and *B. terrestris;* range of expected heterozygosity = 0.67 – 0.84) and the single declining species (*B. ruderatus,* expected heterozygosity = 0.75) sampled by Dreier *et al.* (2014b) in the UK. Maebe *et al.* (2016)

reported expected heterozygosity for four range-restricted and four widespread *Bombus* species sampled in 2015 from sites across Belgium. All the range-restricted species (*B. humilis, B. ruderarius, B. soroeensis, B. sylvarum;* range, 0.31 – 0.43) and one widespread species (*B. pascuorum*, 0.46) had lower expected heterozygosity than did our study population of *B. hypnorum.* Conversely, three of the widespread species (*B. hortorum, B. lapidarius, B. pratorum;* range, of expected heterozygosity 0.57 – 0.74) had higher expected heterozygosity than our estimate for *B. hypnorum*. In separate studies, three different *Bombus* species that have greatly reduced UK ranges (*B. distinguendus, B. muscorum, B. sylvarum;* expected heterozygosity = 0.39, 0.44 and 0.39, respectively) (Darvill *et al.* 2006; Ellis *et al.* 2006; Charman *et al.* 2010) had lower expected heterozygosity than did our study population of *B. hypnorum.* Overall, the level of genetic diversity in the study population of *B. hypnorum* is higher than those of rarer range-restricted species, but lower than those of most common or widespread species.

Since *B. hypnorum* in the UK is not range-restricted, but rather is rapidly expanding its range, it is possible that its reduced genetic diversity relative to other widespread species is due to its recent range expansion. A species expanding its range into new regions would typically be expected, after successive generations each establishing new populations further from the original source, to lose alleles and heterozygosity (Hewitt 1996, 2000; Ibrahim, Nichols & Hewitt 1996). This is because many organisms, including *B. hypnorum* (Chapter 2), have a leptokurtic pattern of dispersal, such that new populations are founded by a small number of long-range dispersers. This means that, at the leading edge of a range expansion, alleles are subject to a sampling effect. Assuming that the rate of numerical population increase at new sites greatly exceeds the rate of new migrants arriving from the source population, clines of genetic diversity can persist along colonisation routes for hundreds of generations (Ibrahim *et al.* 1996). This effect could potentially explain the moderate difference in heterozygosity between *B. hypnorum* and widespread *Bombus* species that have not expanded their ranges.

In summary, we have shown that *B. hypnorum*'s successful colonisation of the UK has occurred without a severe genetic bottleneck and against a background of only moderately reduced levels of genetic diversity. In addition, it is likely that *B. hypnorum*'s arrival in the UK was part of a long-term westwards range expanasion of the species within Europe and that there is continued between-population gene flow through ongoing immigration from the continental European to the UK *B. hypnorum* population. Hence *B. hypnorum* is not an example of a eusocial insect that can overcome severe bottlenecking and still be exceptionally invasive, such as *B. terretris* in Tasmania (Schmid-Hempel *et al.* 2007). Correspondingly, *B. hypnorum*'s colonisation of the UK is not an example of the genetic paradox of invasion (Schrieber & Lachmuth 2017). This is because, as has been demonstrated for other organisms previously hypothesized to exemplify the genetic paradox of invasion (Estoup *et al.* 2016), *B. hypnorum* in the UK is not as genetically depauperate as previously expected.

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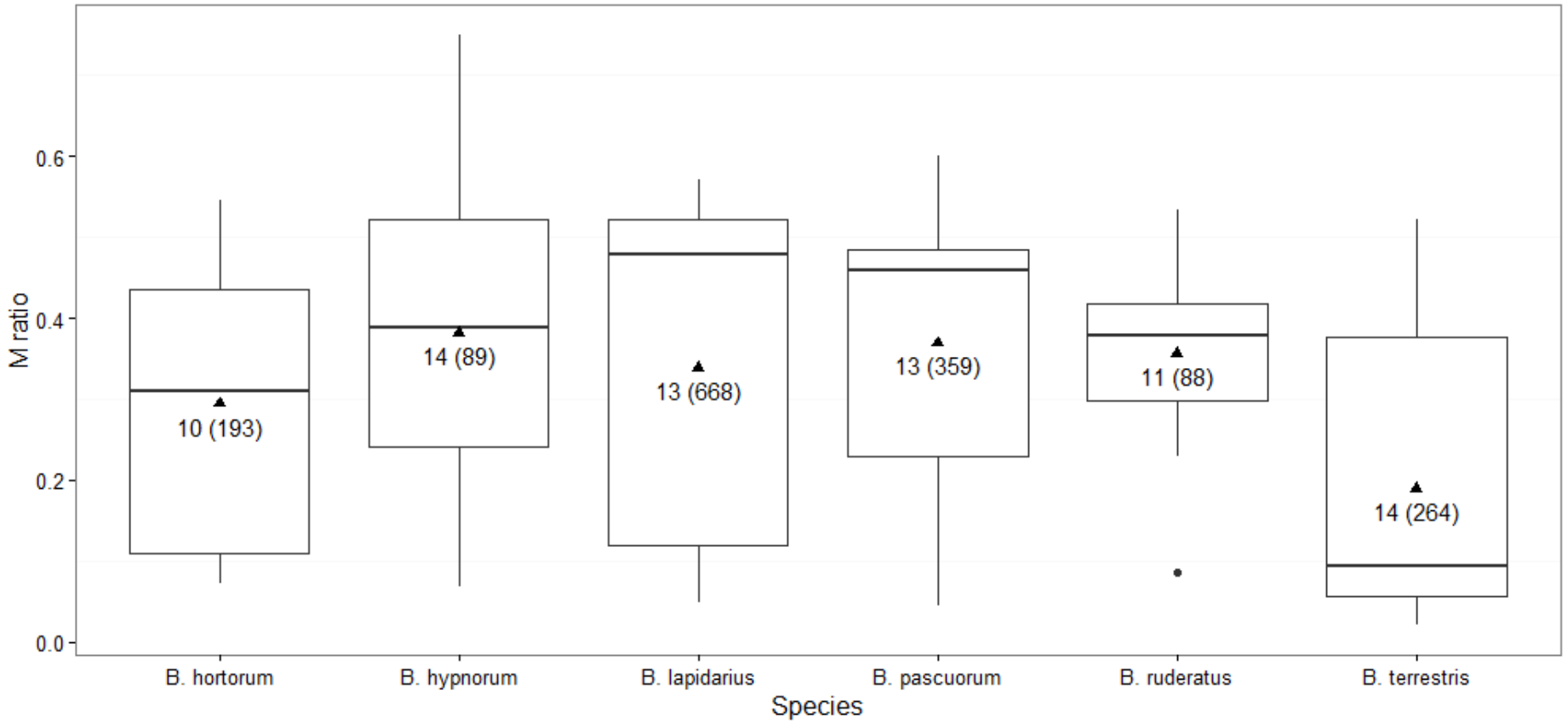

Figure 3.1. M-ratios *sensu* Garza and Williamson (2001) across microsatellite loci in single populations of six *Bombus* spp., based on unrelated workers. *B. hynorum* workers were collected across two years (2014-15) for the current study and were sampled from distinct lineages and workers of all other species were collected in one year (2011) and were sampled from distinct colonies (Dreier *et al*. 2014b). Thick horizontal bar, median; box, interquartile range (IQR); whiskers, range (not including outliers); filled circles, outliers, defined as points more than 1.5 IQR below lower quartile; filled triangles, means. Sample sizes shown within boxes as number of loci and, in parentheses, number of workers included in the analysis.

Table 3.1. Conditions for multiplex PCRs to co-amplify microsatellite fragments from *Bombus hypnorum* template DNA for 20 loci in three multiplexes (A, B, C). Marker, microsatellite locus identifier; dye, fluorescent molecule added to 5' end of forward primer; size range, min – max size of amplicons in base pairs; primer concentration, molar concentration of each oligonucleotide for forward and reverse priming in final reaction volume (2 µl).

Multiplex	Marker	Dye	Size range	Primer concentration
A	B131	6-FAM	118 - 130	0.08
	B132	HEX	159 - 179	0.50
	BL03	6-FAM	144 - 160	0.20
	BT26	HEX	98 - 110	0.08
	BTMS0125	ATTO-550	110 - 149	0.20
	BTMS0132	HEX	134 - 146	0.20
B	B10	6-FAM	178 - 200	0.40
	B11	6-FAM	158 - 164	0.20
	B121	ATTO-550	153 - 208	0.35
	B96	6-FAM	243 - 255	0.50
	BT05	HEX	153 - 162	0.12
	BTMS0033	HEX	201 - 204	0.30
	BTMS0056	HEX	254 - 256	0.20
	BTMS0057	HEX	104 - 113	0.08
C	BL01	ATTO-550	134 - 148	0.20
	BL08	HEX	145 - 149	0.35
	BT10	6-FAM	118 - 124	0.10
	BTERN01	HEX	114 - 127	0.08
	BTERN02	6-FAM	157 - 179	0.25
	BTMS0083	6-FAM	277 - 306	0.10

Table 3.2. Summary of microsatellite marker data by locus for sampled *Bombus hypnorum* individuals. The queen data were used to assign workers to lineages (present chapter, Chapter 4) and for additional analyses (Chapter 4). k, number of alleles; N, number of individuals for which a genotype was successfully obtained at that locus; H Obs, observed frequency of heterozygotes; H Exp, expected frequency of heterozygotes; HWE 2014, HWE 2015, result of Bonferroni-corrected test of the null hypothesis that the locus is not significantly out of Hardy-Weinberg equilibrium for workers sampled in 2014 or 2015, respectively; p 2014, p 2015, p value for corresponding HWE hypothesis test calculated using chi-squared test for associations; F(Null), estimated frequency of null alleles across all workers; Action, decision on use of locus in further population genetic analyses.

Locus	Queens				Workers									
	k	N	H Obs	H Exp	k	N	H Obs	H Exp	HWE 2014	p 2014	HWE 2015	p 2015	F(Null)	Action
B10	7	44	0.659	0.67	9	595	0.615	0.615	TRUE	0.132	TRUE	0.855	0.0013	Retain Marker
B11	4	44	0.568	0.668	4	584	0.62	0.644	TRUE	0.196	TRUE	0.024	0.0203	Retain Marker
B121	5	44	0.523	0.744	5	434	0.576	0.648	TRUE	0.321	TRUE	0.034	0.0569	Retain Marker
B131	3	44	0.432	0.452	5	605	0.364	0.403	TRUE	0.04	FALSE	< 0.001	0.0543	Retain Marker
B132	6	44	0.477	0.501	7	309	0.469	0.635	TRUE	0.448	FALSE	< 0.001	0.1586	Drop Marker
B96	5	44	0.295	0.683	4	467	0.45	0.662	FALSE	< 0.001	FALSE	< 0.001	0.1928	Drop Marker
BL01	5	44	0.25	0.592	8	328	0.46	0.722	TRUE	0.694	FALSE	< 0.001	0.2343	Drop Marker
BL03	5	44	0.477	0.526	9	582	0.498	0.491	TRUE	0.003	TRUE	0.543	-0.0134	Retain Marker
BL08	3	44	0.045	0.39	3	362	0.155	0.153	TRUE	0.645	TRUE	0.004	-0.0086	Retain Marker
BT05	5	44	0.636	0.599	4	605	0.57	0.581	TRUE	0.476	TRUE	0.226	0.0106	Retain Marker
BT10	4	44	0.432	0.515	4	389	0.391	0.599	FALSE	< 0.001	FALSE	< 0.001	0.213	Drop Marker
BT26	5	44	0.545	0.569	7	587	0.578	0.578	TRUE	0.047	TRUE	0.342	0.0037	Retain Marker
BTERN01	4	44	0.25	0.4	7	392	0.156	0.567	FALSE	< 0.001	FALSE	< 0.001	0.5861	Drop Marker
BTERN02	6	44	0.227	0.696	8	346	0.754	0.749	TRUE	0.615	TRUE	0.026	-0.0085	Retain Marker
BTMS0033	3	44	0.159	0.402	2	558	0.238	0.326	TRUE	0.004	FALSE	< 0.001	0.1556	Drop Marker
BTMS0056	4	44	0.136	0.172	3	568	0.164	0.158	TRUE	0.814	TRUE	0.824	-0.0221	Retain Marker
BTMS0057	5	44	0.614	0.592	5	613	0.618	0.65	TRUE	0.023	TRUE	0.722	0.0214	Retain Marker
BTMS0083	8	44	0.682	0.755	6	281	0.598	0.694	TRUE	0.479	FALSE	< 0.001	0.0754	Retain Marker

BTMS0125	2	44	0.023	0.023	11	580	0.669	0.72	TRUE	0.034	TRUE	0.002	0.0337	Retain Marker
BTMS0132	1	44	0	0	3	536	0.011	0.015	TRUE	0.005	TRUE	0.99	0.0986	Retain Marker

Chapter 4: Mating system, worker foraging distance and nest density of the Tree Bumblebee (*Bombus hypnorum*) in its recently expanded UK range

Abstract

Molecular methods have revealed several aspects of bumblebee ecology, including worker foraging distances, that were until recently poorly understood. An important recent finding is that worker foraging distances are plastic with respect to the availability of high-quality foraging habitat and that the availability of such habitat is likely to be linked to the population-level productivity of new sexuals. This finding predicts that, in habitats producing the large numbers of colonising queens associated with rapid range expansion, worker foraging distances should be short. *Bombus hypnorum,* the Tree Bumblebee, has recently colonised the UK and expanded its range by 900 km over 16 years. Unlike queens of most other bumblebee species, *B. hypnorum* queens can mate with multiple males (facultative polyandry). In the current study, we sampled queens and workers of *B. hypnorum* from a suburban landscape in the core of its UK range, typical of the habitats thought to be facilitating the range expansion. We used a panel of 14 microsatellite markers to assign workers of *B. hypnorum* to their colonies based on shared maternity and estimated the colony-specific foraging distance of 62 colonies. We also estimated the level of polyandry by genotyping stored sperm dissected from the spermathecae of the sampled queens. We found that the mean colony-specific worker foraging distance was 103.6 m, which is considerably shorter than those estimated using similar methods from most other bumblebee populations. We estimated that 66% of queens had mated with more than one male and that, across all queens, the mean minimum mating frequency was 1.7 males per queen. Estimated nest density was 2.56 and 0.72 colonies ha^{-1} in 2014 and 2105, respectively Overall, our findings add to our knowledge of the ecology of the UK *B. hypnorum* population and support the prediction that this population should exhibit a short mean worker foraging distance.

4.1 Introduction

Bumblebees are key pollinators of many wild plants (Ollerton, Winfree & Tarrant 2011) and economically important crops (Garratt *et al.* 2014). Along with those of other insects, their pollination services support food security (Klein *et al.* 2007; Gill *et al.* 2016) and account for around 10 % of global agricultural production (Gallai *et al.* 2009). However, across both Europe and North America many bumblebee species are declining (Williams & Osborne 2009; Potts *et al.* 2010; Cameron *et al.* 2011).

While bumblebees are well studied compared with other pollinators (Rader *et al.* 2016), there are considerable gaps in our knowledge base. For example, due in large part to bumblebees forming relatively small colonies that are cryptic and hard to locate, major components of bumblebee ecology and life history have historically proved very difficult to study. Specifically, without being able to locate the nests of a large, unbiased sample of colonies, researchers cannot directly measure key components such as the spatial use of foraging resources, whereas in other ecological systems this would be one of the first things investigators would seek to quantify (Sutherland 1996). Fortunately, advances in molecular methods, and their recent application to wild populations of bumblebees, have revealed substantial components of bumblebee ecology that were previously

obscure (Woodard *et al.* 2015). One particularly informative technique has involved the censusing of colony numbers and reconstruction of colony memberships by inferring sisterhoods among sampled workers using neutral genetic markers. Grouping observations of worker sisters into colonies has provided estimates of species-specific nesting densities (Chapman, Wang & Bourke 2003; Darvill, Knight & Goulson 2004; Knight *et al.* 2005; Charman *et al.* 2010) and worker foraging distances (Darvill *et al.* 2004; Knight *et al.* 2005, 2009; Charman *et al.* 2010). It has also highlighted that urban habitats can support high nest densities of bumblebees (Chapman *et al.* 2003) and that targeting of agri-environment schemes is linked to higher nesting densities (Wood *et al.* 2015).

Refinements to these approaches, in which the nest position or resource use of individual colonies are estimated, have allowed hypothesis-driven research into the previously cryptic spatial ecology of bumblebees. An important finding of this research is that bumblebee worker foraging distance, the distance that workers fly from their nest to forage at plants for nectar and pollen, is plastic with respect to resource availability (Carvell *et al.* 2012; Jha & Kremen 2013; Redhead *et al.* 2016). Rather than being an autecological trait, foraging distance is best understood as a function of the density and spatial arrangement of the resources that are available in the landscape. This insight has been critical in the design of interventions to support pollination services in agricultural ecosystems (Dicks *et al.* 2015; Carvell *et al.* 2016). For example, the agri-environment scheme for pollinators in England (Countryside Stewardship's Wild Pollinator and Farm Wildlife Package) stipulates that farmers maintain 3 - 5 % of their farmed area as patches sown with pollinator-attracting plants. It assumes that, at these densities, floral resources facilitate a reduction in the worker foraging distances of bumblebees. This assumption was supported by results of research using genetic methods to estimate colony-specific worker foraging distances as described above (Dicks *et al.* 2015; Redhead *et al.* 2016). Further research applying these methods has shown that, in field populations, a higher density of floral resources around individual nests is linked to colonies being more likely to have daughter queens surviving to the spring emergence stage in the following year, i.e to greater lineage survival (Carvell *et al.* 2017). Therefore, there is an emerging synthesis indicating that high-quality resources at sufficient densities can lead to reduced worker foraging distances and enhanced rates of queen survivorship and hence, by inference, of population increase. If correct, a prediction is that in a rapidly-expanding population, i.e. one inferred to be characterised by high productivity, bumblebee colonies should encounter resources at densities that facilitate short worker foraging distances.

In the current study we investigated the landscape-scale foraging and nesting ecology of an unequivocally range-expanding bumblebee population, the UK population of *Bombus hypnorum,* the Tree Bumblebee. Our aim was to investigate whether specific features of *B. hypnorum*'s ecology, including worker foraging distance, have contributed to its rapid population and range expansion. *B. hypnorum* has a very large Palaearctic distribution, which extends from Western Europe in the west to Japan in the east, and from the Kola Peninsula in arctic Russia in the north to the Himalayan Mountains in Nepal in the south (Goulson & Williams 2001). It has recently colonised the UK and has undergone a rapid expansion of its new British range since it was first recorded near the southern coast of England in 2001 (Goulson & Williams 2001). Specifically, since 2001 *B. hypnorum* has expanded its range by 900 km and it now occurs throughout all of England and Wales and in much of Scotland. Unlike other widespread species of bumblebees whose ranges have remained stable, the British *B. hypnorum* population must have greatly increased in abundance. Across an urban-rural

gradient typical of southern England, *B. hypnorum* workers occurred much more frequently in suburban landscapes (Crowther, Hein & Bourke 2014). It is therefore highly likely that suburban and similar habitats are facilitating the population increase that underlies this range expansion.

Unlike the queens of most species of bumblebee, which mate singly, *B. hypnorum* queens are facultatively polyandrous, i.e. can mate with multiple males, with studies estimating a mating frequency of 1-6 males per queen (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000; Paxton *et al.* 2001; Brown, Schmid-Hempel & Schmid-Hempel 2003). There is some evidence that the degree of polyandry varies geographically. In particular, studies of *B. hypnorum* queens sampled from different locations in Europe have found the degree of polyandry (percentage of polyandrous queens) to range from 0% to 67% (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000; Paxton *et al.* 2001; Brown, Schmid-Hempel & Schmid-Hempel 2003). In the application of the genetic methods described above, facultative polyandry poses two challenges to assigning workers to colonies based on molecular markers. Firstly, the level of relatedness between workers within colonies can be lower than under single queen mating as they may be half-sisters instead of full sisters, i.e. maternal but not paternal sisters. Secondly, *a priori* information on the mating frequency of queens is needed to validate and inform the assignment of half-sisters (Wang 2004; Wang & Santure 2009).

Therefore, to investigate the landscape-scale foraging ecology and nesting ecology of the UK *B. hypnorum* population using genetic methods, we addressed the following four questions: 1) What is the degree of polyandry in the UK *B. hypnorum* population? 2) Of queens mating multiply, what is the frequency distribution of the numbers of males that they mate with and hence what is the mean mating frequency per queen? 3) Over what distances do workers from *B. hypnorum* colonies forage in a typical suburban landscape in southern UK? 4) What are the nesting density and between-year lineage survival of *B. hypnorum* in the study landscape? Additionally we sought to use the data collected to investigate whether this *B. hypnorum* population was genetically structured at the scale studied?

4.2 Methods

Bumblebee sample collection

As described in Chapter 3, which reports data from the same samples, *Bombus hypnorum* workers were collected from a 2 x 2 km sampling area, on the western edge of Norwich, UK. The position of the sampling area's southwestern corner was: 52°36'56.12"N, 001°14'00.39"E. The sampling area comprised a mix of suburban residential areas, parks, woodland, semi-natural areas and university campus which was taken to typify lowland areas of the UK not used for agriculture. Workers were sampled in two successive summers during 15th of May – 16th June 2014 and 28th May – 1st July 2015 This time period straddles the peak of observed worker abundance for *B. hypnorum* for the locality (Crowther *et al.* 2014). *B. hypnorum* was first recorded in the area, i.e. the same 10 x 10 km grid square, in 2008 (BWARS). In order to evenly distribute both sampling effort in time and space and the locations of sampled workers in space the sampling area was split into 16 evenly sized (25 ha) divisions, hereafter: 'sampling squares'. In each year *B. hypnorum* workers were sampled by free-searching all the publicly accessible suitable habitat of every sampling square, using a net to capture

all encountered workers (from flowers or while free-flying) until either 40 workers had been caught from that sampling square or three two hour searches on separate days had been completed. Sampling took place during dry weather when air temperature was 15^{o}C or higher, during the hours 1000 - 1700. Tissue for DNA extraction was non-lethally sampled by temporarily restraining the worker and clipping the tarsal tip of a mid-leg (Holehouse, Hammond & Bourke 2003) and storing in 100% ethanol in a 1.5 ml tube at room temperature until later extraction. Additionally, for each worker sampled the exact location of capture was recorded using a Garmin eTrex handheld GPS receiver, with an accuracy of approximately 4 m.

Whole *B. hypnorum* queens were collected from five sites, which were public parks selected for their high density of early-season flowering plants, all within 10 km of the worker sampling area (Appendix 4.1). All queen sampling took place during March - April in 2014 and 2015. Each site was searched freely for 2 - 4 hours and all encountered queens captured and frozen live at -20^{o}C and kept frozen until later dissection.

Spermathecal dissection

Queens were dissected under a stereomicroscope at 40 x magnification, in order to isolate the spermatheca (Appendix 4.2). While still frozen, the gaster (major part of the abdomen) was cut from the rest of the body and each abdominal sternite was cut using micro-dissection scissors such that an incision ran centrally along the full length of the gaster, on the ventral side. Care was taken not to incise the still-frozen soft tissues beneath. The cut sternites were then removed by manipulating them with forceps to tear them free from their corresponding tergites. By this stage the soft tissues exposed by the removal of the sternites had usually thawed, and the gut was teased out using forceps without displacing the ovaries. The spermatheca was then visually located. If the ovaries had not been displaced it could be found attached to the junction of the ovaries at the bursa, to which the spermatheca is attached by a short duct usually extending towards the apex of the gaster. If the sting was in a retracted position, the sting was extended by pressing it with a needle to expose the bursa and spermatheca. The spermathaca was held with fine forceps by the sperm duct and torn from its attachment to the bursa. The isolated spermatheca was suspended in a small drop of distilled water on a slide and manipulated with fine needles to separate the mass of stored sperm from any spermathecal (queen) tissue as completely as possible. To minimise contamination between samples, tools and slides were cleaned using bleach after every individual dissection. Dissections were carried out in batches of five, with each batch including a negative control in which the same tools, slides and water source were used to isolate a droplet of the distilled water. DNA extraction from the isolated sperm and from the negative control samples was performed immediately, with no intervening storage period. Wing muscle was also dissected from each queen, to provide tissue for genotyping of unequivocally queen origin.

DNA extraction and genotyping

As described in Chapter 3, DNA was salt-extracted using a modified ammonium-acetate ethanol precipitation (Richardson *et al.* 2001) from worker (tarsal tip), queen (wing muscle) and sperm (isolated sperm) samples. Tarsal tips were first frozen using liquid N for 2 minutes and crushed to a powder before digestion. In order to increase the reliability of DNA yield the ethanol precipitation

step included incubation at -20°C for 3 hours. Extracted DNA was suspended in weak TE buffer (10 mM Tris.HCL, 0.1 mM EDTA) and kept at -20°C until further use.

Twenty four microsatellite primer pairs, previously characterised from other *Bombus* species (Estoup *et al.* 1995; Reber Funk, Schmid-Hempel & Schmid-Hempel 2006; Stolle *et al.* 2009) were tested on a small number of individuals (10 - 20) to ascertain whether the associated primers could amplify polymorphic loci using *B. hypnorum* DNA as template. This gave a set of twenty polymorphic microsatellite markers used for later analyses, while four loci found to be monomophic or to not amplify at all were excluded (Appendix 3.2). For PCR amplification, the 20 polymorphic loci to be screened were divided into three multiplexes. The multiplexes were designed using Multiplex Manager v1.2 (Hollely & Geerts 2008), with the minimum distance between same-dye markers as 14 base pairs and a complementarity threshold of 7 base pairs. Multiplex characteristics and fluorescent dyes used are summarised in Chapter 3, Table 3.1. PCR was carried out in a 2 µl microlitre reaction volume in 96 well plates. Where extraction yields permitted, up to 15 ng of sample DNA was added to each reaction well. In order to maintain consistent concentrations of all reagents in the small reaction volume, all liquid buffer was evaporated from the DNA solution at 50°C leaving a dry pellet of DNA before the addition of aqueous reagents. Each reaction contained 1 µl of Qiagen Multiplex Master Mix and 1 µl of primer mix with primer pairs at 0.08 – 0.50 M concentrations for queen and worker samples. All reagent volumes were doubled for PCRs using template DNA from sperm samples. Each reaction volume was covered with a droplet (ca. 10 µl) of mineral oil to prevent evaporation. In addition to the dissection controls previously mentioned, each plate included (a) a negative control for the reaction, consisting of all the reagents and primers but no template DNA, and (b) two positive controls using DNA from *B. hypnorum* queens whose multi-locus genotype had been ascertained using multiple single-locus PCRs.

Presumably because of the very small amounts of tissue available, the DNA yields of extractions from sperm were considerably lower than those from worker and queen tissue. Therefore, for the sperm samples, one eighth of the total extraction yield was used as a template for each of the three multiplex PCRs, with the remaining five eighths being kept in reserve.

For queen and worker samples, amplification conditions comprised: an activation step for 15 min at 95 °C, 30 cycles of denaturing for 30 s at 94 °C, annealing for 90 s at 50 °C and extension for 1 min at 72 °C; with a final extension of 5 min at 72 °C. Amplifications of DNA from sperm samples used 45 cycles but otherwise identical conditions.

PCR products were visualised using a 48-well capillary ABI 3730 DNA analyser and a ROX-500 internal size standard (Applied Biosystems). Fragments were sized using GeneMapper 4.0 software (Applied Biosystems, Paisley, UK). Alleles were only accepted when confirmed in two or more individuals. To quantify genotyping error, extracted DNA from 80 - 120 workers (i.e. 12 % - 19 % of samples) were re-run in each of the three multiplexes so as to repeat the PCR and analysis steps for 1,880 locus-level genotypes, covering all loci. These repeated genotypes were compared to the original genotypes to calculate locus-specific allelic mistyping rates. The per-locus mean (range) allelic mistyping rate was found to be 2.26% (0.91 – 3.17 %). No negative controls contained peaks that corresponded to any amplified alleles. Four workers were excluded from further analyses that had peaks corresponding to three alleles at one or more loci; it was not possible to determine whether they were triploid or the original samples were contaminated. In total, 44 queens and their

corresponding sperm samples and 645 workers (375 from 2014 and 270 from 2015) were sampled and genotyped.

All data handling and statistical analysis was executed in R version 3.1.2 unless otherwise stated (R Development Core Team 2011). The genotypes of all worker samples were tested for deviations from Hardy-Weinberg equilibrium (HWE) within both years, corrected for multiple comparisons, using the R package 'adegenet' (Jombart 2008). Years were treated as separate subpopulations because, due to the recent colonisation of the study site and surrounding area, it is possible that the local population was structured temporally. This is because between-year genetic mixing within a population that was structured at larger spatial scales could be expected to create temporal structuring of the population at the study site. These analyses used all workers, some of which would have been full or half-sisters. This should not have introduced bias, as offspring genotypes can be taken to be a random sample of parental genotypes. Rather, this potentially made the test more conservative by inflating degrees of freedom. The frequencies of null alleles for all loci were estimated using the program Cervus 3.0 (Kalinowska, 2007) Pairwise tests for linkage across all combinations of the twenty loci were implemented using functions from the R package 'pegas' (Paradis 2008). To meet the assumptions of the analysis, loci were excluded from the colony assignment (below) that exhibited one or more of: (a) significant deviation from HWE in both years after correction for multiple comparisons; (b) null allele frequencies in excess of 0.1 (Dakin and Avise, 2004); or (c) significant linkage with another, more informative locus, after correction for multiple comparisons.

Mating frequency of queens

Confidently assigning colony membership requires *a priori* information on the likely population-level mating frequency of queens. Since there is no estimate of queen mating frequency for the UK *B. hypnorum* population, we estimated this frequency by comparing the genotypes of the sperm samples to the genotypes of the queens from which the spermathecae were dissected and estimating the likely number of males that contributed to each sperm sample.

Although care was taken during the spermathecal dissections to separate the sperm from all queen tissue (ducts, glands, membranes etc.), contamination of the sperm samples with queen tissue cannot be excluded. However, if it occurred the amount of queen tissue contamination would have been low relative to the amount of male tissue (sperm). Hence queen DNA would have been present in the sperm samples at low copy number relative to sperm DNA and so, during PCR, should not have amplified to the same extent as the sperm DNA. Nonetheless, if the sperm sample genotype was found to contain any allele shared with the corresponding queen (henceforth a 'shared allele'), then before estimating the queen's mating frequency we needed to assess whether the allele was more likely to have originated from the sperm or the queen. For this purpose, we made two assumptions. First, if a shared allele arose from queen contamination, then both the queen's alleles for that locus (assuming the queen was a heterozygote) should have amplified and been present in the sperm sample genotype. Therefore a shared allele that was not accompanied in the sperm genotype by an allele identical to a heterozygote queen's other allele at that locus was deemed to be a true male allele. Second, we assumed that queen contamination, if present, would result in a higher frequency of shared alleles than would be expected by chance, given random mating. This assumption was applied using independent information regarding the queen genotypes (from

genotyping the queen wing muscle samples) and population allele frequencies (from genotyping the worker tarsal tip samples). We implemented this procedure via simulation, and so identified which sperm samples were likely to be have been contaminated as the ones in which the corresponding queen's alleles appeared at a rate across loci higher than would be expected by chance assuming that they really had come from her mates.

To perform the simulation (Simulation 1), we calculated, for every locus of every queen, assuming double mating (the commonest mating frequency of polyandrous *B. hypnorum* queens [Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000; Paxton *et al.* 2001]), the probability that her genotype would be matched by the combined genotypes of her two mates. We then ran 10,000 Bernoulli trials of each of these probabilities and, within queens across loci, counted the number of matches. For each queen, the mean number of matching loci across the 10,000 replicates is hereafter referred to as the 'expected number of matches' and, when divided by the number of loci for that queen, gives the 'expected rate of matching'. The mean and variance across queens of the expected rate of matching were then used to calculate a critical value equal to the mean plus two standard deviations. Any sperm sample that matched its corresponding queen sample's genotype at a proportion of loci larger than the critical value was assumed to be contaminated, because matching the queen's genotype at such a high rate would be unlikely due to chance.

For each queen, we then estimated the minimum number of males with which she had mated. This was estimated as the greatest number of alleles from her sperm sample that were (a) not attributable to the queen and (b) supported across two or more loci. Confirmation at two or more loci was required to limit the potential effect of any genotyping errors in the sperm samples as it was not possible to estimate error rates with these samples due to the limited DNA yields. These estimates, averaged across all 44 queens, provided a conservative estimate of the mean (per queen) mating frequency. In order to estimate the uncertainty around this mean, a further simulation was then constructed (Simulation 2).

In Simulation 2, sampled queen genotypes were combined with simulated male genotypes, randomly generated using the population allele frequencies of the workers. The simulated 'true' number of matings was allowed to vary from 1 to 9. Each queen genotype was then paired with 10,000 replicates of simulated sperm genotypes based on each 'true' number of males. The number of male mates of each queen was then counted using a procedure identical to the one described earlier for actual sperm samples. This allowed us to estimate the probability of counting an observed number of males in the sperm samples given the simulated 'true' number. These probabilities allowed us to infer the range of actual mating frequencies that could have led to the observed pattern of mating frequencies.

Colony assignment

The program COLONY v2 (Jones & Wang 2010) was used to assign sampled workers to colonies, on the basis of being full or half-sisters (i.e. maternal but not paternal sisters) with, following Dreier *et al*. (2014), an inclusion probability of 0.8 or more. *Bombus* species follow an annual colony cycle so (even if there is bivoltinism) workers sampled in one year cannot be full or half-sisters of any workers sampled the following year, allowing sisterhoods spanning 2014 and 2015 to be excluded *a priori*. The male mating system was specified as monogamous. This assumption was as made by similar sibship reconstruction studies of bumblebees (Dreier *et al*., 2014). Its justification is that,

while male bumblebees are not obligately monogamous, most *Bombus* populations have a highly male-biased numerical sex ratio and consequently sampling queens mated by the same male is very unlikely. The female mating system was specified as polygamous. Because COLONY v2 does not allow female mating frequency to be specified directly, it was specified indirectly by setting prior values on the relative sizes of maternal and paternal sisterhoods. For a population with female mating frequency *m*, for every *n* offspring who share the same father, on average *mn* offspring will share the same mother. Under the assumption that matrilines and patrilines are sampled independently at rates based on their frequency in the population, our sample should therefore have contained *mn* maternal sisters for every *n* paternal sisters. To estimate *n*, workers were initially assigned to colonies without using *a priori* information on the queen mating frequency, and, based on these assignments, the average size of a full sisterhood was then calculated. The size of full sisterhoods was taken to be reliably estimated by this procedure, as, under haplodiploidy, full sisters will always share a single paternal allele and have one of only two maternal alleles. Consequently, full sisters should be assigned with high accuracy compared to half-sisters. This estimate of *n*, along with the value of *m* estimated above (in 'Mating frequency of queens'), were used within COLONY v2 to set priors of weight 0.25 on the expected size of sampled maternal (*mn*) and paternal (*n*) sisterhoods. This procedure, including the prior weight, followed that recommended within COLONY v2 when the level of confidence in *a priori* knowledge of the mating frequency is relatively low (Jones and Wang 2010). With respect to workers that were assigned to families with multiple patrilines, a maximum number of patrilines per colony was set based on the range of possible individual mating frequencies that Simulation 2 (above) indicated could have been represented by the sampled queens. Colonies that contained patrilines in excess of this maximum were assumed to have been reconstructed in error and therefore reconstructed colonies were only accepted if they contained fewer than the maximum number of patrilines. As an additional test of the robustness of the colony reconstructions, we tested whether, across the sample as a whole, the pairwise distance between the sampling locations of reconstructed full sisters was significantly different from that of reconstructed half-sisters. If half-sisters were reconstructed with appreciably greater error, then this distance should have been greater for half-sisters than for full sisters, because reconstructed half-sisters would then have included a greater proportion of workers that were not in fact from the same colony.

Lastly, to determine whether any of the collected queens were full or half-sisters, a COLONY v2 analysis identical to the one used to assign workers to colonies was performed on the queens' genotypes at the same loci as those used in the worker analysis.

Colony-specific worker foraging distance

To estimate colony-specific worker foraging distance, we first estimated the most likely physical location of a colony (i.e. the position of its nest). This was done by calculating the mean centre of the GPS-determined locations at which all of the workers assigned to a given colony were sampled. Colonies represented by two or more workers with sampling locations separated by more than 4 m (i.e. the precision of the GPS receiver) were used in this analysis, although this resulted in no further exclusions of accepted colonies. A mean centre approach was chosen as it has been shown to produce predicted colony locations very similar to those predicted by alternative but less

parsimonious methods (Redhead *et al*., 2016). However, unlike in the method used by Redhead *et al*. (2016), predicted colony locations were not 'snapped' to suitable land cover types as most types of land cover present in the study area (e.g. gardens, buildings, trees) were suitable for nesting by *B. hypnorum*. The Euclidean distance between the location of each sampled worker and its predicted nest location was then calculated. The colony-specific worker foraging distance was then estimated as the mean of these distances for all workers assigned to a given colony.

Nesting density and lineage survival

Previous studies of bumblebee nest density using assignment to colonies based on microsatellite markers have taken two different approaches to estimating the number of unsampled nests. One method is to use a truncated Poisson distribution to estimate how many colonies were represented in the sample by zero workers, which assumes all colonies are equally detectable (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2009). The second method uses a mark-recapture approach in which colonies can belong to two groups with two different detection probabilities (the two-innate-rates-model; Wood *et al.* 2015). The present study differed in its sampling strategy from these studies, which sampled workers from discrete sites across a landscape. By contrast, in the present study we sampled intensively and continuously across the landscape (via a grid design), with the aim of detecting as many of the colonies present as possible. In addition, it is likely that, instead of there being one or two constant rates of colony detection, actually the detectability of every colony is different. This is because many traits that are presumably associated with the detectability of a colony, such as colony size, vary across colonies. It follows that, with greater sampling intensity, the sampled colonies will present a greater range of detectability and hence that the assumptions of the previous studies will be less applicable.

We therefore applied a method originally devised for estimating species richness from samples that vary in completeness, specifically an 'abundance coverage estimator', hereafter ACE (Chiu *et al.* 2014) This represents the first application of this approach to estimating bumblebee nesting density, and is justified because it is statistically directly analogous to the established use of the ACE for estimating species richness. Moreover, he ACE was specifically devised for estimating species richness in communities of species that vary in abundance and hence in detectability and gives a conservative estimate of the total number of species (Chiu *et al*., 2014). The ACE produces an estimate of species richness based on counts of the individuals detected, using resampling to estimate the 'completeness' of the sample, i.e. the proportion of all the species that have been detected. In the current study we treated each colony as a 'species' and the number of workers that were sampled from that colony as its individual counts. On this basis, estimates of the number of colonies were produced using the R package 'vegan' (Oksanen *et al*., 2016). Sampled workers that were not assigned to colonies were assumed to belong to distinct colonies that were only represented by one sampled worker. For calculating nesting density, the estimated number of colonies was then divided by the area of sampling plus the area of the buffer around its periphery defined by the mean worker foraging distance.

COLONY v2 is able to infer the genotypes of the unsampled mothers of maternal sisterhoods, i.e. the genotypes of unsampled queens from those of workers within sampled colonies. We therefore used COLONY v2 to infer the genotypes of the mothers of the 2015 workers. Following Marshall *et al.* (1998), we then filtered them to include only loci where the genotype was known with a probability

> 0.8. We refer to these genotypes hereafter as the 'inferred queen genotypes'. A further colony assignment using identical settings, but with 2014 worker samples and the inferred 2015 queen genotypes, was used to test whether the queens that founded the colonies sampled in 2015 were full or half-sisters of the workers sampled in 2014. The assignment of a 2015 queen as an inferred sister of a colony of 2014 workers with a probability > 0.8 was taken to indicate that both belonged to a lineage surviving across years, i.e. that the 2015 colony had been founded by a daughter queen produced by the 2014 colony. The metric 'lineage survival' (Carvell *et al*. 2017) was then estimated as the fraction of 2014 colonies that contributed to a colony lineage surviving until 2015.

Isolation by distance

Finally, following Dreier *et al*. (2014), we used the inferred queen genotypes obtained as described above to investigate the fine-scale spatial distribution of *B. hypnorum* nests within the study area. First, based on these inferred queen genotypes, we estimated pairwise relatedness between all inferred colony queens with COANCESTRY (Wang 2011). Second, using the reconstructed positions of the nests of the inferred queens, we ran a linear model to test whether relatedness of queens covaried with the geographic distance between their nests (isolation by distance).

4.3 Results

Hardy-Weinberg equilibrium, null allele frequencies and linkage disequilibrium

Three of the twenty loci significantly deviated from HWE after correction for multiple comparisons, across both 2014 and 2015 worker samples. In addition a further four loci significantly deviated from HWE across only 2015 worker samples (Table 3.2). Six of the twenty loci returned estimated null allele frequencies greater than 0.1. Of these, three were the same loci that deviated from HWE in both years and so these six loci were not used for colony assignment (Table 3.2). No pairwise combination of loci showed significant evidence for linkage disequilibrium after correction for multiple comparisons (400 pairwise comparisons, corrected alpha value = 0.00125, minimum p value = 0.0073). At the 14 retained loci the 645 workers had a median coverage of 11 (interquartile range, 10 - 14) loci.

Mating frequency of queens

None of the 44 collected queens were assigned as full sisters with a probability of greater than 0.8 (range, 0.001 – 0.731), and only two collected queens were assigned as likely half-sisters (probability, 0.832). Therefore, the estimates of mating frequency were conducted using queen genotypes that were largely independent of one another.

For the estimation of queen mating frequency alone, all loci were used. This was because, in this analysis, all inference depended on simulated haploid males and so would not have been affected by deviation from HWE or the presence of null alleles. On this basis, multi-locus genotypes were obtained for all of the 44 sperm samples, at a median (range) of 17 (6 - 19) loci (Appendix 4.3). None of the dissection or reaction negative controls contained any allelic peaks.

The results of Simulation 1 were that, across the sampled queens, the mean expected number of loci at which a queen genotype would be matched by a combination of two random male mates by

chance was 6.03. This gave a locus-level expected rate of matching of 0.369 (standard deviation, 0.075), which in turn gave a critical value of 0.520. This meant that, if a queen's alleles were found in the genotype of the sperm taken from her spermatheca at more than 52% of the loci, then it is unlikely that they were genuinely shared and conversely it is more likely they arose from contamination (Figure 4.2a). For all but one of the sperm samples, the observed rate of matching was above the critical value (Figure 4.2b). Therefore it was assumed that all of the sperm samples may have been contaminated with their corresponding queen's DNA. Therefore, where both of the queen's alleles were present in the sperm sample, they were inferred to be contaminants.

Counting only those alleles in the sperm genotypes that were not inferred to be contaminants across loci for each queen showed that the sample of 44 queens had a minimum mean (range) mating frequency of 1.7 (1 – 3) (Figure 4.3). This is a conservative estimate of the actual mean mating frequency as the power to count further males is dependent on both the queen's and the males' genotypes. The results of Simulation 2 indicated that, as an estimate of the frequency at which queens mate multiply (i.e. once versus twice or more), our methodology is likely to be highly accurate. Only 1.2% of doubly-mated queens were likely to be have been miscounted as singly mated. Triply- and quadruply-mated queens were even less likely to have been miscounted as singly mated, with the estimated proportion of queens in which this would have occurred being 0.02% and 0.0002%, respectively (Table 4.1). The method becomes less accurate and more likely to underestimate mating frequency as the true number of male mates rises. For example, 22% of triply-mated queens would be counted as only doubly mated. This meant that it was not possible to determine the true underlying frequency distribution of levels of queen multiple mating. However, it was possible to estimate the maximum number of mates that a queen may have had in our sample of 44 queens as the largest number of simulated 'true' males that were likely to have been miscounted as the maximum observed number (i.e. 3). This indicates a maximum likely mating frequency of 5, as 6 true males would have been counted as 4, 5 or 6 observed males in 95% of cases (Table 4.1).

Colony assignment

Initial runs of the COLONY analysis without using *a priori* information on the queen mating frequency produced an estimate of the average number of worker representatives of a patriline in the sample of 1.44. This estimate of *n* and the estimate of queen mating frequency (*m* = 1.7) were used as prior values of the estimates of sampled sizes of maternal and paternal sibships (i.e. *mn* = 1.7 x 1.44 and *n* = 1.44, respectively) in the COLONY analysis. In this analysis, a total of 528 of the 645 workers were assigned to 78 colonies with a probability greater than 0.8. Sixteen of these assigned colonies were rejected as they had more than five patrilines (range, 6 - 8), leaving 62 accepted colonies. The pairwise distances between the sampling locations of full sisters were not significantly different from those of half-sisters (*t*-test not assuming equal variances, $t = -1.53$, df = 11.97, $p = 0.152$), which suggests that unrelated workers had not been erroneously over-assigned as half-sisters to the reconstructed colonies.

Colony-specific worker foraging distance

The mean (range) colony-specific worker foraging distance over the 62 accepted colonies was 103.6 m (13.5 m – 460.6 m) (Figure 4.3). The maximum individual worker foraging range was 601 m.

Nesting density and lineage survival

In total, 189 and 89 distinct colonies were sampled in 2014 and 2015, respectively, including colonies represented by just one sampled worker, i.e. the 62 accepted colonies, broken down by year, plus singletons. From the ACE analysis, the total number of colonies present at the study site was estimated to be 1,244 and 350 in 2014 and 2015, respectively (Table 4.2). Significantly more colonies were estimated to be present at the study site in 2014 (95% confidence interval, 1,204 – 1,283) than in 2015 (95% confidence interval, 329 – 372). these values yielded estimated nesting densities of 2.56 and 0.72 colonies ha^{-1} in 2014 and 2015, respectively (Table 4.2).

Fifteen of the 189 colonies sampled in 2014 had one or more of the 2015 mother queens assigned to them, based on the inferred queen genotypes, which suggests a lineage survival probability of 0.07 (i.e. 15/189) between 2014 and 2015.

Isolation by distance

The relationship between pairwise relatedness of the unsampled colony queens and the geographical distance between the estimated positions of their nests was not significant ($F_{1,1709}$ = 1.173, p = 0.279, R^2 = 0.0007; Figure 4.4). Therefore, at the spatial scale studied, there was no evidence for isolation by distance or spatial genetic structure of queen choice of nesting location.

4.4 Discussion

Our results suggest that 34% of *B. hypnorum* queens mate with just one male, that queens overall mated with a mean of 1.7 males and that some individual queens may have mated with up to five males (questions 1, 2). They also show that the mean colony-specific worker foraging distance of *B. hypnorum* in a suburban landscape typical of those in the southern UK was 103.6 m (question 3). *B. hypnorum* appears to nest in suburban areas at potentially high densities that may vary greatly from year to year, with estimated densities of 2.56 and 0.72 colonies per hectare in 2014 and 2015, respectively (question 4). In addition, we found a rate of lineage survival of 0.07 at the study site between 2014 and 2015 and no evidence of spatial genetic structure at the site scale (2 x 2 km).

Queens in the UK population of *B. hypnorum* mate multiply more frequently than was found in *B. hypnorum* queens collected from continental Europe. Across studies from continental Europe with sample sizes of 10 or more queens, the mean mating frequency of *B. hypnorum* queens ranged from 1 to 1.5 (Schmid-Hempel & Schmid-Hempel 2000; Paxton *et al.* 2001; Brown *et al.* 2003). Combined, these and the present findings support the conclusion that the mating frequency of *B. hypnorum* queens may vary geographically (Brown *et al.* 2003), but as yet there is no evidence that the higher mating frequency in the UK either contributes to or is a consequence of the UK range expansion. Polyandry might facilitate range expansion by increasing the effective population size at newly-colonised sites. This is because a given number of colonising queens that are multiply-mated will, on average, have more alleles contained in the stored sperm of their male mates than the same number of singly-mated queens. Nonetheless, even if population-level rates of polyandry were shown to increase closer to the expanding range edge, it would not unequivocally support this hypothesis. This is because causation might actually apply in the opposite direction, as it is not unreasonable to assume that some queens, for example larger queens, might be more likely to both disperse over

large distances and mate multiply. Compared to other *Bombus* species, the rates of multiple mating observed in the current study are high (Schmid-Hempel and Schmid-Hempel 2000). However, while single mating is typical of *Bombus*, several North American species of the subgenus *Pyrobombus*, to which *B. hypnorum* belongs, have also been documented to mate multiply (Payne, Laverty & Lachance 2003).

As far as we are aware no other study has rigorously quantified worker foraging distances of any bumblebee population and found them to be so short. For comparison, Redhead *et al.* (2016) used a worker sampling protocol very similar to the one in the current study to quantify the colony-specific foraging distances of five UK bumblebee species (*B. hortorum, B. lapidarius, B. pascuorum, B. ruderatus* and *B. terrestris*) in an agricultural landscape, and found that they were all much higher (range of species means: 272 – 551 m). One study of four North American alpine bumblebee species has reported very short foraging ranges of 25 - 110 m (Geib, Strange & Galen 2015). However, Geib *et al* (2015) used four discreet sampling sites of 0.79 ha area, with minimum separation of 255 m, so since their estimates of worker foraging distance are less than the minimum resolution to which they could have been measured their low estimates are necessarily an artefact of their sampling design. In addition, it needs recognising that nearly all estimated worker foraging distances, including the present one for *B. hypnorum*, come from single populations, and combining different estimates for single species from different studies shows that worker foraging distance may exhibit considerable within-species, between-population variation (Charman *et al*. 2010).

While the mean *B. hypnorum* worker foraging distance was found to be notably low, the maximum individual worker foraging distance of 601 m was relatively similar to previous estimates of other species' maximum foraging distances. For example, Darvill *et al.* (2004) estimated a maximum foraging distance of *B. terrestris* of 625 m and Knight *et al.* (2005) estimated maximum foraging distances for *B. pascuorum*, *B. pratorum*, *B. lapidarius* and *B. terrestris* of 449 m, 674 m, 450 m and 758 m, respectively. These values, combined with the strong evidence that bumblebee foraging distances are plastic (Carvell *et al.* 2012; Jha & Kremen 2013; Redhead *et al.* 2016), support an interpretation that the density of foraging resources is driving the short-range foraging we observe. This is because our finding that some *B. hypnorum* workers forage at distances similar to the distances reported for other species suggests that the low mean foraging distance estimated in the present study is not an autecological characteristic (i.e. species-level trait) of *B. hypnorum*. Rather, it indicates that, while capable of foraging profitably (e.g. in terms of net energy return) at the longer distances observed in other species, *B. hypnorum* workers in the study population are able to opt to forage more profitably by covering shorter distances.

The UK population of *B. hypnorum* is one of the most rapidly expanding bumblebee populations documented anywhere in the world. Its rapid expansion is likely to be facilitated by habitats similar to the suburban landscape used in the present study (Crowther *et al.* 2014). Hence our results support the predictions of the emerging synthesis (see Introduction) in which bumblebee population dynamics are linked to the local density of foraging resources (Dicks *et al.* 2015; Carvell *et al.* 2016, 2017; Redhead *et al.* 2016). Expending less energy on flying, while foraging profitably at shorter distances, ought to result in potentially higher productivity due to enhanced rates of energy return (Goulson 2010). As we can expect the UK population of *B. hypnorum* to have a high rate of numerical

increase, in sites like the current study site we would expect foraging resources to be at sufficient density to support both a high colony productivity in terms of new queens produced and short worker foraging distances. By documenting a notably short mean worker foraging distance in *B. hypnorum*, the current study supports this interpretation because, at the national scale, the bumblebee population studied is unequivocally successful and has expanded in recent history, and this has most likely occurred by its using habitats similar to the landscape in the current study (Crowther *et al.* 2014).

While it is possible that our estimates of worker foraging distance could be subject to some biases, it is unlikely that the difference between our estimates and the higher estimates for other species' foraging distances from previous studies are attributable to biases. A possible source of bias is overassignment, as the relatively high level of polyandry in the study population of *B. hypnorum* could have led to workers being erroneously assigned to colonies more frequently than in other studies in which queens are monandrous. Relatedness among half-siblings (0.5) is lower than that of full siblings (0.75), making the colony assignments of half-sisters less certain. However, it is most likely that this factor would have biased the estimation of foraging distance upwards. The estimated mean worker foraging distance in the present study (103.6 m) was much smaller than the dimensions of the study area (2 km x 2 km). This means that a worker assigned to a colony in error is more likely to have been sampled further away from the estimated nest position than a worker that had actually originated from the colony. Regardless, since half-sisters were not sampled at significantly greater pairwise separation distances than full sisters, it is unlikely that overassignment had any effect on the estimates of foraging distance. Underassignment cannot be excluded, but again it is unlikely that this biased the estimates of worker foraging distance. This is because a worker not being assigned to its colony in error is likely to have happened at random with respect to the worker's position in the distribution of worker foraging distances.

Our estimates of nesting density are notable as they vary greatly between years and in 2014 are very high compared to estimates for other *Bombus* species in other years (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2005; Charman *et al.* 2010). High nesting density could stem from the high availability, within the suburban study landscape, of the artificial cavities favoured by *B. hypnorum* for nesting (Crowther et al. 2014). Large between-year variation in nesting density points to large demographic flucuations in *B. hypnorum* numbers at a local scale, though such a phenomenon clearly requires further study. In addition, since we are unable to exclude the possibility of underassignment in the colony assignments and since the *Bombus* species in previous studies of nest density are monandrous and therefore less likely to be underassigned, it is possible that the apparently far greater nest density of *B. hypnorum* is at least partly a statistical artefact. This is because underassignment is more likely to produce singletons, i.e. workers from colonies with only one sampled member, which is likely to inflate the estimate of the number of unsampled colonies and hence of total colony number (Chapman *et al.* 2003; Darvill *et al.* 2004; Knight *et al.* 2009; Chiu *et al.* 2014; Wood *et al.* 2015). Nonetheless, our evidence of very high nesting densities in *B. hypnorum* is consistent with its range expansion being associated with high population-level productivity and with the view that suburban habitats are important in the ecology of this species in the UK.

The estimated lineage survival rate between years in *B. hypnorum* (0.07) was low compared to the only other estimate of site-level *Bombus* lineage survival, which was 0.25 across three established

UK *Bombus* from a site in southern England (Carvell *et al.* 2017). However, differences between the studies make it difficult to compare these rates. First, since they exclude lineages of queens that left the study areas, the estimates of lineage survival would only be properly comparable across sites of similar sizes, yet the site used in the current study is much smaller than that of Carvell *et al*. (2017), which was 1950 ha. Furthermore, (Carvell *et al.* 2017) used data from more colony cycle stages and were therefore able to adjust their estimate for imperfect rates of lineage recapture.

The finding that the *B. hypnorum* population at our site exhibited no significant genetic isolation by distance matches the findings of similar analyses of other *Bombus* species (Dreier *et al.* 2014). A lack of genetic structure at this scale (2 x 2 km) is consistent with gene flow and genetic mixing at larger scales. Such larger-scale gene flow is to be expected as in a previous chapter (Chapter 2) we estimated that *B. hypnorum* queens in the UK have an average dispersal distance of 4.3 km and often colonise new sites at distances well in excess of this.

In conclusion, we have applied recent molecular approaches to elucidate some basic ecological parameters for a *B. hypnorum* population within the newly-colonised UK range. At the same time, our findings support the hypothesis that range expansion, population-level productivity and short worker foraging distances are associated with one another and, moreover, characteristic of the expanding UK *B. hypnorum* population.

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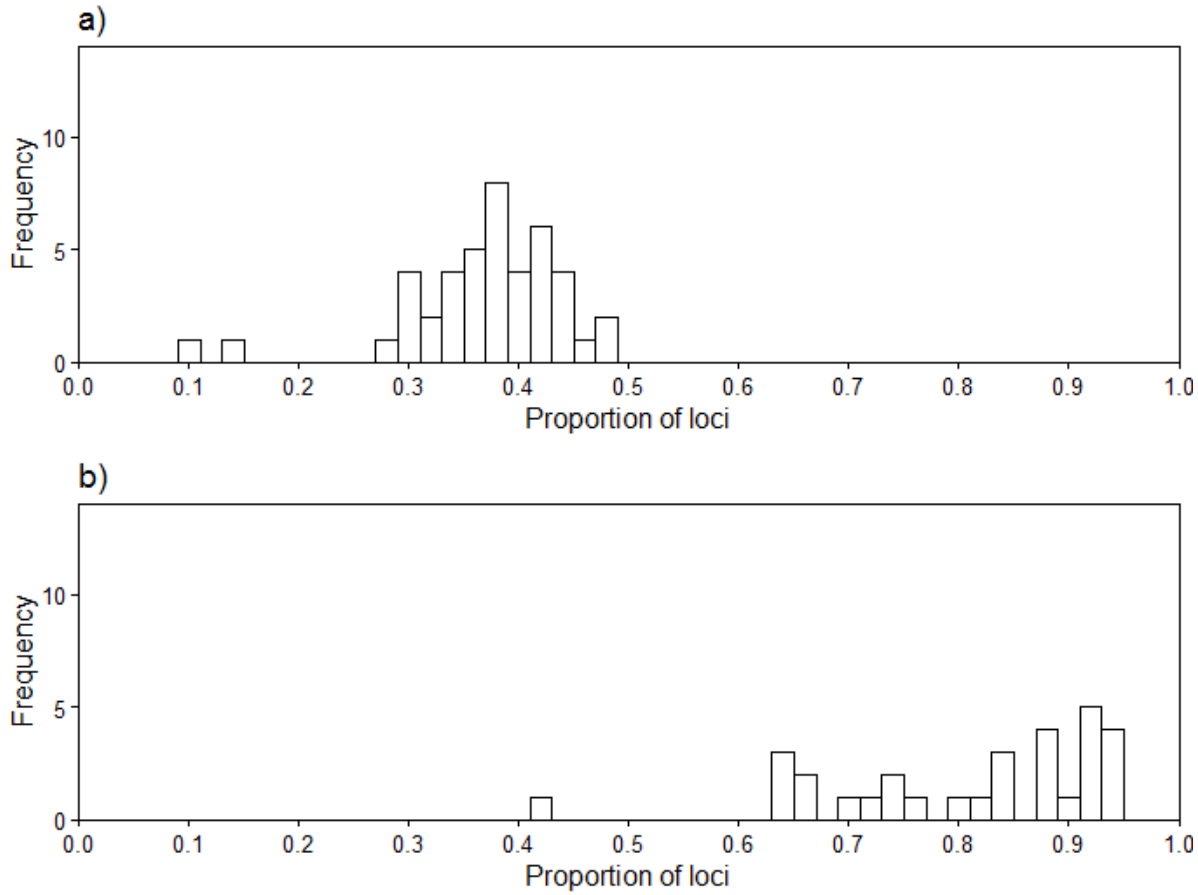

Figure 4.1. Frequency distributions of the proportion of microsatellite loci at which a *Bombus hypnorum* queen's alleles were found, for a given locus, in the genotype of the sperm sample dissected from her spermatheca (*n* = 44 queen and corresponding sperm samples). a) Expected distribution, assuming double mating, based on simulation of 10,000 random pairs of males drawn from population allele frequencies of 645 *B. hypnorum* workers; b) Observed distribution from actual sperm samples.

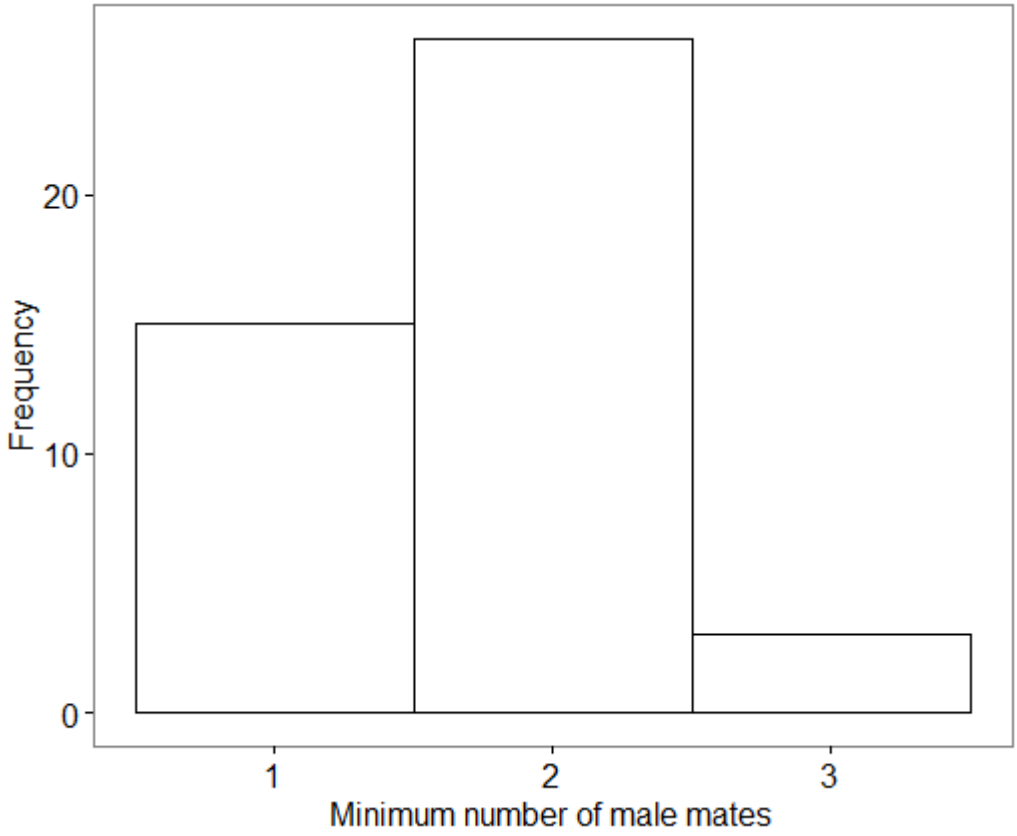

Figure 4.2. The frequency distribution of the minimum mating frequency of 44 *Bombus hypnorum* queens, estimated from the maximum number of non-queen microsatellite alleles, supported by more than one locus, present in sperm samples dissected from the queens' spermathecae.

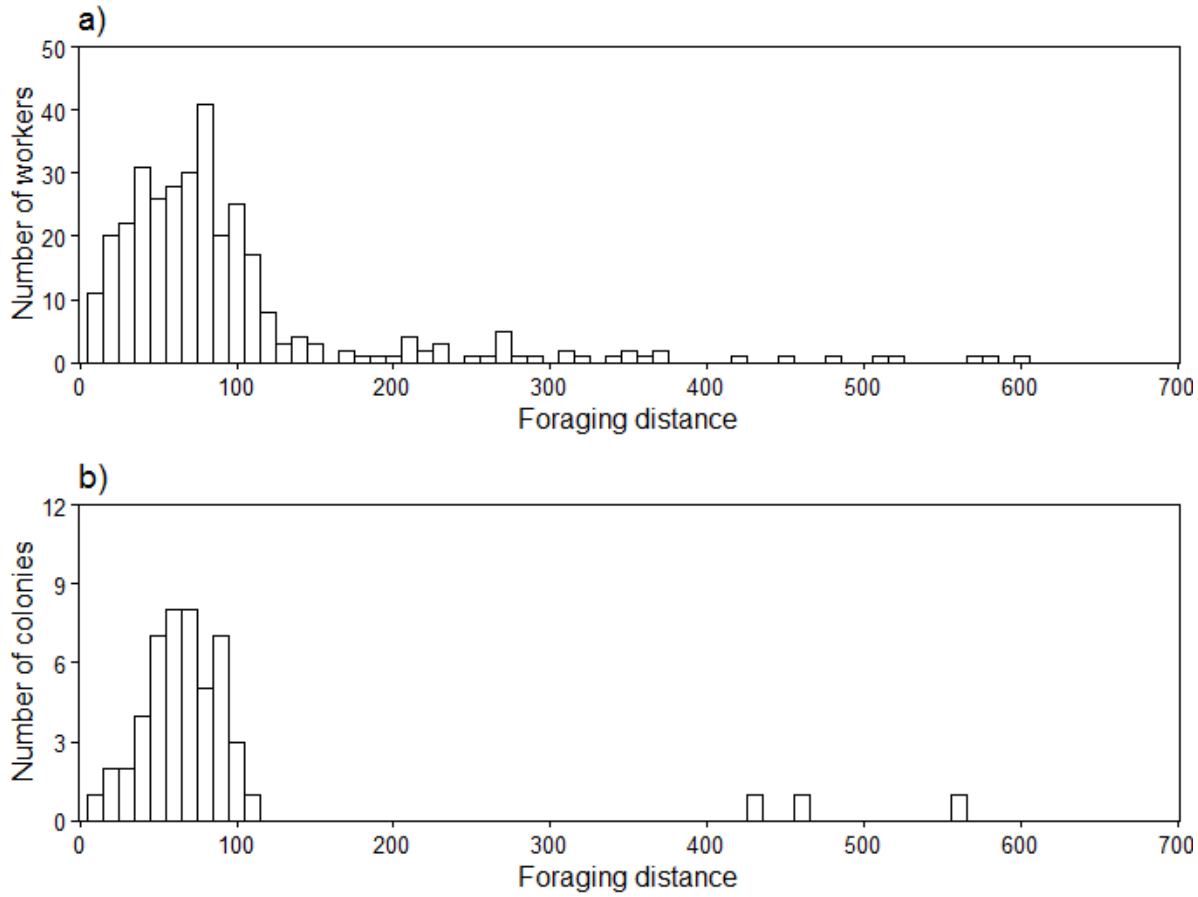

Figure 4.3. Foraging distances (m) of workers of *Bombus hypnorum*. Frequency distribution of a) individual foraging distances of workers (n = 347 workers) and b) estimated colony-specific foraging distances averaged over all sampled workers in accepted colonies (n = 62 colonies).

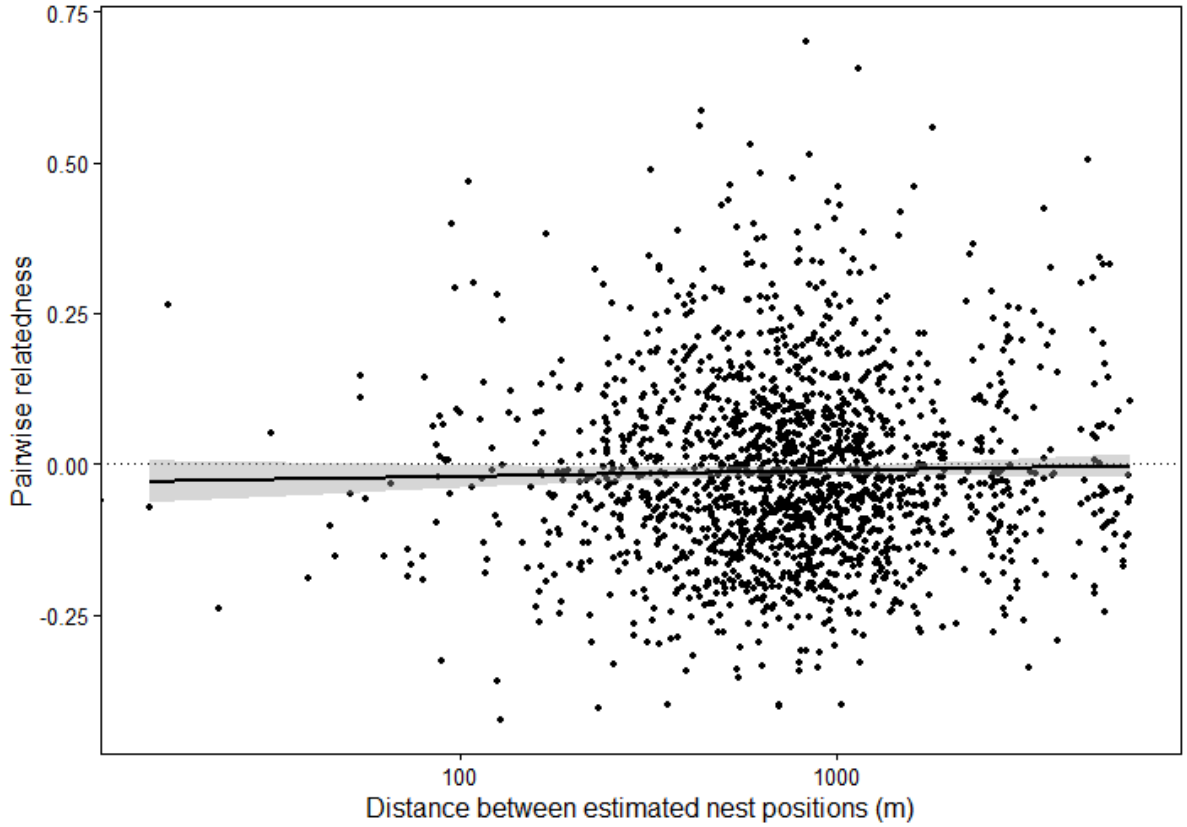

Figure 4.4. Relationship between pairwise relatedness of 62 *Bombus hypnorum* colony queens, whose genotypes were constructed from worker sibships, and distance between the estimated positions of their nests, on a log.$_{10}$ transformed scale, in a suburban 2 x 2 km study area in Norwich, UK. Black line, regression equation (y = [-1.63 x 10^{-2}] + [3.94 x 10^{-6}]x); grey ribbon, 95% confidence interval; dotted line, null hypothesis (y = 0). The slope is not significant ($F_{1,1709}$ = 1.173, p = 0.279, R^2 = 0.0007).

Table 4.1. Results of Simulation 2 for estimating the queen mating frequency of *Bombus hypnorum*, i.e. probabilities of observing 1 – 9 males in sperm samples, given the simulated 'true' number of males. Forty four *Bombus hypnorum* queens were genotyped at a median (range) of 17 (6 - 19) microsatellite loci. Population allele frequencies from 645 *B. hypnorum* workers in the study population were used to simulate sperm genotypes based on 1 - 9 contributing males. Each queen genotype was paired with 10,000 simulated sperm genotypes for each of the 1 - 9 simulated values of the 'true' number of males. Observed number of males was then counted from the combined queen and sperm sample genotype, assuming that alleles shared with the queen were contamination.

	'True' number of males								
Observed number of males	1	2	3	4	5	6	7	8	9
1	0.999852	0.011964	0.000225	2.27E-06	0	0	0	0	0
2	0	0.988036	0.223486	0.01652	0.001395	0.00013	6.82E-06	2.27E-06	0
3	0	0	0.776289	0.685734	0.22685	0.053093	0.012289	0.002914	0.000732
4	0	0	0	0.297743	0.718698	0.638714	0.363143	0.16508	0.069068
5	0	0	0	0	0.053057	0.303498	0.575214	0.655068	0.560993
6	0	0	0	0	0	0.004566	0.049136	0.173875	0.35263
7	0	0	0	0	0	0	0.000211	0.003055	0.016498
8	0	0	0	0	0	0	0	6.82E-06	7.95E-05
9	0	0	0	0	0	0	0	0	0

Table 4.2 Estimates of the number of *Bombus hypnorum* colonies present in and around the 2 x 2 km (400 ha) study site by year of survey. Colonies detected, number of different colonies workers were assigned to; Estimated number of colonies, detected colonies plus estimated number of undetected colonies using an 'abundance coverage estimator' (Chiu *et al*., 2014), standard error in parentheses; Nesting density, colonies per hectare, standard error in parentheses, number of colonies divided by area of sampling area (400 ha) plus area of buffer within the mean colony specific worker foraging distance (103.6 m) of the periphery of the sampling area (86.25 ha); Sample completeness, proportion of estimated number of colonies that were sampled.

Year	Colonies detected	Estimated number of colonies	Nesting density	Sample completeness
2014	189	1244.21 (20.07)	2.56 (0.05)	0.15
2015	91	350.38 (10.86)	0.72 (0.03)	0.25

Chapter 5: Efficient foraging contributes to the ecological success of the Tree Bumblebee, *Bombus hypnorum*, a naturally colonising insect pollinator

Abstract

Insects provide vital pollination services both to ecosystems and, via the pollination of agricultural crops, to human society. Many insect pollinator taxa, including bumblebee (*Bombus*) species, are declining , and widespread changes in the availability of forage plants are implicated in these declines. However, some insect pollinators are expanding their ranges and the extent to which foraging ecology underpins these ecological successes is unknown. *B. hypnorum*, the Tree Bumblebee, has recently rapidly colonised the UK. In the current study we investigated whether the ecological success of *B. hypnorum* is underpinned by low flower constancy, i.e. by foraging over a relatively broad range of food plants (low-constancy scenario), or by an absolute advantage in foraging efficiency relative to other *Bombus* species (absolute advantage scenario). We combined surveys of flower visitation by seven *Bombus* species, estimates of flowering plant community composition and data on handling times of 12,418 individual flower visits captured from digital films. Firstly, we tested whether *B. hypnorum* workers handle the same forage plants faster than workers of other *Bombus* species with either the same or opposite foraging preferences. Secondly, we tested whether, across all *Bombus* species and forage plants, workers handle their preferred forage plants faster. Lastly, we tested whether *B. hypnorum* workers forage on a broader range of forage plants, relative to workers of other species, given the same choice of forage plants. We found evidence that *B. hypnorum* workers handle both their preferred and non-preferred forage plants significantly faster than workers of other *Bombus* species with the same preferences. Across all *Bombus* species and plant taxa, flower handling times were significantly lower on the plant taxon that was preferred by a given *Bombus* species. *B. hypnorum* workers did not forage on a broader range of plants than workers of other *Bombus* species. These results indicate that an advantage in foraging efficiency (absolute advantage scenario) rather than low flower constancy is likely to contribute to the ecological success of *B. hypnorum* in the UK and represent the first evidence linking *B. hypnorum*'s rapid expansion to foraging ecology.

5.1 Introduction

Bumblebees are key pollinators of many wild plants (Ollerton, Winfree & Tarrant 2011) and economically important crops (Garratt *et al.* 2014). Along with those of other insects, their pollination services support food security (Garratt *et al.* 2014; Gill *et al.* 2016), and account for around 10 % of global agricultural production (Gallai *et al.* 2009). However, across both Europe and North America, many bumblebee species are declining (Williams & Osborne 2009; Potts *et al.* 2010; Cameron *et al.* 2011). Understanding the foraging ecology of bumblebees is critical because changes in forage plant abundances are thought to be driving population declines in many species (Carvell *et al.* 2006; Knight *et al.* 2009) and because their ecosystem function as pollinators depends on interactions with their forage plants. Insect-mediated pollination systems are predominantly generalist in that most pollinating insects visit multiple flowering plant species and most insect-pollinated flowering plants are visited by multiple insect species (Waser *et al.* 1996). However, the efficiency of foraging insects is likely to be under strong positive selection as lower handling time

(i.e. the faster manipulation of forage plants) has been shown to be strongly correlated with the net rate of energy return and hence with fitness (Pyke 1978, 1980). Specialisation might therefore be expected to yield better fitness returns if a specialist can handle its preferred food plant faster than a generalist. For example, naïve workers of one of the few specialist *Bombus* species, *B. consobrinus*, have been found to handle flowers of *Anconitum*, their specialised food plant, much faster than naïve workers of generalist *Bombus* species (Laverty & Plowright 1988).

Levels of generalism and specialism are species-level autecological traits. Within different habitats, with different choices of food plants available, a given generalist species might, across sites, exhibit quantitatively different forage preferences (Williams 2005). Specifically, a generalist species might have a higher or lower preference for a given food plant depending on the availability of alternative food plants or it might forage on a broader or narrower suite of food plants depending on the available range of food plants. In bumblebees, such considerations call for site-level measures of workers' foraging ecology.

Clearly, site-level measures such as forage preferences or diet breadth are the result of individual worker's decisions to select plants to visit rather than visit plants at random. Studies of individual worker floral handling times have revealed that, after repeatedly handling flowers of the same plant species, bumblebee workers handle individual flowers faster (Woodward & Laverty 1992; Keasar *et al.* 1996; Heinrich 2004; Raine & Chittka 2007). This effect is not exclusive to bees and has also been demonstrated in a flower-visiting Lepidopteran, *Pieris rapae* (Lewis 1986). The proposed mechanism behind this relationship is hypothesised to be learning mediated by imperfect memory and was originally proposed by Darwin (1876). It is therefore known as Darwin's interference hypothesis (Waser 1986). Under this hypothesis, flower constancy, i.e. foraging preferentially on one flower taxon rather than on alternatives, is rewarded by higher rates of return of energy per unit time due to learned, faster handling of flowers. Low flower constancy, i.e. foraging across flowering taxa in proportion to their rate of encounter, fails to yield this increase because of the cognitive interference created by the requirement to learn to handle different flowers simultaneously (Goulson 2010). As part of the evidence for Darwin's interference hypothesis, there is considerable support for individual-level learning underpinning the foraging preferences of flower visitors (Lewis 1986; Woodward & Laverty 1992; Keasar *et al.* 1996; Raine & Chittka 2007). Differences in learning speed have also been shown to correlate with colony-level performance metrics and therefore are likely to correlate with fitness (Raine & Chittka 2008; although see Evans, Smith & Raine 2017). However, by forgoing flowers of other plant taxa to seek out the preferred taxon, foragers have to travel further, and hence there is a trade-off between the rate of energy return as a function of flower-handling times and energy expended on flight as a function of foraging distance.

This study focuses on the foraging ecology of *B. hypnorum* workers and those of co-occurring *Bombus* species in the United Kingdom. *B. hypnorum* is a recent natural colonist of the UKand has undergone a rapid expansion of its new British range. *B. hypnorum* has a very large Palaearctic distribution, which extends from Western Europe in the west to Japan in the east, and from the Kola Peninsula in arctic Russia in the north to the Himalayan Mountains in Nepal in the south (Goulson & Williams 2001). Since it was first recorded near the southern coast of England in 2001 (Goulson and Williams, 2001), in just 16 years *B. hypnorum* has expanded its range by 900 km and it now occurs

throughout all of England and Wales and in much of Scotland. It is therefore reasonable to assume that colonies of *B. hypnorum* are on average successful at producing enough new queens to consistently colonise new sites. Across an urban-rural gradient typical of southern England, *B. hypnorum* workers occurred much more frequently in suburban and wooded landscapes (Crowther, Hein & Bourke 2014). Recent evidence suggests that, in suburban UK landscapes, *B. hypnorum* has short worker foraging distances, relative to other UK *Bombus* species in rural landscapes, with a mean colony specific worker foraging distance of just 104 m (Chapter 4). If *B. hypnorum* colonies can be provisioned by foraging, on average, over such a small distance, their ability to use the available food plants effectively within this foraging radius may also contribute to the association with suburban landscapes found in Crowther *et al*. (2014), so representing an important factor in the success of the UK population. More generally, given the *B. hypnorum* population in the UK is increasing its range, the foraging ecology that underpins its short worker foraging distances is likely to an important factor in its ecological success.

Given that Darwin's interference hypothesis predicts that flower constancy creates a trade-off between flower-handling times and worker foraging distances, the short worker foraging distances of *B. hypnorum* reported in Chapter 4 could be underpinned by one of two scenarios. These two scenarios can be termed 'low-constancy' and 'absolute advantage'. Under the *low-constancy scenario*, *B. hypnorum* workers forage on a broad range of plant taxa and, through exploiting a wider range of food plants than workers of other *Bombus* species, do not have to fly relatively far from their nests. With respect to the trade-off between handling times and foraging distance, *B. hypnorum* workers forgo the opportunity to reduce handling times by foraging from a taxonomically broader range of flowers. Under the *absolute advantage scenario*, *B. hypnorum* workers can handle their food plants more efficiently than workers of other *Bombus* species and show the same level of flower constancy. With respect to the trade-off between handling times and foraging distance, they are subject to the trade-off individually, but having an absolute handling time advantage relative to other species means that they can forage over relatively short distances and handle flowers relatively quickly.

To differentiate which of these two possible foraging ecology scenarios underpins *B. hypnorum*'s success in the UK, in the current study we sought to test three hypotheses. Hypothesis 1 was that *B. hypnorum* workers handle flowers faster than expected, based on their relative forage preference ordering, compared to workers of other *Bombus* species. This hypothesis predicts that *B. hypnorum* has lower handling times on its preferred plants relative to other *Bombus* species for which the same plants are also preferred; and the same or lower handling times on its non-preferred plants relative to *Bombus* species that do prefer them. The *low constancy scenario* predicts no support for Hypothesis 1 whereas the *absolute advantage scenario* predicts support for the hypothesis (Table 5.1). Hypothesis 2 was that, across all food plants and *Bombus* species, handling times are lower on preferred plants relative to non-preferred plants. The *absolute advantage scenario* predicts support for Hypothesis 2 (Table 5.1). Finally, Hypothesis 3 was that, relative to workers of other *Bombus* species, *B. hypnorum* workers forage on a broader range of flowering plant taxa, given the same range of flowering taxa to choose from. The *low constancy scenario* predicts support for Hypothesis 3 (Table 5.1).

5.2 Methods

Site selection

Sites selected for the study were those having a high density and diversity of flowering plants that are known to be foraged on by bumblebees. This was done to maximise both the rate at which we could sample foraging workers and the range of plant taxa available for them to forage on, given that a quantitative comparison of *Bombus* species' foraging choices is only valid when all the individual bees are exposed to the same foraging resources (Williams, 2005). On this basis, three sites in or near Norwich, Norfolk, UK, were selected for sampling, and were further chosen as publicly accessible sites approximately 1 ha in area. They comprised two urban parks, Eaton Park (site centre; 52.62072, 1.26103) and Waterloo Park (site centre; 52.64525, 1.28976), and one rural site planted with annual and perennial flower mixes aimed at supporting insect pollinators, High Ash Farm (site centre; 52.57774, 1.29837). The three sites had pairwise separation distances from the nearest other site of 3.2 km, 5.4 km and 7.5 km, respectively

All sampling and digital filming took place between 13 June 2016 and 15 July 2016 between the hours of 0900 and 1800 in dry weather with a minimum air temperature of 15^{o}C.

Foraging preferences

On each site ten transects of 50 x 2 m were placed so as to cover the plants in flower at the time of the site visit. Each transect was walked at a slow pace (approx. 1.5 km h^{-1}) and the species, sex, caste, and forage plant taxon were recorded for all foraging bumblebees observed. When necessary, bees were caught temporarily with a handheld net to confirm species and sex. Species were identified using Edwards and Jenner (2005), workers of *B. terrestris* and *B. lucorum* are impractical to separate in the field so were all recorded as *B. terrestris*. The identification of *B. ruderatus* (one male), a species very similar to the more common *B. hortorum*, was confirmed by an individual with extensive field experience of the species (Nick Owens, personal communication).

To assess the coverage of forage plants, a 2 x 2 m quadrat was placed randomly within three metres of the start, mid-point and end of each transect (i.e. 3 quadrats $transect^{-1}$ or 30 quadrats $site^{-1}$). The quadrat was subdivided into 25 equal area divisions and, for each plant taxon present and currently in flower, the number of divisions containing open flowers was recorded. Plants were identified to genus level or, in some cases, to species. Forage plant coverage for a given site was then measured as the proportion of squares, averaged across all thirty quadrats, occupied by open flowers of each plant taxon.

For each site, the floral preferences of *B. hypnorum* workers and those of workers of all other *Bombus* species recorded on the site's transects, were quantified for each of a set of flowering plant taxa following the method described by Crowther *et al*. (2014). Preferences were calculated using the flower visits of workers only, i.e. not of males or queens, on the site's transects. Preferences were calculated for all pairwise combinations of the selected *Bombus* species and flowering plant taxa that met some minimum conditions to avoid small-number encounter rates of workers in the handling time analyses. These conditions were that, for a flowering plant taxon to be included as a visited forage plant species, it had to appear in four or more of the site's quadrats and to have been observed being foraged at by at least one *Bombus* worker on the site's transects.

For the included pairwise combinations of *Bombus* species and plant taxa, we then compared the proportion of a given *Bombus* species' worker foraging visits that were to a focal plant taxon to the proportion of available flowers that were represented by that plant taxon. When these proportions were the same, we inferred that workers of the given *Bombus* species had no preference for that plant taxon, whereas any deviation of the proportion of visits above or below the plant's relative abundance indicated a foraging preference or non-preference (avoidance), respectively. Foraging preference was then calculated as: (observed – expected) / expected, where observed = proportion of visits and expected = relative abundance. Comparisons between such preference estimates are only valid where all of the foragers are exposed to the same suite of available forage plants (Williams, 2005), and hence all preferences were calculated and compared only at the site level.

Handling times

To inform the handling time analyses, for each site two plant taxa identified in the foraging preference analysis were selected to meet the following conditions: 1) one taxon was the most preferred forage plant of *B. hypnorum*, i.e. it returned the highest foraging preference for *B. hypnorum* within the site; 2) the other taxon was a less preferred forage plant of *B. hypnorum*, i.e. it returned a lower foraging preference for *B. hypnorum* within the site compared to the first taxon, and also was the most preferred by one of the two most abundant other *Bombus* species (i.e. other than *B. hypnorum*). Within all three sites, two plant taxa collectively met the conditions, except in Eaton Park, where the other two most abundant *Bombus* species both had identical preference ordering to *B. hypnorum*. Therefore at Eaton Park the third most abundant other *Bombus* species, *B. pascuorum*, was selected instead of *B. terrestris*, which was the second most abundant. Overall, selecting a pair of plant taxa meeting these conditions meant that, on each site, handling-time comparisons could be made between *B. hypnorum* and two *Bombus* species one of which had an identical preference ordering for the two plant taxa and another which had an opposite preference ordering (Table 5.2). The pair of *Bombus* species used for the comparison of handling times with *B. hypnorum* on a particular site are hereafter referred to as the 'selected *Bombus* species' and the pair of plant taxa selected for comparison are hereafter referred to as the 'selected plant taxa'.

Within each site, four patches of area 4 m^2, with minimum between-patch separations of 20 m, of each of the selected plant taxa were then marked out. Where individual plants were large, these patches included only a portion of the flowers belonging to an individual plant and when individual plants were small, the patches included the flowers of several individual plants.

Using a handheld digital camcorder (Sony Handycam DCR-SR32E), we digitally filmed ten workers of *B. hypnorum* and ten workers of each of the selected *Bombus* species as they were foraging on the given plant taxon within each of the four marked patches. Workers were selected for filming as and when they were observed to be foraging on a patch. Each filming bout covered sequential flower visits by an individual worker, hereafter a 'foraging sequence', including 10-15 visits. This procedure yielded 40 foraging sequences per *Bombus* species per plant taxon per site, resulting in a maximum sample size of 720 foraging sequences for the study as a whole (40 foraging sequences x 3 *Bombus* spp. x 2 plant taxa x 3 sites). In order to avoid biases from temporally correlated confounding effects (e.g. weather conditions), filming at a given marked patch went on for no more than 20 minutes and when possible the taxon of plant on which filming was conducted was alternated. While filming, investigators kept between 1 m and 1.5 m from the worker being filmed in order to minimise

disturbance to the workers while also ensuring film of sufficient quality for efficient data capture. If the worker being filmed left the marked patch, then the film was retained if the foraging sequence included at least 10 flower visits. When multiple workers of the same species as the focal worker were foraging within the marked patch during filming, a second investigator watched them to minimise the chance of filming the same worker for more than one foraging sequence. All digital filming of foraging sequences took place within five days of the observations of foraging preference at that particular site. Filming took place only in dry weather with a minimum air temperature of 15^{o}C.

Following completion of digital filming in the field, the digital film of each foraging sequence was played back on a monitor in the laboratory and the event-logging software BORIS (Friard & Gamba 2016) was used to extract the handling time of every flower visit and intervening flight time within each foraging sequence. Events during playback were logged by a single observer watching the digital films at half speed and keying each event as it occurred on the film to a keyboard as specified by the software. The observer was not blind to the treatment, as the *Bombus* species and plant taxa were readily identifiable on the digital films. Handling time was defined as the time to the nearest 0.01 seconds between the worker landing on a floral unit and leaving the same floral unit, and flight time was defined as the time to the nearest 0.01 seconds between the worker leaving a floral unit and landing on the next floral unit within the foraging sequence. For the purposes of this study a floral unit was defined as all of the inflorescences on a single flower spike, and so in some flower taxa the floral unit may have comprised multiple inflorescences and in others just a single inflorescence. A worker was considered to have landed when any part of her body was in contact with the flower and the handling time of the flower visit was only used if the worker was seen to probe the flower with her proboscis. No distinction was made between nectar foraging and pollen foraging.

Diet breadth

Diet breadth was calculated as rarefied diet breadth, i.e. by using resampling with replacement to estimate the number of plant taxa foraged on by twenty workers of a given *Bombus* species on a site's transects. This procedure was used so that estimates of diet breadth were not biased by variation in the abundances of the different *Bombus* species, which might have arisen because a species that was locally less abundant would have been observed foraging fewer times and would therefore be likely to have been observed foraging on fewer species of flowering plants. Diet breadth was only calculated for *Bombus* species for which at least twenty workers were recorded at a site. Since the species identities and relative abundances of available forage plants varied across sites, diet breadth across *Bombus* species was only compared within sites. Estimates with standard errors were calculated using the 'rarefy' function from the R package 'vegan' (Oksanen *et al.* 2016).

Statistical analysis

In total, 12 h 36 min of usable digital film was recorded covering 675 foraging sequences, which contained a total of 13,725 flower visits (Some filmed foraging sequences were not usable because of poor image quality). Of the 13,725 visits, 1,307 either could not be accurately timed or could not be confirmed to include probing of the flower with the proboscis, leaving final sample sizes of 675 foraging sequences containing 12,418 flower visits, each of which yielded an individual flower handling time .

Hypothesis 1: differences in handling time across Bombus species within plants

To test for significant differences in handling time across *Bombus* species within selected plant taxa and sites, a separate linear mixed effects model was fitted, using R package 'lmerTest'(Kuznetsova, Brockhoff & Bojesen Christensen 2016), to data for each plant species and site combination. Models were fitted with handling time per floral unit as the response variable, *Bombus* species as a fixed predictor and nested random effects for the plant patch and foraging sequence. A further analysis was conducted to estimate the effect sizes of any significant differences found in the test of Hypothesis 1 to the currency of overall rate of flower visiting. Specifically, an additional model was fitted to predict the total of handling time and the preceding flight time, hereafter 'latency', with identical fixed and random predictors. Since the parameters of this model represent the estimated time in seconds from the beginning of an average flower visit to the beginning of the next flower visit, the linear predictor could be used to calculate the expected number of flowers visited in a given period. In order to ensure that these differences were due to faster handling of flowers and not faster flight between flowers, identical models were also fitted with flight time as the response.

Hypothesis 2: differences in handling time across preferred and non-preferred plant taxa

We tested for significant differences in handling time across preferred and non-preferred plant taxa. This necessitated making comparisons across flower taxa with different morphologies, such as multiple versus single inflorescences per flower spike. Therefore, to control for differences in the mean handling time and variance of handling time across different plant taxa, within each flower taxon handling time was standardised so that the within-flower taxon mean handling time was set to zero and its variance was set to one, hereafter 'standardised handling time'. A linear mixed effects model was then fitted, using R package 'lmerTest'(Kuznetsova *et al.* 2016), to all of the handling time data with the standardised handling time as the response variable, preference/non-preference of the *Bombus* species for the plant taxon as the fixed predictor, and plant patch and foraging sequence as nested random effects.

Hypothesis 3: differences in diet breadth across Bombus species

Hypothesis 3 was tested by using the rarefied estimates of diet breadth and their standard errors to calculate confidence intervals for the diet breadth of each *Bombus* species at each site.

5.3 Results

In total 930 *Bombus* individuals from nine species were recorded on the transects across the three sites from approximately 20 minutes of transect-walking time per site (Appendix 5.1). In addition, 34 different plant taxa were recorded in flower across the quadrats (Appendix 5.2).

Twenty-one of the recorded plant taxa met the minimum conditions to be included in the initial analysis of foraging preferences (Appendix 5.3). The preferences allowed us to select five plant taxa for observations of handling time. Two *Bombus* species were selected per site providing comparisons within plants across *Bombus* species with both the same and opposite preference ordering (Table 5.2).

Hypothesis 1: differences in handling time across *Bombus* species within plants

At Eaton Park, on its preferred plant, *Geranium*, *B. hypnorum* had a handling time significantly lower than that of *B. pascuorum* (p = 0.007), for which *Geranium* was also its preferred plant, and did not have a handling time significantly different from that of *B. pratorum* (p = 0.090), for which *Geranium* was not its preferred plant (Table 5.3, Figure 5.1). On its non-preferred plant, *Salvia*, *B. hypnorum* had a handling time not significantly different from that of either *B. pascuorum* (p = 0.210), for which *Salvia* was its preferred plant, or *B. pratorum* (p = 0.300), for which *Salvia* was not its preferred plant (Table 5.3, Figure 5.1). Neither of these results supported the predictions of Hypothesis 1 (Table 5.1).

At High Ash Farm, on its preferred plant, *Phacelia tanacetfolia*, *B. hypnorum* had a handling time not significantly different than that of either *B. terrestris* (p = 0.382), for which *P. tanacetfolia* was also its preferred plant, or *B. lapidarius* (p = 0.327), for which *P. tanacetfolia* was not its preferred plant (Table 5.3, Figure 5.1). On its non-preferred plant, *Onobrychis viciifolia, B. hypnorum* had a handling time not significantly different from that of either *B. lapidarius* (p = 0.076), for which *O. vicifolia* was its preferred plant, or *B. terrestris* (p = 0.606), for which *O. vicifolia* was not its preferred plant (Table 5.3, Figure 5.1). Neither of these results supported the predictions of Hypothesis 1 (Table 5.1).

At Waterloo Park, on its preferred plant, *Pentaglottis sempervirens*, *B. hypnorum* had a handling time significantly lower than that of both *B. terrestris* (p < 0.001), for which *P. sempervirens* was the preferred plant, and *B. lapidarius* (p = 0.011), for which *P. sempervirens* was not its preferred plant (Table 5.3; Figure 5.1). On its less preferred plant, *Geranium, B. hypnorum* had a handling time not significantly different from that of either *B. lapidarius* (p = 0.082), for which *Geranium* was its preferred plant, or *B. terrestris*, for which *Geranium* was not its preferred plant (Table 5.3; Figure 5.1). Both these results supported Hypothesis 1 (Table 5.1).

In summary, two out of six possible comparisons supported Hypothesis 1 and four did not. Overall, the models indicated that, on the same plant patches, handling times were highly variable between individuals of different *Bombus* species. Parameter estimates of the significant effects summarised above are the estimated differences between species mean handling times and ranged from 0.31 s (standard error, SE, = 0.12) to 1.05 s (SE = 0.38) (Table 5.3). Over none of the comparisons in which *B. hypnorum*'s handling time was significantly different from those of the other *Bombus* species was there a significant difference between *Bombus* species in the flight time between flower visits (Appendix 5.4). Therefore, parameter estimates from models with latency (i.e. handling time + flight time) as the response variable can be used to estimate effect sizes in terms of overall foraging rate. *B. hypnorum's* fast handling of *P. sempervirens* (at Waterloo Park), relative to that of *B. terrestris* and *B. lapidarius*, was equivalent, respectively, to an extra 297 and 121 flower visits per hour of foraging (Table 5.3). *B. hypnorum's* fast handling of *Geranium* (at Eaton Park), relative to that of *B. lapidarius*, was equivalent to an extra 115 flower visits per hour of foraging (Table 5.3). Since these estimates are based on the fixed effects of models for which the random components are fitted to the variation across plant patches used in this study, as predictions they are valid for plant patches with characteristics (e.g. spacing, flower density) similar to those used in the current study.

Hypothesis 2: differences in handling time across preferred and non-preferred plant taxa

Within sites and *Bombus* species, the standardised handling time was significantly lower for workers foraging on their preferred forage plant species than for workers foraging on their non-preferred forage plant species (Table 5.4, Figure 5.2). The effect size was small, since on a preferred plant the mean handling time was just 0.092 within-plant taxon standard deviations lower (β = -0.092, SE =

0.034, d.f. = 540, t = -2.68, p = 0.009). The proportion of variance explained by the fixed component, i.e. foraging preference, was also small (R^2_M = 0.002). The random component of the model, i.e. the component stemming from differences between plant patches and differences between individual *Bombus* workers, explained a much higher proportion of variance, as the conditional R squared (proportion of variance explained by both fixed and random components) was R^2_C = 0.163. Nonetheless, the finding of significantly lower standardised handling times of *Bombus* workers on their preferred forage plants was consistent with the prediction of Hypothesis 2 (Table 5.1).

Hypothesis 3: differences in diet breadth across *Bombus* species

At none of the three sites did *B. hypnorum* have a diet breadth significantly higher than that of any of the other *Bombus* species and at no site did *B. hypnorum* have the highest diet breadth (Figure 5.3). At Eaton Park, *B. terrestris* had a significantly higher diet breadth than both *B. hypnorum* and *B. pratorum* (Figure 5.3). These results did not support Hypothesis 3 (Table 5.1).

5.4 Discussion

To elucidate whether and in what manner foraging ecology might contribute to *B. hypnorum*'s recent range expansion in the UK, we investigated the relationship between the worker flower-handling times of *B. hypnorum* and of other co-occuring *Bombus* species and their foraging preferences. We found some evidence that *B. hypnorum* workers, relative to workers of other species, handled flowers faster than predicted based upon their preference for the focal plant taxon, relative to other plant taxa at the site (i.e., at most, limited support for Hypothesis 1). Specifically, we found this relationship across both the plant taxa we investigated at one of three sites. Firstly, *B. hypnorum* workers handled their preferred plant's flowers, *P. sempervirens*, significantly faster than *B. terrestris* workers that also preferred *P. sempervirens* at the site. Secondly, at the same site, we found that *B. hypnorum* workers' handling times on their non-preferred plant, *Geranium*, were significantly lower than those of *B. terrestris*, which also did not prefer *Geranium* at that site, and were not significantly different from those of *B. lapidarius* which preferred *Geranium* at that site. However, on the four plant taxa we investigated at the two other sites, we found no evidence that *B. hypnorum* workers handled flowers of their preferred forage plant significantly faster. Across workers of all *Bombus* species, handling times were lower when workers were foraging on a plant taxon that was preferred at that site (i.e. support for Hypothesis 2). There was no evidence of *B. hypnorum* workers exhibiting diet breadths significantly greater than those of any co-occurring *Bombus* species (i.e. no support for Hypothesis 3).

Taken as a whole, these findings strongly suggest that *B. hypnorum*'s successful foraging ecology, , cannot be explained by the *low constancy scenario*. The *low constancy scenario* predicts that, at least at some of the sites, *B. hypnorum* workers would have a significantly broader diet than some or all of the other co-occurring *Bombus* species. Furthermore, were this the case then we would not expect to find support for hypothesis 1. This is because, according to the predictions of Darwin's interference hypothesis, foraging with low flower constancy means that a forager is less likely to learn how to handle the flowers of any particular plant taxon faster (Darwin, 1876; Lewis, 1986, Raine and Chittka, 2006).

Conversely, we have found some evidence that *B. hypnorum*'s foraging success is due to its advantage in handling efficiency relative to other *Bombus* species that share similar foraging preferences. This constitutes partial support for the *absolute advantage* scenario. Firstly, we have identified two instances where *B. hypnorum* workers were handling flowers significantly faster than we would expect given the predictions of Darwin's interference hypothesis (Darwin, 1876). Although this results was found on only one of three sites, none one of the four other *Bombus* species studied showed this pattern on any site. Secondly, we have shown that, across all of the *Bombus* species included in the study, on average workers handled their preferred flowers significantly faster than their non-preferred flowers (i.e. support for Hypothesis 2). This supports our interpretation of the results of testing hypothesis 1, as it demonstrates that the general pattern of handling efficiency and floral preferences revealed in the current study are otherwise consistent with the predictions of Darwin's interference hypothesis (Darwin, 1876).Thirdly, we failed to find any support for Hypothesis 3, as at none of the sites did *B. hypnorum* workers have a significantly greater diet breadth than workers of any of the co-occurring *Bombus* species, a result which cannot be explained by the *low constancy scenario*.

These results represent, to our knowledge, the first evidence for links between the ecological success of *B. hypnorum* in the UK and its use of floral resources for foraging. Taken together with earlier findings, specifically that *B. hypnorum* workers in suburban landscapes in the UK have notably short worker foraging distances compared to those of other species in rural landscapes in the UK (Chapter 4), and that suburban landscapes are associated with higher densities of *B. hypnorum* (Chapter 4; Crowther *et al*., 2014), the findings of the current study could indicate one of the underlying mechanisms contributing to *B. hypnorum*'s successful range expansion.

However, at two of the three sites we found no support for Hypothesis 1. One possible explanation for finding only partial support for Hypothesis 1 could be biases resulting from the criteria we used for site selection. In order to maximise both the rate at which we encountered *Bombus* workers foraging and the range of plant taxa across which we could measure *Bombus* workers' preferences, we intentionally selected sites that had a high density and range of flowers that *Bombus* workers forage on. Therefore, by selecting sites that are likely of particularly high value for worker foraging to all *Bombus* species, it is possible that we have selected against the sorts of habitats in which *B. hypnorum*'s hypothesised advantage in flower handling could be expected to be most apparent. Crowther *et al.* (2014) measured *B. hypnorum*'s foraging preferences for a relatively narrow set of plants that occurred on transects placed randomly within urban and rural habitats in Norfolk, UK, and none of those plants were found in the quadrats or observed being visited on the transects at the sites used in the current study. It is therefore possible that, in sites more closely resembling the typical habitat matrix in suburban landscapes, we would find a different result.

Another important caveat is that this interpretation of these results relies on the assumption from Darwin's interference hypothesis that a trade-off exists between handling times and foraging distance. Although this hypothesis is well supported by empirical evidence (Lewis 1986; Woodward & Laverty 1992; Keasar *et al*. 1996; Raine & Chittka 2007; Raine and Chittka, 2008), in the current study or system it has not been explicitly tested. We have shown a relationship between site-level floral preferences and handling times. The more specific question of whether individual-level flower constancy leading to lower handling times via learning is indeed the mechanism behind this relationship was beyond the scope of this study. An alternative model that can explain foraging

patterns of generalist flower visitors is known as the search image hypothesis (Tinbergen 1960). Under this hypothesis, which is not necessarily mutually exclusive with Darwin's interference hypothesis, foragers learn to locate their preferred forage faster, resulting in more rapid encounters with their food plants. While there is little empirical support for the search image hypothesis in general (Guilford & Dawkins 1987), there is some evidence that bumblebees may take longer to locate flowers depending on the background against which they are presented (Goulson 2000). This suggests that bumblebee foraging preferences may be related to foraging success and hence fitness without necessarily having any effect on handling times. Were this the case then it is possible that the differences in handling times we have observed stemmed simply from certain *Bombus* species being suited in some manner to a flower taxon. If so, any marginal returns to specialising on that plant taxon would be a product of shorter searches and not reduced handling times. However, providing that foragers specialise on food plants they can already handle quickly, then we would still expect a positive relationship between handling times and worker foraging distances. Therefore the interpretation of our results with respect to the ecological success of *B. hypnorum* in the UK would still be valid, although with a different causal mechanism.

A possible, but misguided, criticism of these analyses, in which handling time is related to the *Bombus* species preferences revealed on the transects, is that the metrics used are not independent. Hypothetically, a preference of a *Bombus* species for a plant taxon might be inferred from the visitation transects, when infact no such preference exists, simply because the *Bombus* species handles that plant taxon faster and hence more visits are recorded per unit time. However this hypothesis necessarily assumes that all the possible flower visits are 'saturated' and clearly this is not the case, even in habitats with high flower visitation rates at any given moment most flowers are not being visited.

If the pattern of *B. hypnorum* having an efficiency advantage compared to other *Bombus* species in handling flowers extends to plant species used agriculturally, this could result in it being a relatively effective pollinator of some crops. Crowther *et al*. (2014) found that, relative to other *Bombus* species, *B. hypnorum* had a higher preference for foraging on flowers of several flowering trees and shrubs, including wild relatives of fruit crops. Higher flower visitation rates due to faster handling relative to other *Bombus* species would boost the level of pollination services that *B. hypnorum* could be expected to supply at a given level of local abundance, as reflected in visitation rate being a key parameter in models of pollination service provision (Garibaldi *et al.* 2014). Future research is needed to quantify for *B. hypnorum* in the UK (a) flower visitation rate with respect to local abundance, (b) rates of flower constancy and (c) whether these translate into enhanced pollination and economic yields of insect-pollinated crops.

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Table 5.1. Predictions for the three hypotheses tested in the current study, given two scenarios for *Bombus hypnorum* worker foraging ecology. Hypothesis 1 makes no specific prediction for contrasts not detailed here.

Hypothesis	Metric	Foraging scenario	
		Low constancy	Absolute advantage
1. *B. hypnorum* workers handle flowers faster than expected, based on their relative forage preference ordering, compared to workers of other *Bombus* species.	Handling time on preferred plants	*B. hypnorum* ≥ other *Bombus* sp. that also prefers the same plant (no support for Hypothesis 1)	*B. hypnorum* < other *Bombus* sp. that also prefers the same plant (support for Hypothesis 1)
	Handling time on non-preferred plants	*B. hypnorum* ≥ other *Bombus* sp. that also does not prefer the same plant (no support for Hypothesis 1)	*B. hypnorum* < other *Bombus* sp. that also does not prefer the same plant (support for Hypothesis 1)
2. Across workers of all *Bombus* species, handling times are faster when foraging on a preferred plant taxon.	Standardised handling time	No prediction	On preferred plants < On non-preferred plants (support for Hypothesis 2)
3. *B. hypnorum* workers have a broader diet than workers of other *Bombus* species.	Diet breadth	*B. hypnorum* > other *Bombus* spp. (support for Hypothesis 3)	No prediction

Table 5.2 Foraging preferences of the selected *Bombus* species for the selected plant taxa at the three study sites. +, preferred plant; -, non-preferred plant.

	Eaton Park			High Ash Farm			Waterloo Park		
	B. hypnorum	*B. pascorum*	*B. pratorum*	*B. hypnorum*	*B. lapidarius*	*B. terrestris*	*B. hypnorum*	*B. lapidarius*	*B. terrestris*
Geranium	+	-	+				-	+	-
Salvia	-	+	-						
Onobrychis viciifolia				-	+	-			
Phacelia tanacetfolia				+	-	+			
Pentaglottis sempervirens							+	-	+

Table 5.3. Parameter estimates for the difference in mean flower handling time and flower visit latency on the same individual plant patches between individual workers of different *Bombus* species and individual *B. hypnorum* workers. Estimates are from the outputs of a linear mixed model (LMM) fitted to each combination of site and plant taxon and to the stated number (n) of flower visits. Each LMM has nested random effects of four plant patches and i foraging sequences of flower visits, each by an individual worker. Comparison, site and plant taxon combination; effect, factor levels for *Bombus* species ordered relative to *B. hypnorum* (intercept); β, parameter estimate in seconds; SE, standard error; df, degrees of freedom; t, test statistic; p, p value; visits h^{-1}, predicted number of flower visits from an hour of foraging – calculated as 3,600 / linear predictor of latency model. Results that support Hypothesis 1 are bolded and underlined.

Comparison	Effect	Handling time					Latency					visits h^{-1}
		β	SE	df	t	p	β	SE	df	t	p	
Eaton Park,	intercept	3.70	0.66	3.30	5.57	0.009	5.15	0.76	3.41	6.78	0.004	699
Geranium	*B. pascuorum*	1.06	0.38	67.89	2.81	0.007	1.10	0.50	69.19	2.22	0.030	576
n = 1,627, i = 95	*B. pratorum*	-0.47	0.28	71.04	-1.69	0.096	-0.48	0.37	71.22	-1.32	0.192	771
Eaton Park,	intercept	4.74	0.47	6.52	10.15	< 0.0001	5.79	0.52	6.24	11.04	<0.0001	622
Salvia	*B. pascuorum*	0.60	0.47	87.51	1.26	0.210	0.41	0.53	82.58	0.78	0.437	581
n = 1,647, i = 119	*B. pratorum*	-0.47	0.44	81.48	-1.05	0.300	-0.40	0.49	76.62	-0.82	0.413	668
High Ash Farm,	intercept	4.23	0.31	6.68	13.81	< 0.0001	5.68	0.40	5.93	14.23	< 0.0001	634
Onobrychis vicifolia	*B. lapidarius*	-0.56	0.31	102.87	-1.80	0.076	-0.21	0.38	99.33	-0.56	0.580	658
n = 2,176, i = 120	*B. terrestris*	0.16	0.31	104.11	0.52	0.606	0.73	0.38	100.20	1.92	0.058	562
High Ash Farm,	intercept	7.56	0.64	9.65	11.89	< 0.0001	9.08	0.76	7.01	11.90	< 0.0001	396
Phacelia tanacetfolia	*B. lapidarius*	-0.72	0.73	99.08	-0.99	0.327	-0.69	0.78	94.61	-0.89	0.378	429

n = 1,676, i = 118	*B. terrestris*	-0.65	0.74	99.24	-0.88	0.382	-0.67	0.79	94.71	-0.85	0.398	428
Waterloo Park,	intercept	2.79	0.18	5.60	15.18	< 0.0001	4.10	0.31	4.48	13.26	< 0.0001	878
Geranium	*B. lapidarius*	**-0.30**	**0.17**	83.08	-1.76	**0.0819**	-0.41	0.24	89.79	-1.71	0.090	976
n = 2,709, i = 123	*B. terrestris*	0.55	0.18	89.94	3.066	0.0029	0.62	0.25	96.13	2.52	0.014	763
Waterloo Park,	intercept	1.37	0.08	82.35	17.41	< 0.0001	2.34	0.09	80.05	25.358	< 0.0001	1538
Pentaglottis sempervirens	*B. lapidarius*	**0.31**	**0.12**	76.90	2.59	**0.011**	0.20	0.14	74.37	1.45	0.152	1417
n = 2,583, i = 100	*B. terrestris*	**0.52**	**0.13**	87.46	4.104	**< 0.0001**	0.56	0.15	85.38	3.81	0.0003	1241

Table 5.4. Summary of a linear mixed effects model (LMM) to predict flower handling time, standardised within plant taxon, fitted to standardised handling times of 12,418 flower visits from sequential foraging visits by 675 *Bombus* workers to 24 patches of flowers from five plant taxa across three sites. Component, whether effects are treated as fixed or random; effect, variable names; β, parameter estimate; SE, standard error of estimate; df egrees of freedom; t, test statistic; p, p value. Groups, number of levels in random effect; Variance, variance of normal distribution that levels are drawn from. Variance explained by fixed component, R^2_M = 0.002; random and fixed component, R^2_C = 0.163.

Component						
Fixed						
	Effect	β	SE	df	t	p
	intercept	0.115	0.053	25.5	2.171	0.039
	preference	- 0.092	0.035	540	-2.632	0.009
Random						
	Effect	Groups	Variance			
	Bombus individual	675	0.117			
	Plant patch	24	0.050			

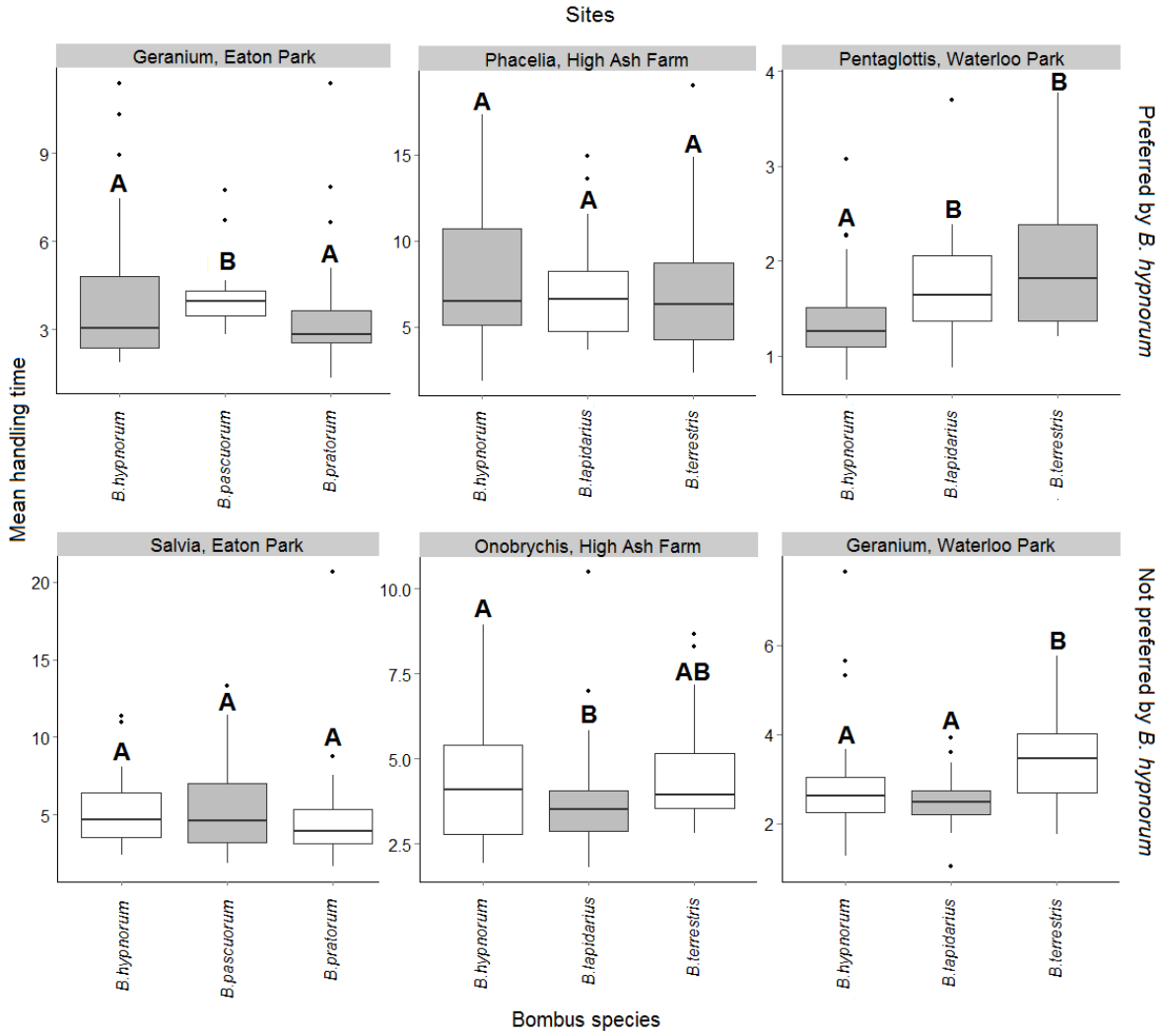

Figure 5.1. Distributions of mean handling times (s) per foraging sequence of flower visits by *Bombus* workers to preferred and non-preferred plant taxa at three different sites. Panel header, taxon of *B. hypnorum*'s preferred or non-preferred plant, followed by name of the site. Upper row of panels, results for *B. hypnorum*'s preferred plant; lower row of panels, results for *B. hypnorum*'s non-preferred plant. Thick black line; median across foraging sequences by individual workers; box, interquartile range (IQR); whiskers, range not including outliers (defined as further than 1.5 x IQR from median); filled circles, outliers; grey box, preferred forage of focal *Bombus* species at that site; white box, non-preferred forage of focal *Bombus* species at that site; shared letters (A/B), no significant difference between subsets shown by LMMs (Table 5.3).

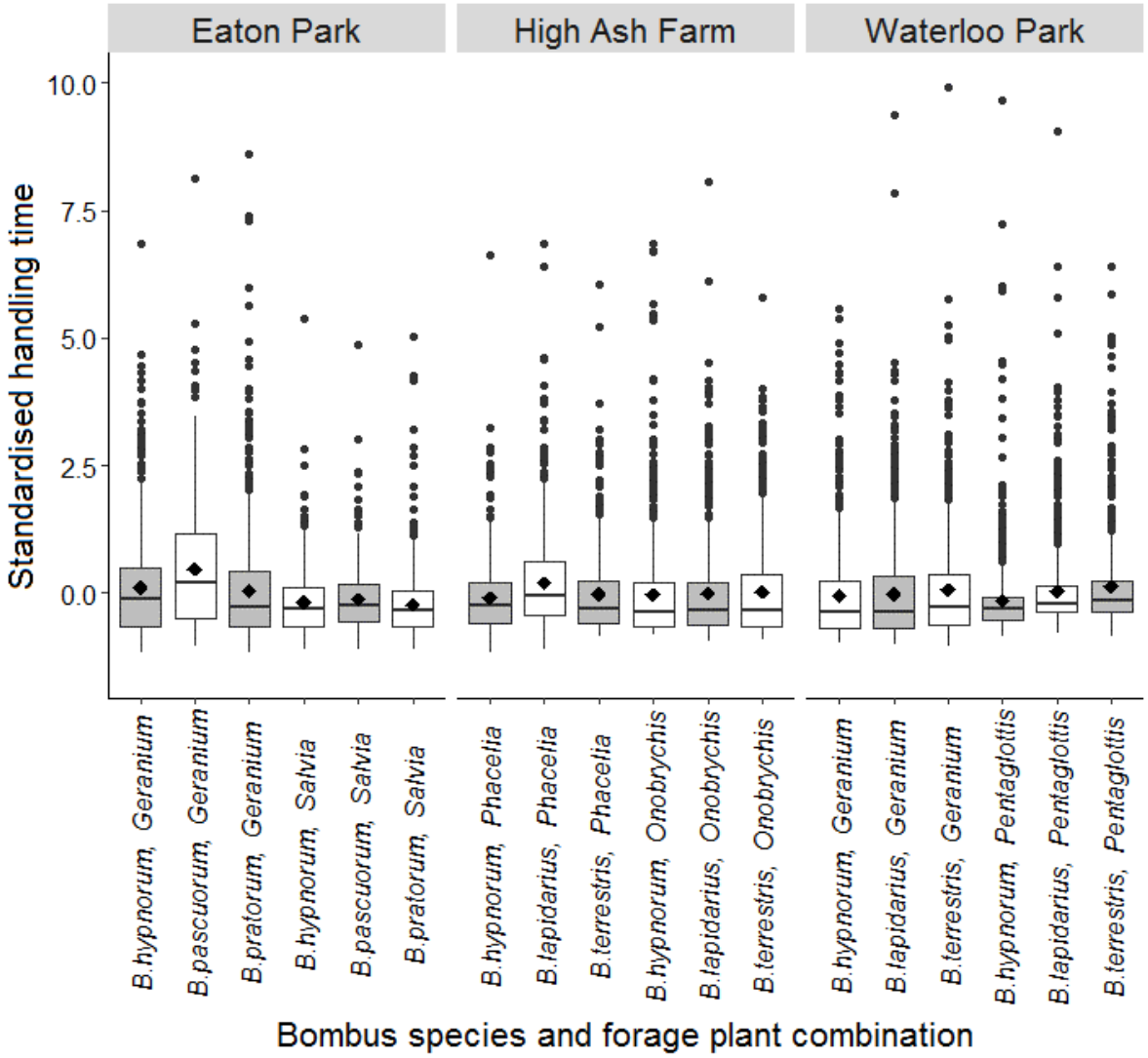

Figure 5.2. Distribution of handling times, standardised within plant taxon (μ = 0, σ = 1), of *Bombus* workers during 12,418 flower visits to preferred (grey boxes) and non-preferred forage plants (white boxes). Standardised handling times on visits to flowers of preferred plant taxa are significantly lower than those on visits to non-preferred flowers (LMM, d.f. = 540, t = -2.632, p = 0.009, Table 5.3). There were 645 visits *Bombus* workers to four marked patches of each of two plant taxa per site. Filled diamond, mean; thick black line; median across individual workers; box, interquartile range (IQR); whiskers, range not including outliers (defined as further than 1.5 x IQR from median); filled circles, outliers. Seven outliers with a standardised handling time greater than 10 are omitted.

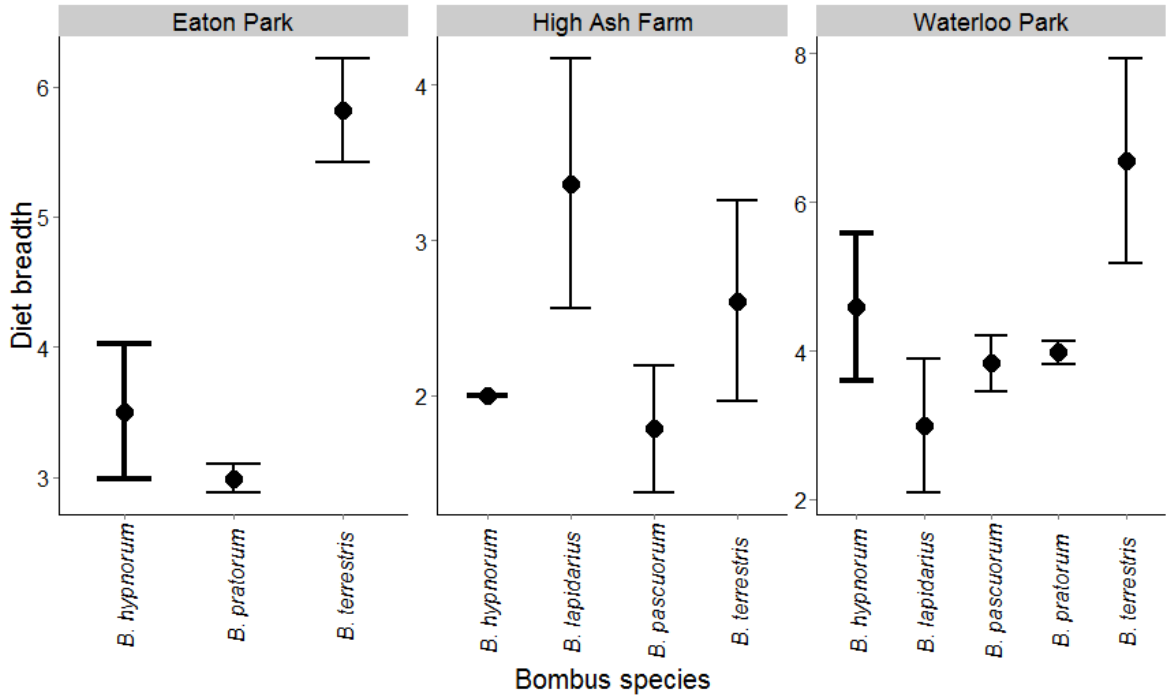

Figure 5.3. Diet breadth (number of plant taxa visited standardised to 20 worker foraging visits, obtained by resampling worker records with replacement) of *Bombus* species on ten 50 x 2 m transects per site. Filled circles, mean diet breadth; error bars, confidence interval of estimate. As shown by overlapping CIs, within sites diet breadth in *B. hypnorum* was never significantly the higher relative to those of other *Bombus* species.

Chapter 6: Concluding remarks

This thesis aimed to investigate the ecology and genetics of the UK population of *Bombus hypnorum*, in order to learn about aspects of bumblebee ecology that will be useful for the conservation of members of the genus as a whole.

In summary, the key findings are as follows. In Chapter 2, we found that *B. hypnorum* has colonised the UK by leptokurtic dispersal, where most new sites are colonised by a subset of individuals that disperse much further than the average. Our analysis suggested that the average colonising queen dispersed 4.3 km between the year of eclosion and the year of colony foundation, that 5 % of colonisers dispersed more than 14.4 km and 1 % of colonisers dispersed more than 23.9 km. In Chapter 3, we found evidence that the UK population of *B. hypnorum* was founded by more than 60 diploid individuals (45 singly mated queens or 30 doubly mated queens) and exhibits levels of genetic diversity intermediate between those of populations of widespread *Bombus* species and those of populations of range-restricted *Bombus* species. In Chapter 4, we found that *B. hypnorum* workers forage over notably short distances in a suburban landscape typical of the habitats that are suspected of facilitating the range expansion in the core of *B. hypnorum*'s UK range. The mean colony-specific worker foraging distance was 103.6 m, which is substantially shorter than the worker foraging distances measured in populations of other *Bombus* species in nearly all previous studies. We also found relatively high levels of polyandry in the sampled queens, with 66% of queens mating more than once. In Chapter 5, we found evidence that an absolute advantage in worker foraging efficiency as measured by flower handling time, rather than high diet breadth, may be contributing to the ecological success of *B. hypnorum* in the UK.

The remainder of this chapter aims to briefly synthesise these results, discuss their broader implications for bumblebee conservation and suggest future directions for research raised by the thesis. A more general discussion of each of the results can be found in each respective chapter's discussion section (Sections 2.4, 3.4, 4.4 and 5.4).

An important implication of the thesis is that the population-level productivity of new queens has major relevance for questions concerning the large-scale ecology of bumblebee populations. For example, the results from Chapter 2, namely that *B. hypnorum* has a leptokurtic dispersal kernel, suggest links between population-level productivity and the meta-population ecology of bumblebees. The proportional increase in the maximum distance one would expect dispersing queens to travel as increasing quantiles of the dispersal kernel are realised is much greater under leptokurtic dispersal than under diffusion dispersal. To illustrate, the 99^{th} percentile of the estimated leptokurtic dispersal kernel was 1.66 times higher than its 95^{th} percentile, whereas the proportional increase in distance dispersed for a diffusion dispersal kernel with the same mean queen dispersal distance was only 1.31 times greater. The effect of population-level production of new queens on the distances over which queens colonise new sites or join other populations is necessarily mediated by the quantiles of the dispersal kernel. This is because the quantiles of the dispersal kernel describe the increase in the expected maximum dispersal distance with greater numbers of potential dispersers.

Similarly, the results from Chapter 4 support the predictions of a synthesis of recent evidence on the effects of landscape-scale habitat quality on bumblebee population processes, specifically the

prediction that landscapes with high-quality habitats can both increase the population-level production and over winter survival probabilityof new queens and permit foraging by workers over shorter distances (Carvell *et al.* 2012, 2015, 2017; Jha & Kremen 2013; Dicks *et al.* 2015; Redhead *et al.* 2016). More precisely, the results of Chapter 4 support the prediction that a range-expanding population, which by definition must have a high population-level production of new queens, should also have low worker foraging distances. However, this assumes that the low worker foraging distance found in Chapter 4 is typical of *B. hypnorum* in the given habitat.

Therefore, combined, the findings of these two chapters suggest that the conservation of fragmented bumblebee populations may be better served by efforts to increase their population-level production of queens, such as by increasing the available area of high-quality habitat, than by increasing connectivity between parts of their distributions, such as with 'stepping stone' habitats. Future work to examine this hypothesis could investigate fragmented populations of species living in island meta-populations (e.g. Darvill *et al.* 2006, 2010; Ellis *et al.* 2006; Goulson *et al.* 2011) in order to determine whether differences in colony-level production of new queens underpin their responses to fragmentation (cf. Carvell *et al.* 2017).

The findings of Chapter 4, taken together with those of Chapter 5, also suggest that the short worker foraging distances and hence the small areas used for foraging by single *B. hypnorum* colonies are not associated with workers foraging over a broad range of plants but rather with workers foraging efficiently over a subset of forage plants. This conclusion has implications for whether *B. hypnorum* and other species are in competition for foraging resources. This is because, if Chapter 5 had found support for the '*low constancy*' scenario, *B. hypnorum* – as a hyper-generalist -- might have the potential to compete with a much larger range of flower-visiting insects. However, our findings indicated that in many situations *B. hypnorum* uses a fairly narrow range of flowering plants and is only more efficient in certain cases. We also found in Chapter 4 that some *B. hypnorum* colonies actually forage over quite large areas (as inferred from their larger foraging distances). It follows that the extent to which *B. hypnorum* and other flower-visiting insects are in competition will be highly context-dependent. This suggests that any studies aiming to address this issue will need to be broad in scope and to study patterns of flower-visiting insect community composition over many different habitats and landscape gradients.

The findings of Chapter 3, specifically that the UK population of *B. hypnorum* is not as genetically depauperate as has been hypothesised (Jones & Brown 2014), raise the possibility that there may not necessarily be anything exceptional about the autecology of the species underpinning its ecological success. Moreover, taken alongside the fact that at least one other range expansion in other *Bombus* species may have been considerably more rapid (i.e. *B. terrestris* in South America; Schmid-Hempel *et al.* 2014), the finding that *B. hypnorum* in the UK is succeeding without a severe genetic load suggests that it is less likely that there is some as yet undiscovered hidden process or factor needed to explain *B. hypnorum*'s ecological success. Nonetheless, the lack of a genetic and hence demographic bottleneck in the UK *B. hypnorum* population, which suggests continuous migration rather than a small founding population, matches the evidence for *B. hypnorum*'s long-term westwards expansion across continental Europe, which would indeed be remarkable. In addition, the findings of Chapters 4 and 5 and those of previous work (Crowther, Hein & Bourke 2014) point to the conclusion that the combination of plentiful foraging resources and nesting cavities in suburban habitats, alongside features of *B. hypnorum*'s spatial and foraging ecology, may

be underpinning *B. hypnorum*'s success. The availability and distribution of floral resources and nesting sites and bumblebees' use of space are widely recognised as key factors connected to population processes of bumblebees more generally (Carvell 2002; Redhead *et al.* 2016; Carvell *et al.* 2017). Overall, therefore, this thesis has made novel scientific advances that will also help inform the conservation of bumblebees and their ecological functions.

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Appendix 2

Appendix 2.1 Uninformative prior distributions for model parameters

Detection sub-models

Both models used a detection sub-model with identical specification; prior distributions of all parameters were uninformative (Expressions 1 -5).

$$\alpha_t \sim normal(\mu_{lp}, \tau_{lp}) \quad (1)$$

$$\mu_{lp} \sim normal(\mu = 0, \sigma = 100) \quad (2)$$

$$\tau_{lp} \sim uniform(0, 0.05) \quad (3)$$

$$\delta_2 \sim uniform(-10, 10) \quad (4)$$

$$\delta_3 \sim uniform(-10, 10) \quad (5)$$

Dynamic dispersal state sub-model

One parameter in the dynamic dispersal state sub-model, $\Psi_{initial}$, was given an informative prior distribution (Chapter 2.2). All other parameters in the dynamic dispersal state sub-model were given uninformative priors (Expressions 6 - 8).

$$\phi \sim uniform(0,1) \quad (6)$$

$$c \sim uniform(0,3) \quad (7)$$

$$\alpha_\gamma \sim uniform(0,12) \quad (8)$$

Static state sub-model

Informative priors were specified on the random walk year effect on occupancy and its precision; all other parameters in the static state sub-model were given uninformative priors (Expressions 9 – 12)

$$\beta_1 \sim normal(\mu_\beta, \sigma = 1000) \quad (9)$$

$$\mu_\beta \sim normal(0, \ \sigma = 100) \quad (10)$$

$$\eta_i \sim normal(0, \ \tau_\eta) \quad (11)$$

$$\tau_\eta \sim t(\mu = 0, \sigma = 1, df = 1)T(0, \infty) \quad (12)$$

Appendix 2.2 Posterior estimates of parameters from the dynamic dispersal model, a Bayesian dynamic occupancy model with colonisation parametrised by a dispersal kernel. The model is fitted to detection/non-detection records of *Bombus hypnorum* on 12,444 visits to 2080 1 x 1 km sites across the UK over 13 years (2001 – 2013). Parameter, term in model and description; Quantiles, 50% is the median estimate and range of 2.5 % - 97.5 % gives 95% credible interval; Rhat, Gelman diagnostic statistic indicates convergence in the range 1 – 1.1. Parameters $\alpha_{1\text{-}13}$ are back transformed by the inverse logistic function to give the conditional detection probability on a visit of list length 1.

Parameter	**Quantile**			**Rhat**
Detection sub-model	**50%**	**2.5%**	**97.5%**	
α_1 yearly detection intercept	0.002018	8.03E-06	0.024741	1.002908
α_2	0.008042	0.000641	0.035571	1.006406
α_3	0.002085	9.02E-06	0.026011	1.001655
α_4	0.001623	7.39E-06	0.018462	1.002082
α_5	0.04867	0.018619	0.10391	1.047321
α_6	0.1054	0.049957	0.19731	1.028633
α_7	0.4286	0.34389	0.5212	1.095564
α_8	0.5095	0.4277	0.59361	1.023854
α_9	0.4987	0.45569	0.5414	1.002614
α_{10}	0.7941	0.7589	0.8268	1.01645
α_{11}	0.4826	0.435	0.5307	1.003339
α_{12}	0.8227	0.7826	0.8575	1.001212
α_{13}	0.6986	0.4691	0.86711	1.001077
δ_2 short list factor effect	-2.391	-2.684	-2.118	1.001626
δ_3 long list factor effect	-1.372	-1.657	-1.109	1.005388
μ_{lp} mean of yearly intercept	-2.005	-4.1311	-0.07675	1.00145
τ_{lp} precision of intercept	0.08511	0.0423	0.21351	1.001951
State sub-model				
Ψ_2 proportion of occupied sites by year	0.2313	0.2106	0.2519	1.02225
Ψ_3	0.2317	0.2115	0.2529	1.022691
Ψ_4	0.2327	0.212	0.2538	1.023354
Ψ_5	0.2332	0.213	0.2548	1.02411

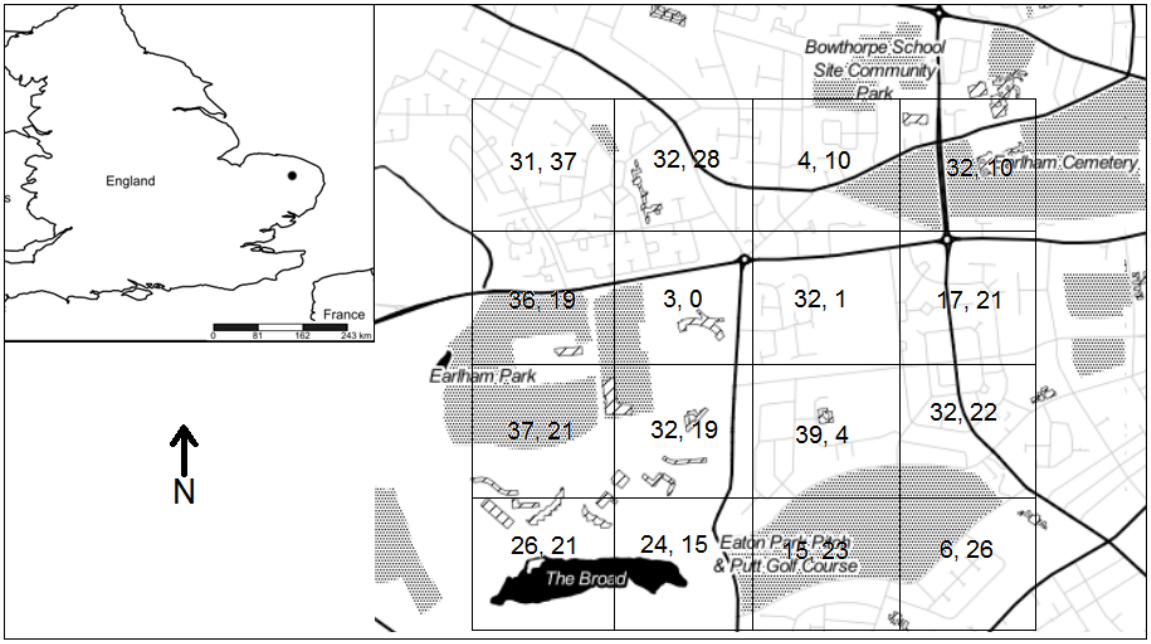
ψ_6	0.2361	0.2159	0.2572	1.031117
ψ_7	0.2413	0.2212	0.2639	1.065923
ψ_8	0.2534	0.2332	0.2764	1.076583
ψ_9	0.276	0.2562	0.2986	1.130802
ψ_{10}	0.3096	0.2913	0.3298	1.067592
ψ_{11}	0.3438	0.3264	0.3644	1.037314
ψ_{12}	0.3688	0.3514	0.3889	1.046253
ψ_{13}	0.3913	0.3736	0.412	1.058166
Φ persistence probability	0.9987	0.9966	0.9997	1.005015
C dispersal kernel shape	0.7715	0.6624	0.87837	1.062014
α_Υ dispersal kernel scale	2.79	2.0988	2.99	1.014345

Appendix 2.3 Posterior estimates of parameters from the static model, a Bayesian occupancy model with a random walk prior on the year to year change in the proportion of occupied sites. The model is fitted to detection/non-detection records of *Bombus hypnorum* on 12,444 visits to 2080 1 x 1 km sites across the UK over 13 years (2001 – 2013). Parameter, term in model and description; Quantiles, 50% gives median estimate and range of 2.5 % - 97.5 % gives 95% credible interval; Rhat, Gelman diagnostic statistic indicates convergence in the range 1 – 1.1. Parameters $\alpha_{1\text{-}13}$ are back transformed by the inverse logistic function to give the conditional detection probability on a visit of list length 1.

Parameter	**Quantile**			**Rhat**
Detection sub-model	**50%**	**2.5%**	**97.5%**	
α_1 yearly detection intercept	0.5941	0.1362	0.9065	1.013324
α_2	0.6352	0.2376	0.9204	1.002236
α_3	0.5895	0.152397	0.9016	1.013955
α_4	0.57875	0.130197	0.898105	1.002462
α_5	0.727	0.4148	0.9478	1.001758
α_6	0.4953	0.2629	0.714802	1.00117
α_7	0.5934	0.479	0.696702	1.000963
α_8	0.6039	0.5057	0.6927	1.001527
α_9	0.4542	0.4073	0.5016	1.001011
α_{10}	0.752	0.7113	0.7894	1.001053
α_{11}	0.4271	0.3795	0.4769	1.001025
α_{12}	0.8097	0.767797	0.8473	1.000975
α_{13}	0.6349	0.435397	0.802	1.001213
δ_2 short list factor effect	-2.4645	-2.76	-2.18498	1.00119
δ_3 long list factor effect	-1.437	-1.71403	-1.162	1.001032
μ_{lp} mean of yearly intercept	0.4618	-0.2271	1.115	1.001863
τ_{lp} precision of intercept	1.136364	1.500582	0.762715	1.001078
State sub-model				
ψ_2 proportion of occupied sites by year	0.001923	0.000481	0.009615	1.001036
ψ_3	0.000962	0	0.008173	1.001486
ψ_4	0.002404	0	0.01298	1.00808

ψ_5	0.01538	0.006731	0.03365	1.002019
ψ_6	0.05	0.02404	0.09471	1.001039
ψ_7	0.1385	0.09904	0.1918	1.001007
ψ_8	0.1933	0.1447	0.2534	1.001132
ψ_9	0.5216	0.4567	0.5904	1.001007
ψ_{10}	0.7207	0.6678	0.7736	1.001396
ψ_{11}	0.7909	0.7202	0.8591	1.002965
ψ_{12}	0.7966	0.7269	0.8644	1.001165
ψ_{13}	0.8567	0.6798	0.9697	1.001557
τ_η *precision of random site effect*	0.03323	0.01444	0.06216	1.030582

Appendix 3



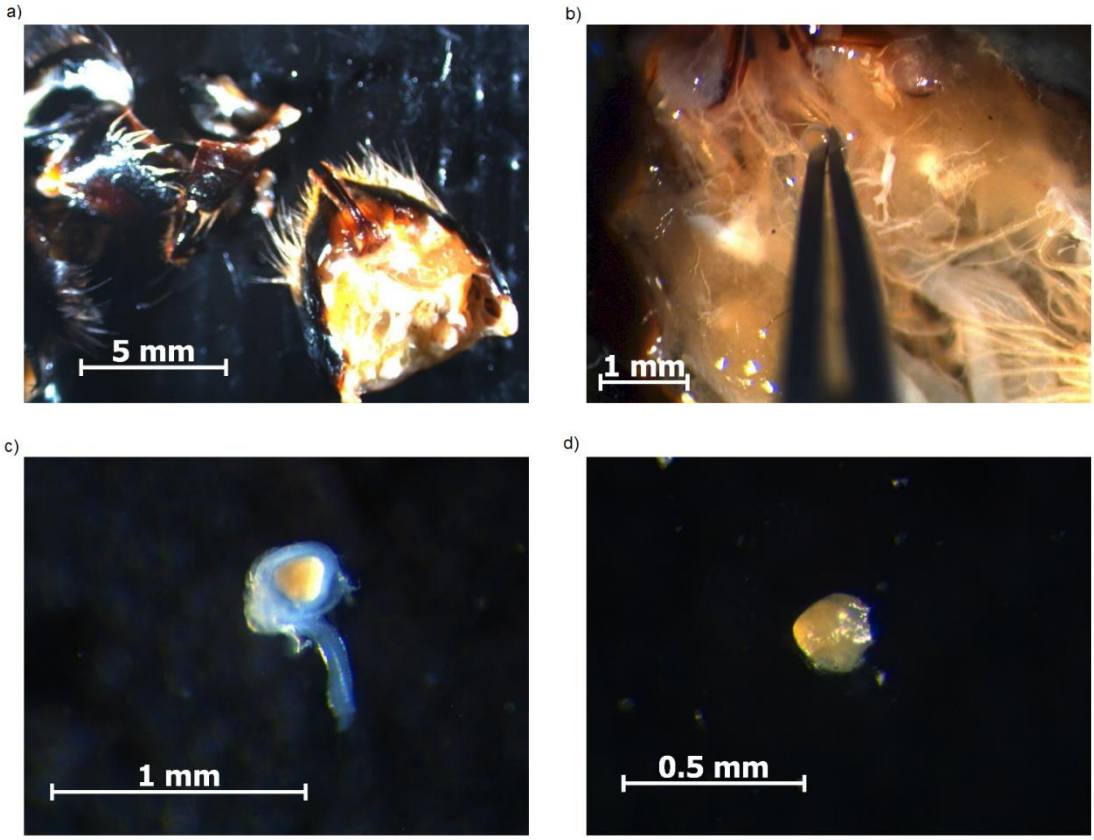
Appendix 3.1. The study site for *Bombus hypnorum* worker sampling, a 2 x 2 km tetrad divided into 16 500 x 500 m sampling squares; numbers represent workers collected in 2014, 2015. Location of study site in south eastern England, UK (inset).

Appendix 3.2. Microsatellite loci tested on *B. hypnorum* but excluded from the current study. Marker, locus identifier designated by authors that originally identified the locus (Estoup et al., 1995; Reber-Funk et al., 2005; Stolle et al., 2009); Reason for exclusion, whether PCRs produced either no amplicons or the amplicons were monomorphic size fragments; Size, amplicon length in base pairs; n, number of *B. hypnorum* workers tested; n/a, not applicable

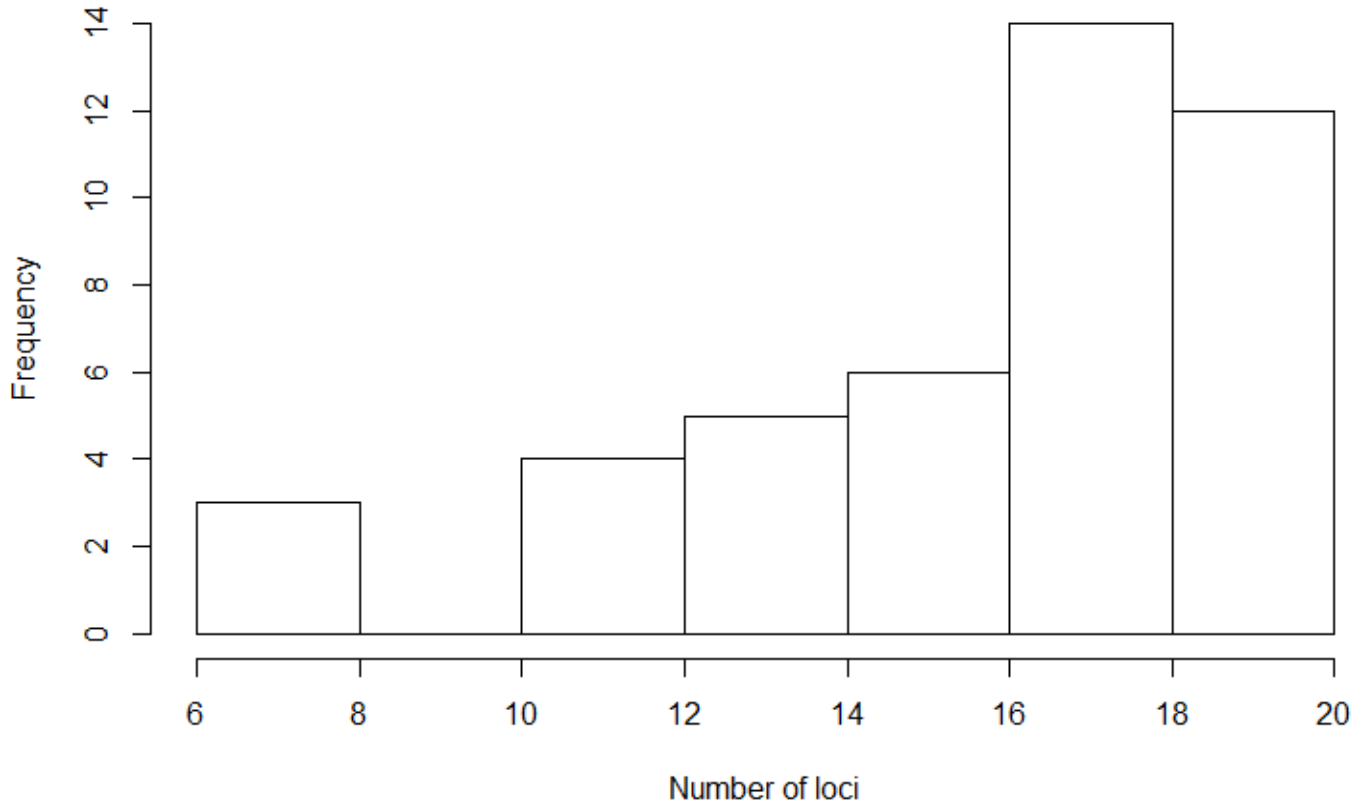
Marker	Reason for exclusion	Size	n
BL13	Monomorphic	163	20
BL15	Failed to amplify	n/a	15
BTMS0126	Failed to amplify	n/a	15
BTMS0151	Failed to amplify	n/a	15

Appendix 4.1. Sites used for collection of *Bombus hypnorum* queens in Norfolk, UK

Site	Latitude	Longitude
Whitlingham Country Park	52° 37' 0.4008''	1° 14' 20.2776''
Drayton	52° 41' 12.1992''	1° 12' 37.8468''
University of East Anglia	52° 37' 5.358''	1° 14' 3.0516''
Spixworth	52° 41' 30.1812''	1° 19' 1.614''
Hethersett	52° 36' 1.998''	1° 10' 40.2384'



Appendix 4.2. Steps in the dissection of the spermatheca from a *Bombus hypnorum* queen. a) Removal of the queen's sternites; b) Isolation of spermatheca using fine forceps; c) spermatheca with sperm duct and glands suspended in droplet of distilled water; d) sperm mass isolated from spermathecal structures.



Appendix 4.3. Frequency distribution of the number of microsatellite loci available in paired queen and sperm samples to estimate the mating frequency of 44 *Bombus hypnorum* queens.

Appendix 5.1. Counts of *Bombus* individuals recorded on ten 50 x 2 m transects on each of the three sites visited, broken down by caste: M, male; Q, queen; W, worker; and species. Worker counts of the selected *Bombus* species, bold.

	Bombus species								
Eaton Park									
	B. hortorum	*B. hypnorum*	*B. lapidarius*	*B. lucorum*	*B. pascorum*	*B. pratorum*	*B. ruderatus*	*B. terrestris* agg.	*B. vestalis*
M	0	25	0	0	0	22	0	7	22
Q	0	1	0	-	0	0	0	0	0
W	3	**38**	5	-	**10**	**23**	0	22	1
High Ash Farm									
M	0	3	3	0	0	5	0	1	0
Q	0	0	0	-	0	0	0	0	0
W	1	**37**	**185**	-	51	8	0	**66**	4
Waterloo Park									
M	0	14	3	4	0	5	1	0	112
Q	0	0	0	-	0	0	0	0	0
W	0	**60**	**50**	-	24	24	0	**89**	1

Appendix 5.2. Summary of the composition of flowering plant communities at the three sites used in the current study. Relative abundances are estimated from the presence/absence of open flowers in the 25 equal area subdvisions of thirty 2 x 2m quadrats per site as the proportional of all flower cover represented by the focal flowering plant.

Plant taxon	Relative abundance		
	Eaton Park	High Ash Farm	Waterloo Park
Achillea millefolium	0.011	0	0
Anemome	0.065	0	0
Aquilegia	0.021	0	0
Bellis perennis	0.135	0	0.031
Brassica napus	0	0.084	0
Centauria	0.033	0	0
Digitalis purpurea	0.010	0	0.002
Echinops	0.002	0	0
Echium vulgare	0	0.001	0
Erysimum linifolium	0.142	0	0.008
Geranium	0.1842	0.001	0.722
Geum	0.018	0	0
Heracleum sphondylium	0	0.017	0
Hypochaeris radicata	0.012	0	0
Lamium album	0	0	0.020
Lavandula	0.091	0	0
Leucanthemum vulgare	0	0.257	0
Lotus corniculatus	0	0.025	0
Medicago sativa	0	0.001	0
Onobrychis viciifolia	0	0.236	0
Papaver	0.011	0.100	0.022
Pentaglottis sempervirens	0	0	0.033

Phacelia tanacetifolia	0	0.191	0
Potentilla	0.012	0	0.010
Pulmonaria	0.021	0	0.006
Ranunculus	0	0.013	0
Salvia officinalis	0.047	0	0.076
Scabious	0	0	0.016
Sisyrinchium striatum	0.039	0	0.039
Sonchus arvensis	0	0.001	0
Symphytum offinale	0	0	0.014
Tradescantia virginiana	0.079	0	0
Trifolium pratense	0	0.029	0
Trifolium repens	0.067	0.043	0

Appendix 5.3. Foraging preferences of nine *Bombus* species for twenty-one plant taxa over three sites in Norfolk, UK. Preferences calculated as: (proportion of *Bombus* visits – relative plant taxon abundance) / relative plant taxon abundance. Proportion of visits calculated from ten 50 x 2 m transects per site, relative abundances estimated from the presence/absence of open flowers in the 25 equal area subdivisions of thirty 2 x 2m quadrats per site. Proportion of visits is the proportion of all *Bombus* species specific worker foraging visits observed which were to the focal plant taxon, with the exception of *B. vestalis* (a workerless *Psithyrus* social parasite) for which visits by females were used. NA, plant taxon not present in four or more separate quadrats on the site. Comparisons selected for handling times, underlined.

	Eaton Park							High Ash Farm							Waterloo Park					
	B. lapidarius	*B. hortorum*	*B. pratorum*	*B. terrestris*	*B. pascorum*	*B. hypnorum*	*B. vestalis*	*B. lapidarius*	*B. terrestris*	*B. pratorum*	*B. pascorum*	*B. hypnorum*	*B. hortorum*	*B. vestalis*	*B. lapidarius*	*B. hypnorum*	*B. pascorum*	*B. terrestris*	*B. pratorum*	*B. vestalis*
Centauria	12	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Digitalis purpurea	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Erysimum linifolium	0	0	0	0.320	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Geranium	1.086	0	**4.248**	1.481	**1.629**	**3.714**	0	NA	NA	NA	NA	NA	NA	NA	**1.218**	**0.992**	0.692	**0.871**	0.865	1.384
Hypochaeris radicata	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Potentilla	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pulmonaria	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Salvia officinalis	0	0	**2.754**	1.152	**12.67**	**2.778**	21.11	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tradescantia virginiana	0	0	0	2.727	0	2	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

	Eaton Park							High Ash Farm							Waterloo Park					
	B. lapidarius	*B. hortorum*	*B. pratorum*	*B. terrestris*	*B. pascorum*	*B. hypnorum*	*B. vestalis*	*B. lapidarius*	*B. terrestris*	*B. pratorum*	*B. pascorum*	*B. hypnorum*	*B. hortorum*	*B. vestalis*	*B. lapidarius*	*B. hypnorum*	*B. pascorum*	*B. terrestris*	*B. pratorum*	*B. vestalis*
Trifolium repens	6	0	0	7.677	0	0	0	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA
Brassica napus	NA	NA	NA	NA	NA	NA	NA	0.386	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA
Leucanthemum vulgare	NA	NA	NA	NA	NA	NA	NA	0.274	0.059	0	0	0	0	0	NA	NA	NA	NA	NA	NA
Onobrychis viciifolia	NA	NA	NA	NA	NA	NA	NA	**3.364**	**2.117**	1.587	3.984	**1.831**	4.233	1.058	NA	NA	NA	NA	NA	NA
Papaver	NA	NA	NA	NA	NA	NA	NA	0.108	0.152	0	0	0	0	0	0	0	0	0.501	0	0
Phacelia tanacetifolia	NA	NA	NA	NA	NA	NA	NA	**0.480**	**2.455**	3.267	0.307	**2.967**	0	3.920	NA	NA	NA	NA	NA	NA
Lamium album	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Pentaglottis sempervirens	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	**0.613**	**2.552**	0	**2.065**	0	0
Scabious	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0
Sisyrinchium striatum	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA	NA	0	0	0	0.580	0	0
Symphytum	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Trifolium pratense	NA	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	NA	NA	NA	NA	NA	NA

Appendix 5.4. Parameter estimates for the difference in mean between flower flight time on the same individual plants between individual workers of different *Bombus* species and individual *Bombus hypnorum* workers. Estimates are from the output of a different linear mixed model (LMM) per combination of site and plant taxon, fitted to n flower visits. Each LMM has nested random effects of four plant patches and i foraging sequences of flower visits by an individual worker. Comparison, site and plant taxon combination; Effect, factor levels for *Bombus* species ordered relative to *B. hypnorum* (intercept); β, parameter estimate in seconds; se, standard error; df, degrees of freedom; t, test statistic; p, p value.

Comparison	Effect	Flight time				
		β	se	df	t	p
Eaton Park, *Geranium* n = 1513, i = 92	intercept	1.57	0.17	5.6	9.07	0.0001
	B. pascuorum	0.09	0.22	63.77	0.43	0.68
	B. pratorum	-0.02	0.17	66.01	-0.16	0.88
Eaton Park, *Salvia* N = 1524, i = 119	intercept	1.04	0.07	73.38	14.30	< 0.0001
	B. pascuorum	-0.03	0.11	67.27	-0.26	0.80
	B. pratorum	-0.14	0.10	63.62	1.47	0.15
High Ash Farm, *Onobrychis vicifolia* n = 2176, i = 120	intercept	1.50	0.10	16.28	14.48	< 0.0001
	B. lapidarius	0.32	0.14	86.48	2.22	0.03
	B. terrestris	0.50	0.14	86.11	3.43	0.0009
High Ash Farm, *Phacelia tanacetfolia* n = 1676, i = 118	intercept	1.79	0.13	9.30	13.62	< 0.0001
	B. lapidarius	-0.31	0.15	98.88	-2.11	0.04
	B. terrestris	0.13	0.15	92.16	-0.83	0.41
Waterloo Park, *Geranium* n = 2709, i = 123	intercept	1.32	0.17	3.96	7.90	0.001
	B. lapidarius	-0.07	0.11	97.27	-0.73	0.47
	B. terrestris	0.06	0.11	108.11	0.48	0.63
Waterloo Park, *Pentaglottis sempervirens* N = 2583, i = 100	intercept	1.04	0.05	6.78	20.75	< 0.0001
	B. lapidarius	-0.10	0.07	86.87	-1.31	0.20
	B. terrestris	-0.02	0.08	106.23	-0.28	0.78