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

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Abstract.

1. Bumblebee nests are difficult to find in sufficient numbers for well replicated studies. Counts of nest-searching queens in spring and early summer have been used as an indication of preferred nesting habitat, but this relationship has not yet been validated; high densities of nest-searching queens may indicate habitat with few nesting opportunities (meaning that queens have to spend longer looking for them).
2. From mid April 2010, queen bumblebees were counted along 20 transects in grassland and woodland habitats in central Scotland, U.K. The number of inflorescences of suitable forage plants were also estimated at each transect visit. The area surrounding each transect was searched for nests in the summer.
3. In total, 173 queen bumblebees were recorded on transects, and, of these, 149 were engaged in nest-searching. Searches subsequently revealed 33 bumblebee nests.
4. The number of nest-searching queens on transects was significantly, positively related to the number of nests subsequently found. Estimated floral abundance along the transect did not correlate with numbers of nest-searching queens or with the number of nests found, suggesting that queens do not target their searching to areas that are locally high in spring forage.
5. The data suggest that counts of nest-searching queens provide a useful positive indication of good nesting habitat, and hence where bumblebee nests are likely to be found later in the year.

Key words. Apidae, Bombus, colony, floral resources, nest founding.

Introduction

Bumblebees usually nest in the abandoned dwellings of other animals, typically those of small mammals, such as mice and voles, but they sometimes use other nests, including those of birds or rabbits (Sladen, 1912; Free & Butler, 1959; Alford, 1975; Fussell & Corbet, 1992; Lye *et al.*, 2012). These nests tend to be subterranean or under thick vegetation such as tussocks of grass. Bumblebees have an annual life cycle and colonies are founded in spring or early summer by a fertilised queen (Sladen, 1912). The queen rears an initial brood of eight to 16 worker bees, which then assist in rearing successive broods (Plowright & Pendrel, 1977). The workforce increases to a maximum of several hundred workers (depending on species (Goulson, 2010). Nonetheless the nests remain well concealed and may only be revealed by sporadic worker traffic to and from the entrance.

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A variety of approaches to locating wild bumblebee nests have been deployed, including training sniffer dogs (Waters *et al.*, 2010; O'Connor *et al.*, 2012) or recruiting volunteers to search for nests following a variety of protocols (Fussell & Corbet 1992; Osborne *et al.*, 2008; Lye *et al.*, 2012). The most effective method is time-consuming diligent searches for worker bee traffic (O'Connor *et al.*, 2012). Because of the labour-intensive nature of this work and the small numbers of nests found per hour, we still have a poor idea of the preferred nesting habitats of different bumblebee species, particularly for the less common species.

The relative suitability of different habitats as nest sites for bumblebees and differences in nesting habitat preferences among bumblebee species can be studied indirectly using counts of nest-searching queens (Svensson *et al.*, 2000; Kells & Goulson, 2003, Lye *et al.*, 2009). In these studies, the abundance of nest-searching queens is used as a positive indicator of the nesting suitability of an area. This approach has been used to demonstrate that nest-searching queens tend to prefer linear features (e.g. hedgerows and fence lines) to open ground, and in some cases they have more specific site preferences. For example, more sheltered sites near forest boundaries may be preferred by *Bombus pascuorum* and *Bombus lucorum*. However, the validity of using such indices has rarely been tested and it is possible that high numbers of nest-searching queens indicate poor habitat where good nest sites are unavailable, leading to prolonged searching by queens. In areas where good nest sites are plentiful, queens might be expected to find them quickly so that few searching queens are observed. On the other hand, if queens aggregate strongly in areas with favourable nest sites but the best sites quickly become occupied, then more favourable areas may have larger numbers of nest-searching queens, particularly in the late season. Overall, it is unclear how we might expect abundance of nest-searching queens to relate to suitability of habitat and subsequent nest density.

Bumblebee queens in spring and early summer must have access to sufficient pollen and nectar to develop their ovaries, fuel their nest site searches and initiate a colony (Cumber, 1953; Stephen, 1955; Alford, 1975; Steffan-Dewenter & Tscharntke, 2001; Suzuki *et al.*, 2007). Lack of forage causes slower colony growth and impacts survival and fecundity (Plowright & Pendrel, 1977; Schmid-Hempel & Schmid-Hempel, 1998). Therefore locations with ample spring flowering plants might be the most suitable (Fye & Medler, 1954; Holm, 1966), and in support of this Suzuki *et al.* (2009) found a positive relationship between floral availability and number of nest-searching queens in *Bombus ardens*, but only during the early morning when it was cool; later in the day nest-searching queens were found far from flowers. They subsequently found that nests tended to be located in flower-rich areas, but only six nests were detected

In this study we aimed to determine whether the number of nests in an area is positively or negatively predicted by the abundance of nest-searching queens during the spring, testing the assumption of a positive relationship that is implicit in Svensson *et al.* (2000), Kells and Goulson (2003) and Lye *et al.* (2009). If reliable, spring queen counts could be used to infer suitability of habitat or land management for conservation purposes and allow researchers wishing to locate bumblebee nests to target resources to areas where greater numbers of bumblebee nests are likely to be found. We also examined whether nest locations are predicted by local (within 50 m) availability of spring forage.

Materials and methods

Bumblebee queens were counted and floral abundance estimated along transects in springtime, from 19 April to 4 June 2010. Transect walks took place in dry conditions between 08.30 and 19.30 hours. The temperature ranged between 6 and 22 °C. All transects were visited once a week, for 7 weeks. Twenty transects were selected – 10 in woodlands and 10 in grasslands – as bumblebees of the six common species in Britain are known to nest in both (Alford, 1975; Osborne *et al.*, 2008). Sites were either on the campus of the University of Stirling (Scotland, U.K.) or on nearby private estates. It was important that sites were accessible to researchers, and so areas with thick undergrowth (e.g. *Rhododendron* spp., *Urtica dioica*), those on steep slopes and those prone to becoming water-logged were avoided. Woodlands were dominated by deciduous species such as *Quercus robur*, *Fraxinus excelsior*, *Fagus sylvatica*, and *Betula pendula*). Grasslands were long-established, tussocky swards (>10 cm) which receive minimal management. There were numerous signs of small mammal and rabbit activity and burrows in both habitats.

The transect protocol followed that of Lye *et al.* (2009). Each was 100 m long, and was walked at a slow, constant pace of approximately 3 km h^{-1}. Bumblebees were counted within 3 m of each side of the path walked by the observer. Bumblebees were identified to species, and their caste and behaviour at the time recorded. Behaviours included 'nest-searching', 'in flight' or 'foraging' for nectar or pollen (as indicated by presence of pollen in pollen baskets). Nest-searching behaviour is distinctive and consists of bees flying in a low, zigzag pattern and/or investigating holes in the ground, tussocks of vegetation, etc. Bees classed as 'in flight' were typically flying higher, on a straighter trajectory and not apparently investigating either potential nesting sites or flowers. In addition, plant species visited by foraging bees were noted.

The amount of forage available to bumblebees was recorded during each visit. Estimations of the number of flowering units of each plant species within 50 m of each transect were made following a brief (~10 min) search of the area, to provide an approximate measure of forage availability at the site. This assessment followed Carvell *et al.* (2007) with one flower cluster (e.g. an umbel, a head, a capitulum) counted as a single unit. Total numbers of floral units per transect were used in subsequent analyses.

To establish the subsequent density of nests, the area within 25 m either side of the 100 m transect (i.e. a rectangle of 0.5 ha) was intensively searched for nests twice; initially for 3 h in early summer, in the period between 9 and 18 June and again in mid-summer for 1 h between 20 and 28 July (80 man-hours in total). The recorder walked very slowly, stopping frequently, passing backwards and forwards across the rectangular area with approximately 4 m between passes. Nests were detected by watching for bumblebee traffic in or out of nests whilst either stationary or moving slowly through the site. Efforts were made to avoid overly trampling the ground (e.g. disturbing leaf litter or flattening long grass) as this can lead to difficulties for bees returning to their nests. Two or more bumblebees flying either in or out of a hole, tussock of grass, or similar potential nest location signified a nest and all were verified at a later date by a further inspection for bumblebee traffic. Searches were carried out in dry conditions between 08.00 and 20.00 hours. Data from the two searches were pooled for analysis. All transects, nest searches, and floral estimates were carried out by SO to ensure consistency.

Analysis

Analysis was carried out in R statistical software version 2.12.2 (R Development Core Team, 2014). A generalised linear mixed

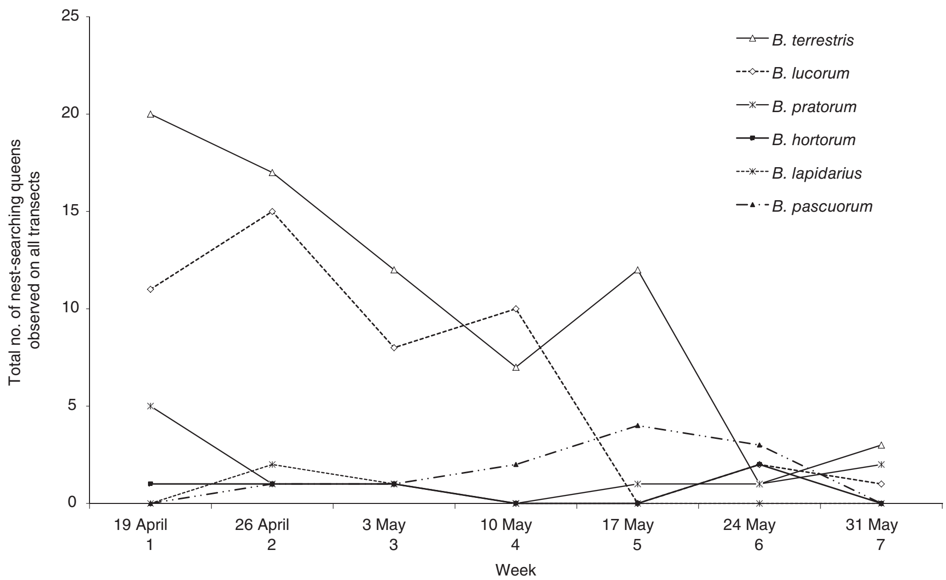

Fig. 1. Total number of nest-searching bumblebee queens ($n = 149$) recorded on all transects during the seven survey periods, separated by species.

model (GLMM) with Poisson errors and a log link was used, with number of nest-searching queens recorded on each transect walk (all species pooled) as the response variable, and the total number of floral units for all known bumblebee forage plant species within each site as a covariate. Time of day was binned into the periods 8.30–11.00, 11.00–14.00, 14.00–17.00, and 17.00–19.30 hours, and included as a fixed factor along with habitat (woodland/grassland). Site was included as a random factor nested within habitat. Bee species were pooled as there were too few of any one species for individual analysis. No model simplification was conducted.

A general linear model (GLM) with Poisson errors and log link was then carried out with the total number of nests detected as the response, and numbers of nest-searching queens and floral abundance (using the total number of floral units for all known bumblebee forage plant species within each site, averaged across visits) as covariates. Habitat (woodland/grassland) was included as a fixed factor. The initial model included all explanatory variables, plus all two-way interactions. The model was simplified by removal of interactions that were not significant.

Results

In total, 173 queens were observed. Of these, 18 were foraging, six were in flight and 149 were nest-searching queens (Fig. 1). The peak of queen nest-searching activity may have occurred before the beginning of the experiment as *Bombus terrestris* and *Bombus pratorum* numbers were at their highest in the first week of recording (week beginning 19 April). *Bombus pascuorum* activity peaked later, during the fifth week of data collection.

In total, 33 nests were subsequently found: 18 in grassland and 15 in woodland. Overall nest density was thus 3.30 nests ha^{-1} (3.60 and 3.00 nests ha^{-1} for grassland and woodland sites, respectively).

There was no significant relationship between the number of nest-searching queens and habitat (GLMM, $F_{1,134} = 1.42$, $P = 0.24$), floral abundance (GLMM, $F_{1,134} = 0.49$, $P = 0.49$) or time of day (GLMM, $F_{3,134} = 0.86$, $P = 0.46$) (Fig. 2). There was a significant, positive association between numbers of nest-searching queens on transects and number of nests subsequently found at sites (GLM, $\chi^2_1 = 6.61$, $P = 0.010$; Fig. 3). There were no significant interactions between explanatory factors (numbers of nest-searching queens, habitat, and floral abundance on transects). Neither habitat nor floral abundance had any appreciable effect on the number of nests (GLM, $\chi^2_1 = 0.23$, $P = 0.63$ and $\chi^2_1 = 0.89$, $P = 0.35$ for habitat and floral abundance, respectively). The 10 sites with the greatest floral availability (100 to >4500 mean floral units) yielded 13 nests, whereas 14 nests were found in the 10 sites with poorest availability of spring flowers (>40 mean floral units) and it can also be noted that seven sites devoid of any floral resources yielded nine bumblebee nests.

Discussion

Our data demonstrate that the density of nest-searching queen bumblebees does positively predict nest density later in the year, thereby confirming the underlying assumption of previous studies which have used queen abundance to infer nesting habitat (Svensson *et al.*, 2000; Kells & Goulson, 2003; Lye *et al.*, 2009). Interestingly, the density of floral resources available in spring had no influence on numbers of bumblebee nests subsequently found. This is in accordance with Lye *et al.* (2009), who found that floral availability of agricultural field margins was not correlated with abundance of nest-searching queens. In contrast, floral resources have been found to predict nest-searching queens (although only in the early morning) and also the location of actual nests of *B. ardens* (Suzuki *et al.*,

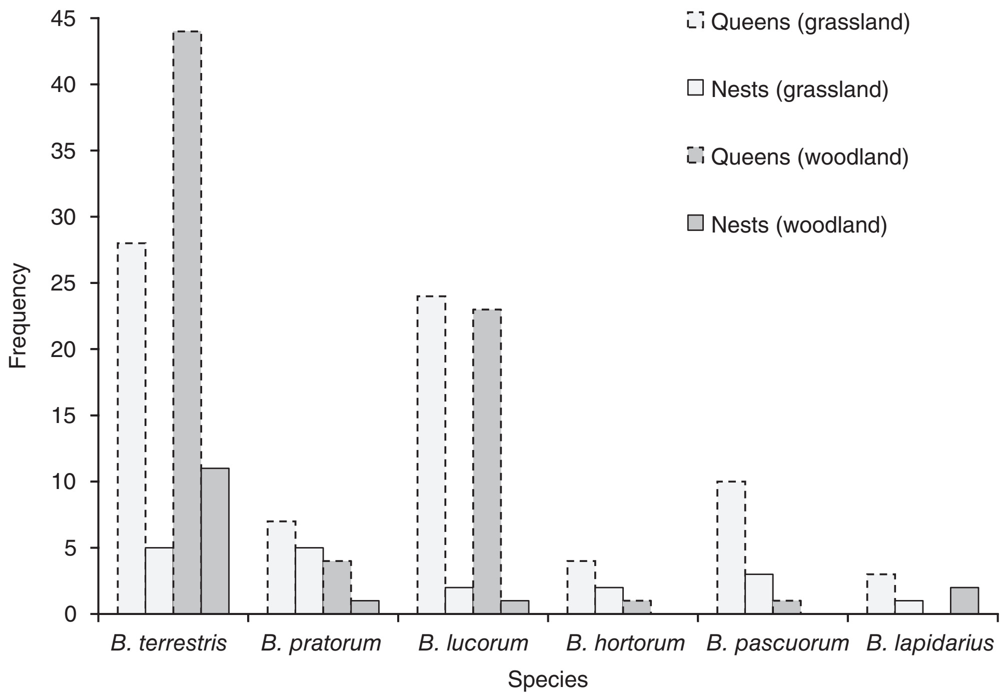

Fig. 2. Total number of nest-searching queens and nests, separated by species and habitat.

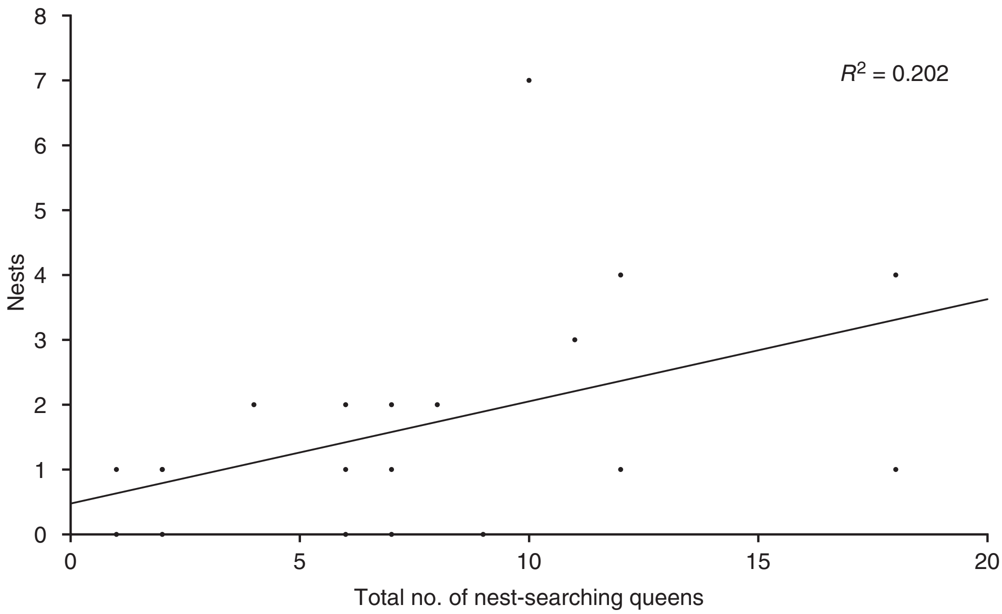

Fig. 3. The total number of nest-searching queens observed during transects is positively correlated with the number of bumblebee nests subsequently found.

2009). However Suzuki *et al.* (2009) assessed floral abundance at a much greater scale, (2.5 km^2). Bumblebee foraging ranges vary depending upon factors such as species and size of bee (Darvill *et al.*, 2004; Knight *et al.*, 2005; Greenleaf *et al.*, 2007). Bumblebee workers rarely forage immediately outside their nest, tending to fly in excess of 100 m before beginning to forage (Dramstad, 1996; Osborne *et al.*, 1999; Dramstad *et al.*, 2003). Although there are no data for queen foraging ranges, it seems likely that the scale of the forage survey used in this study was smaller than that on which queen bees operate. In addition, the survey provides only a crude estimate of available forage, as flowers of those species surveyed are not equal in terms of the quantity and quality of pollen and nectar they provide and their preferred use by bumblebees (Carvell, 2002; Goulson &

Darvill, 2004; Goulson *et al.*, 2005; Williams & Osborne, 2009). Regardless of these limitations, our data strongly suggest that the availability of high densities of floral resources in springtime within close proximity is not essential for nest establishment of the common British bumblebee species. However, workers of some rarer species of bumblebees forage over a smaller area (Connop *et al.*, 2011), and if this trend is the same for queens of such species, availability of spring forage within 100 m of nests may be essential for successful nest establishment.

The nest density averaged across both habitats was 3.30 nests ha^{-1}. This is comparable with molecular studies which have estimated nest density for four common British bumblebee species. Estimates for *B. pascuorum* were 1.93 nests ha^{-1} (Darvill *et al.*, 2004), 0.26 nests ha^{-1} (Knight *et al.*, 2005) and 0.35–1.73 nests ha^{-1} (Knight *et al.*, 2009). *Bombus terrestris* nests were estimated to nest at lower density: 0.13 nests ha^{-1} (Darvill *et al.*, 2004) and 0.29 nests ha^{-1} (Knight *et al.*, 2005). Knight *et al.* (2005) estimated densities for nests of *B. lapidarius* and *B. pratorum* of 1.17 and 0.26 nests ha^{-1} respectively. If we take the mean estimate for these four species and sum them, this gives a total of approximately 2.70 bumblebee nests ha^{-1} for these common British bumblebee species. There are no molecular estimates for nest density of *B. hortorum* or *B. lucorum*.

In contrast, our estimates of nest densities are lower than those obtained when small areas of ground are exhaustively searched; Osborne *et al.* (2008) recorded nest densities of 14.6 and 10.8 nests ha^{-1} for long grassland and woodland, respectively, and O'Connor *et al.* (2012) estimated a woodland nest density of 27.8 nests ha^{-1}. Molecular studies can be expected to provide lower densities as they integrate estimates across a mixture of habitats, including those that are unfavourable for nesting, such as ploughed fields. Osborne *et al.* (2008) used satellite imagery and geographic information system software to estimate the areas of habitats observed in their study (such as woodland, gardens, hedgerows, etc,) for an area of Hertfordshire (U.K.), and proposed that there were approximately 7 nests ha^{-1} averaged across the landscape. The discrepancy may arise simply because nest searches in these studies involved spending more than five times as long per unit area searched (46 h ha^{-1}) as we spent in the present study (8 h ha^{-1}). It is highly likely that we did not find every nest.

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

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