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1 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 twenty transects in grassland and woodland habitats in Central Scotland, UK. The number of inflorescences of suitable forage plants were also estimated at each transect visit. The area surrounding each transect was searched for nests in the summer.
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 the number of nests found, suggesting that queens do not target their searching to areas locally high in spring forage.
5. The data suggest that counts of nest-searching queens do provide a useful positive indication of good nesting habitat, and hence where bumblebee nests are likely to be found later in the year.

Introduction

Bumblebees usually nest in the abandoned dwellings of other animals, typically those of small mammals such as mice and voles but sometimes using 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 8-16 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.

A variety of approaches to locating wild bumblebee nests have been deployed, including training sniffer dogs (O'Connor *et al.*, 2012; Waters *et al.*, 2012), or recruiting volunteers to search for nests following a variety of protocols (Fussell & 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 indicates 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 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 & Tschardt, 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 aim 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 examine whether nest locations are predicted by local (within 50 m) availability of spring forage.

Materials and Method

Bumblebee queens were counted and floral abundance estimated along transects in springtime, from 19th April to 4th June 2010. Transect walks took place in dry conditions between 08:30 and 19:30. The temperature ranged between 6 °C and 22°C. All transects were visited once a week, for seven weeks. Twenty transects were selected; ten in woodlands and ten in grasslands as bumblebees of the six common species in Britain are known to nest in both (Alford, 1975; Osborne *et al.*, 2008). Sites were either on the campus of the University of Stirling (Scotland, UK) or on nearby private estates. It was important that sites were accessible to researchers, and so areas with thick undergrowth, (e.g. *Rhododendron* spp., *Urtica dioica*), those on steep slopes or 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 Lye *et al.* (2009). Each was 100m long, and was walked at a slow, constant pace of approximately 3 km per hour. Bumblebees were counted within 3m each side of the path walked by the observer. Bumblebees were identified to species, and their caste and behaviour at the time 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 50m 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 25m either side of the 100m transect (i.e. a rectangle of 0.5ha) was intensively searched for nests twice; initially for three hours in early summer, in the period between June 9th and 18th and again in mid-summer for one hour between July 20th and 28th (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 trampling the ground overly (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. Data from the two searches were pooled for analysis. All transects, nest searches and floral estimates were carried out by S.O. to ensure consistency.

Analysis

Analysis was carried out in ‘R’ Statistical Software Version 2.12.2 (R Development Core Team, 2011). A Generalised Linear Mixed 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, with 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 8:30h to 11h; 11:00h to 14:00h; 14:00h to 17:00h; 17:00h to 19:30h, 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, 6 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 19th April). *Bombus pascuorum* activity peaked later, during the 5th 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 nests ha⁻¹ 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, $F_{3,134} = 0.86$, $p=0.46$). 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 ten sites with greatest floral availability (100 to >4500 mean floral units) yielded 13 nests, whereas 14 nests were found in the ten sites with poorest availability of spring flowers (>40 mean floral units) and it may 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 and 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 (though only in early morning)

and also the location of actual nests of *B. ardens* (Suzuki *et al.*, 2009). However Suzuki *et al.* (2009) assessed floral abundance at a much greater scale, (2.5km²). 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 100m before beginning to forage (Dramstad, 1996; Dramstad *et al.*, 2003; Osborne 1999). Although no data exists 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 spring time 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 100m of nests may be essential for successful nest establishment.

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* have ranged from 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) estimate 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 nest densities are lower than those obtained when small areas of ground are exhaustively searched; Osborne *et al.* (2008) recorded nest density at 14.6 nests ha⁻¹ and 10.8 nests ha⁻¹ for long grassland and woodland respectively and O'Connor *et al.* (2012) estimated woodland nest density at 27.8 nests ha⁻¹. Molecular studies can be expected to provide lower 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 GIS software to estimate the areas of habitats observed in their study (such as woodland, gardens, hedgerows, etc.) for an area of Hertfordshire (UK) and proposed that there were approximately seven nests ha⁻¹ averaged across the landscape. The discrepancy may be simply because nest searches in these studies involved spending more than five times as long per unit area searched (46h/ha) as we spent in the present study (8h/ha). 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 do provide a useful tool in studies of bumblebee nesting ecology.

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Conflicts of Interest

There were no conflicts of interest.

Referenced Literature

Alford, D.V. (1975) *Bumblebees*. Davis-Poynter Ltd., London.

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

Carvell, C., Meek, W.R., Pywell, R.F., Goulson, D. and Nowakowski, N. (2007) Comparing the efficacy of agri-environment schemes to enhance bumblebee abundance and diversity on arable field margins. *Journal of Applied Ecology*, **44**, 29-40

Connop, S., Hill, T., Steer, J. and Shaw, P. (2011) Microsatellite analysis reveals the spatial dynamics of *Bombus humilis* and *Bombus sylvarum*. *Insect Conservation and Diversity* **4**, 212–221.

Cumber, R.A. (1953) Some aspects of the biology and ecology of the humble-bees bearing upon the yields of red clover seed in New Zealand. *New Zealand Journal of Science and Technology* **34**, 227-240.

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

Dramstad, W. (1996) Do bumblebees (Hymenoptera: Apidae) really forage close to their nests? *Journal of Insect Behaviour* **9**, 163-182.

Dramstad, W.E., Fry, G.L.A. and Schaffer, M.J. (2003) Bumblebee foraging – is closer really better? *Agriculture, Ecosystems and Environment* **95**, 349-357.

Free, J.B. and Butler, R.F. (1959) *Bumblebees*. Collins Press; London, UK.

Fussell, M. and Corbet, S.A. (1992) The nesting places of some British bumblebees. *Journal of Apicultural Research* **31**, 32-41.

Fye, R.E. and Medler, J.T. (1954) Field domiciles for bumblebees. *Journal of Economic Entomology* **47**, 672-676.

269 Goulson, D. (2010) *Bumblebees; their behaviour, ecology and conservation*. Oxford
270 University Press; Oxford, UK.

271 Goulson, D. and Darvill, B. (2004) Niche overlap and diet breadth in bumblebees; are rare
272 species more specialised in their choice of flowers? *Apidologie* **35**, 55-63.

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

275 Greenleaf, S.S., Williams, N.M., Winfree, R. and Kremen, C. (2007) Bee foraging ranges and
276 their relationship to body size. *Oecologia* **153**, 589-596.

277 Holm, S.N. (1966) The Utilisation and management of bumblebees for red clover and alfalfa
278 seed production. *Annual Review of Entomology* **11**, 155-182.

279 Kells, A.R. and Goulson, D. (2003) Preferred nesting sites of bumblebee queens
280 (Hymenoptera: Apidae) in agroecosystems in the UK. *Biological Conservation*
281 **109**, 165-174.

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

285 Knight, M.E., Hale, R.J., Sanderson, R.A., Martin, A.P., Osborne, J.L., and Goulson, D.
286 (2009) Bumblebee nest density and the scale of available forage in arable
287 landscapes. *Insect Conservation and Diversity* **2**, 116-124.

288 Lye, G.C., Park, J.K., Osborne, J., Holland, J.M. and Goulson, D. (2009) Assessing the value
289 of Rural Stewardship schemes for providing foraging resources and nesting habitat
290 for bumblebee queens (Hymenoptera: Apidae). *Biological Conservation* **142**,
291 2023-2032.

292 Lye, G.C., Osborne, J.L., Park, K.J. and Goulson, D. (2012) Using citizen science to monitor
 293 *Bombus* populations in the UK: nesting ecology and relative abundance in the
 294 urban environment. *Journal of Insect Conservation* **16**, 697-707.

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

297 Osborne, J.L., Clark, S.J., Morris, R.J., Williams, I.H., Riley, J.R., Smith, A.D., Reynolds,
 298 D.R. and Edwards, A.S. (1999) A landscape-scale study of bumblebee foraging
 299 range and constancy, using harmonic radar. *Journal of Applied Ecology* **36**, 519-
 300 533.

301 Osborne, J.L., Martin, A.P., Shortall, C.R., Todd, A.D., Goulson, D., Knight, M.E., Hale, R.J.
 302 and Sanderson, R.A. (2008) Quantifying and comparing bumblebee nest densities
 303 in gardens and countryside habitats. *Journal of Applied Ecology* **45**, 784-792.

304 Plowright, R.C. and Pendrel, B.A. (1977) Larval growth in bumblebees (Hymenoptera:
 305 Apidae). *Canadian Entomologist* **109**, 967-973.

306 Schmid-Hempel, R. and Schmid-Hempel, P. (1998) Colony performance and
 307 immunocompetence of a social insect, *Bombus terrestris* in poor and variable
 308 environments. *Functional Ecology* **12**, 22-30.

309 Sladen, F.W.L. (1912) *The Humble-bee*. Macmillan and Co. Ltd., Hereford, UK.

310 Steffan-Dewenter, I. and Tscharntke, T. (2001) Succession of bee communities on fallows.
 311 *Ecography* **24**, 83-93.

312 Stephen, W.P. (1955) Alfalfa pollinator in Manitoba. *Journal of Economic Entomology* **48**,
 313 543-48.

314 Suzuki, Y., Kawaguchi, L.G., Toquenaga, Y. (2007) Estimating nest locations of bumblebee
 315 *Bombus ardens* from flower quality and distribution. *Ecological Research* **22**, 220-
 316 227.

317 Suzuki, Y., Kawaguchi, L.G., Munidasa, D.T. and Toquenaga, Y. (2009) Do bumblebee
318 queens choose nest sites to maximize foraging rate? Testing models of nest site
319 selection. *Behavioural Ecology and Sociobiology* **63**, 1353-1362.

320 Svensson, B., Lagerlof, J. and Svensson, B.G. (2000) Habitat preferences of nest-seeking
321 bumblebees (Hymenoptera: Apidae) in an agricultural landscape. *Agriculture,*
322 *Ecosystems and Environment* **77**, 247-255.

323 Waters, J., O'Connor, S., Park, K. and Goulson, D. (2010) Testing a detection dog to locate
324 bumblebee colonies and estimate nest density. *Apidologie* **42**, 200-205.

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

327

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

330

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

332

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

335