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An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species

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Abstract

Bumblebees are major pollinators of crops and wildflowers in northern temperate regions. Knowledge of their ecology is vital for the design of effective management and conservation strategies but key aspects remain poorly understood. Here we employed microsatellite markers to estimate and compare foraging range and nest density among four UK species: *Bombus terrestris, Bombus pascuorum, Bombus lapidarius*, and *Bombus pratorum*. Workers were sampled along a 1.5-km linear transect across arable farmland. Eight or nine polymorphic microsatellite markers were then used to identify putative sisters. In accordance with previous studies, minimum estimated maximum foraging range was greatest for *B. terrestris* (758 m) and least for *B. pascuorum* (449 m). The estimate for *B. lapidarius* was similar to *B. pascuorum* (450 m), while that of *B. pratorum* was intermediate (674 m). Since the area of forage available to bees increases as the square of foraging range, these differences correspond to a threefold variation in the area used by bumblebee nests of different species. Possible explanations for these differences are discussed. Estimates for nest density at the times of sampling were 29, 68, 117, and 26/km^2 for *B. terrestris, B. pascuorum, B. lapidarius* and *B. pratorum*, respectively. These data suggest that even among the most common British bumblebee species, significant differences in fundamental aspects of their ecology exist, a finding that should be reflected in management and conservation strategies.

Keywords: *Bombus*, foraging range, kinship, microsatellites, nest density

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Introduction

There is a wide consensus that many populations of pollinators have declined in much of the developed world with potentially serious economic and ecological implications (Kosior 1995; Rasmont 1995; Buchmann & Nabhan 1996; Westrich 1996; Kearns *et al*. 1998; Memmott *et al*. 2004). Improved understanding of the ecology of pollinator species is vital if we are to design appropriate management and conservation strategies.

Bumblebees (*Bombus* spp.) are a major pollinator group in the Northern Hemisphere (Goulson 2003a). Nesting density and foraging range are key parameters of their ecology but neither is currently well documented. Some evidence suggest that at least some species may differ quite dramatically in these traits (Darvill *et al*. 2004), but what factors determine those differences and how those are limited both within and between species is unknown. Knowing the extent of intraspecific variation in foraging range and nesting density, and how heavily these ecological parameters are dependent on specific habitat features, such as nest site availability and temporal and spatial availability of forage, is also vital for successful management schemes. The present study aimed to quantify these parameters by estimating the distribution of sister pairs found in the field using microsatellite genotypes.

Over the past two decades, molecular markers have been increasingly employed to estimate kinship, allowing ecological issues that had proved intractable using more traditional techniques to be quantified. Both range size and dispersal are two such parameters now commonly targeted

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(e.g. Feldheim *et al*. 2004; Hazlitt *et al*. 2004; Vidya *et al*. 2005). Population size itself is in many species easily measured using more direct survey techniques but small organisms such as insects that often exist ephemerally in large numbers present particular difficulties, and especially eusocial insects where most individuals are not reproductively active. Both colony densities and foraging ranges have been addressed using microsatellite markers in eusocial insects, notably in termites (Goodisman & Crozier 2002; Deheer & Vargo 2004). Typically in these studies the analysis of genotypes has allowed individuals to be directly assigned to a relatively small number of identified colonies in a limited area for which ecological data have also been collected. Here we instead analyse the spatial patterning of nest mates to infer population-level processes using a more indirect analysis approach.

As eusocial insects, the effective population size of bumblebees is determined not by the number of individuals in an area, but by the number of successful nests. The number of nests that (increasingly fragmented) habitats can support is critical in terms of the long-term viability of populations. Nests, however, are extremely difficult to locate and consequently quantitative data are scarce. Cumber (1953) provided an actual count of nests of several species after an intense search of a small area in Buckinghamshire, UK. While making an invaluable contribution to our knowledge of ecology and nesting behaviour of several species, there are problems in extrapolating from such studies to habitat scales because of the variability that exists at small scales between locations due to land use and history, both of which may have profound effects on local nesting densities (Cumber 1953). Similar large-scale nest counts are unfeasible due to the difficulties in locating nests. Methods to estimate nest density must therefore employ other techniques. Using microsatellite markers to reconstruct sibships, Darvill *et al*. (2004) provide the only published habitat scale data, to our knowledge, which are neither largely based on qualitative information (e.g. Fussell & Corbet 1992), nor founded on anecdotal, although educated, guesses (M. Edwards, 2000, UK Biological Action Plan, Bumblebee Working Group Report, personal communication; see also Chapman *et al*. 2003). It remains to be seen whether the nest density estimates of Darvill *et al*. (2004) of 13/km^2 for *Bombus terrestris* and 193/km^2 for *Bombus pascuorum* for arable agricultural landscapes in southern England can be confirmed as generally applicable figures, or whether substantial variation exists even within broadly comparable habitats. Similar empirical data for all other *Bombus* species are non-existent with estimates remaining essentially conjectural.

Foraging range is another central aspect of bumblebee ecology, determining the area of habitat (and therefore the amount and variety of forage) that a nest utilizes (Bronstein 1995; Westrich 1996). In concert with nesting density, it indicates appropriate scales of habitat management for bee population conservation, and the distances over which pollen can be transported (Proctor & Yeo 1973; Corbet *et al*. 1991). This has become particularly topical with regard to the spread of genes from transgenic crops (Cresswell & Osborne 2004).

It has long been assumed that bumblebees forage as close to their nest as possible (reviewed in Goulson 2003a). This view is strengthened by optimality models, which predict that choosing the closest foraging sites is the best strategy, all else being equal (Heinrich 1979). Despite the wealth of literature on bumblebee foraging behaviour, there are few hard data available on bumblebee foraging ranges (Bronstein 1995; Osborne *et al*. 1999; Cresswell *et al*. 2000) principally because they have proved difficult to quantify.

The obvious method is to mark bees at the nest and then to observe where they forage. In practice, few marked bees are observed on forage (Kwak *et al*. 1991; Saville 1993; Schaffer & Wratten 1994; Dramstad 1996; Saville *et al*. 1997; Walther-Hellwig & Frankl 2000), reflecting the difficulty in finding bees distributed across a large area. For example, if 50 marked bees are actively foraging from a nest, if they all remain within 500 m of the nest (a reasonably conservative estimate), they will be present at a density of just 0.6 bee/hectare. Finding substantial numbers of marked bees at such low density is problematic. For this reason researchers have turned to a range of alternative approaches. Modelling the economics of foraging suggests that a bumblebee could profitably travel up to 10 km to patches of very rewarding forage (Cresswell *et al*. 2000). Homing experiments have found that *B. terrestris* are able to return to their nests after being artificially displaced by up to 9.8 km (Goulson & Stout 2001); but whether homing ability can be related to natural foraging range is debatable. The use of harmonic radar allowed individual bumblebee movements to be tracked directly for the first time (Osborne *et al*. 1999). Unfortunately this technique has a major limitation with respect to determining foraging range, for bees can only be detected up to 700 m, and then only if they remain within a direct line of sight of the radar equipment. Using *B. terrestris*, Osborne *et al*. (1999) recorded foraging up to 631 m from the nest, but many bees went further and were lost from view on the radar.

Most recently, the foraging ranges of two bumblebee species (*B. terrestris* and *B. pascuorum*) have been estimated using molecular (microsatellite) markers. Both Chapman *et al*. (2003) and Darvill *et al*. (2004) used an approach based on identifying sisters among sampled workers although their methodologies and analyses differed. In the former study, focusing on urban habitats, foraging ranges were estimated as 0.6–2.8 km (*B. terrestris*) and 0.5–2.3 km (*B. pascuorum*). In Darvill *et al*.'s study, the distribution of sisters along a linear transect was used to directly estimate the foraging range of *B. terrestris* as greater than 312 m and that of *B. pascuorum* as less than 312 m. With hindsight, the spacing of sample points along this transect was too wide (17 sample points 625 m apart along a 10-km transect) relative to bee foraging ranges to reveal the details of foraging ranges (no *B. pascuorum* sisters were detected in adjacent sites). Here we refine and expand on this approach by using a much shorter transect and more closely spaced sample points to compare the forage range and nest densities of four UK bumblebee species.

Materials and methods

Sample collection

Individuals of four common bumblebee species (*Bombus pascuorum*, *Bombus terrestris*, *Bombus lapidarius*, and *Bombus pratorum*; see, e.g. Goulson 2003a) were collected from seven sites (A–G) spaced approximately 250 m apart along a 1.5-km linear transect across arable farmland on the estate of Rothamsted Research, Hertfordshire, UK, during the summers of 2002 and 2003 (Fig. 1, Table 1). Sampling was conducted over a period of approximately 1 month in each year [July–August 2002 (*B. pascuorum*), June–July 2003 (*B. terrestris*, *B. lapidarius*, *B. pratorum*)]. The sampling period differed slightly between years to allow sampling to take place when worker numbers were highest; *B. pascuorum* workers peak in August, slightly later than most species, with *B. terrestris* and *B. lapidarius* peaking in July and *B. pratorum* in June (Prys-Jones & Corbet 1991; Goulson *et al*. 2005). Although ideally all species would have been sampled from the same year, the method was initially employed with *B. pascuorum* as a pilot test of previous data (Darvill *et al*. 2004), which was then expanded to include the other species in the following year. Bees of each of the four species were collected from suitable forage within a 50-m radius of each sampling point. Suitable forage included flowering crops: oilseed rape (*Brassica napus*), borage (*Borago officinalis*), and field beans (*Vicia faba*); and also various hedgerow and field margin flowers, notably bramble (*Rubus fruticosus agg.*), white clover (*Trifolium repens*), field poppy (*Papaver rhoeas*), comfrey (*Symphytum officinale*), and bird's foot trefoil (*Lotus corniculatus*). Potential bumblebee nesting sites were probably largely limited to banks, hedgerows, and small areas of woodland (Fig. 1). Samples were immediately preserved in 100% ethanol for later DNA extraction.

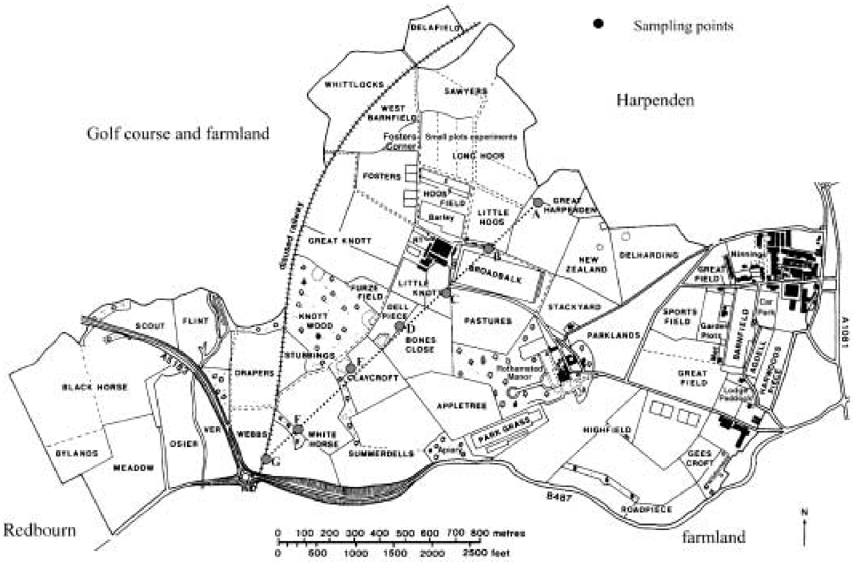

Fig. 1 Rothamsted estate with the transect location and sample sites A–G.

Table 1 Samples sizes for each transect point (A–G, with UK ordnance survey map reference) and species. Entries in brackets denote samples that did not amplify for all loci that were therefore excluded in subsequent analysis

Species	A	B	C	D	E	F	G	Total
OS grid ref.	TL124138	TL122137	TL121135	TL118133	TL117132	TL114129	TL113128	
B. pascuorum	72	15	11	18	8	43 (+ 1)	17	184
B. terrestris	75 (+ 1)	31	46	21	9 (+ 1)	12	14	203
B. lapidarius	48 (+ 1)	56	49	52	53	62	59	379
B. pratorum	48	45	35	23	11	8	33	203

Microsatellite genotyping

DNA was extracted from thoracic muscle tissue using the HotSHOT protocol (Truett *et al*. 2000) and amplified using FAM-, HEX- or NED-labelled forward primers. Polymerase chain reaction (PCR) products were resolved on an ABI Prism 377 automated sequencer (Applied Biosystems) with internal size standards (Genescan ROX 350, Applied Biosystems). Identical sample controls were used throughout for each species. Alleles were sized using GENESCAN and GENOTYPER software (Applied Biosystems). Any cases of scoring ambiguity or non-amplification were reprocessed for confirmation of allele sizes. A total of four samples could not be amplified at all loci and were excluded from subsequent analysis (Table 1).

All samples were scored at either eight (*B. pascuorum* and *B. pratorum*) or nine (*B. terrestris* and *B. lapidarius*) variable microsatellite loci (Estoup *et al*. 1995, 1996). All species were scored at loci *B10*, *B11*, *B96*, *B124*, *B126*, *B132* and *B100* (*B100* monomorphic in *B. pascuorum*). *Bombus terrestris* and *B. pascuorum* individuals were additionally typed at *B118* and *B121*, *B. lapidarius* at *B118* and *B131*, and *B. pratorum* at *B131* and *B121*. Homozygous genotypes were amplified and scored twice independently for *B. terrestris* to gauge the extent of scoring error and/or allelic dropout.

Data analysis

Genotypes were checked for typographic errors using MSA (Dieringer & Schlötterer 2002; http://i122server.vu-wien.ac.at). Current evidence suggests that queens of all of the study species mate only once (Estoup *et al*. 1995; Schmid-Hempel & Schmid-Hempel 2000). In the absence of polyandry any pair of bumblebee workers from the same nest has an expected relatedness of 0.75 (Hamilton 1964). Sister relationships among the individuals sampled were established within each species using the likelihood function of KINSHIP 1.3.1 (http://www.gsoftnet.us/GSoft.html; Goodnight & Queller 1999) where $Rm = 0.5$ and $Rp = 1.0$. Confidence in sister pair assignment was calculated from 1 000 000 simulations, the number of iterations determined by repeating analysis runs using variable numbers of simulations and establishing the point after which results reached a plateau. To minimize type I errors, given the high number of pairwise comparisons within each data set, only sisters designated at $P \leq 0.001$ (the most stringent value that KINSHIP will return) were used in further analysis. Wang's recently published COLONY 1.1 program (2004) is an alternative likelihood option for detecting colonies with precisely such data sets that can additionally account for specified rates of genotyping error. This package has given colony number estimates similar to the described method when tested on previous, similar data sets (from Darvill *et al*. 2003). We here used KINSHIP primarily to keep the general analysis consistent with a previous study (see introduction, Darvill *et al*. 2003) in order for resultant data to be comparable.

KINSHIP assumes linkage equilibrium and no inbreeding. Data for each species were therefore tested for linkage and deviations from Hardy–Weinberg equilibrium using GENEPOP (Raymond & Rousset 1995; http://wbiomed.curtin.edu.au/genepop/). Sequential Bonferroni corrections were applied for multiple tests (Rice 1989).

Estimating foraging range

Foraging ranges were estimated for each species from an analysis of the distribution of identified sisters along the transect. Sister pairs were grouped into separate categories on the basis of being found within the same site, 1 site apart, 2 sites apart, and so on up to the maximum of 6 sites apart. To preclude bias from the variation in sample sizes across sites, the number of sister pairs found was then compared to the total 'possible' number of sister pairs caught within the same site, 1 site apart, 2 sites apart, etc., giving a relative frequency of sister pairs found at each separation distance. This then also controls for any bias introduced by the sample site position along the transect (an 'end' effect) because these sites contribute fewer possible sister pairs than sites nearer the centre of the transect. Relative frequencies were then plotted and the best-fit curve fitted. The confidence level as given by KINSHIP represents the probability of reporting a sister pair erroneously. Here, we expect false sister pairs to be reported at frequency of 0.001 (see above). Given this, once the relative number of sister pairs (the number of sister pairs found/the total potential number of sister pairs) reported falls below 0.001, the expected error rate, we cannot assume these to be 'true' sister pairs. To calculate the distance at which the relative frequency of detected sister pairs fell to this level, 0.001 was used as the value of y in the equation of the best-fit curve for each species to calculate x, the separation distance. The resultant x was then halved to give a minimum estimate of the maximum foraging range for each species (the most conservative assumption being that two detected sisters were both foraging equidistant from their nest, and that the nest was situated on the transect). Nest size is known to vary between these four species, with *B. pratorum* nests ranging in size from approximately 30 to 50 individuals, *B. pascuorum* 30 to 100 individuals, and *B. lapidarius* and *B. terrestris* 30 to 250 individuals (Sladen 1912). Nest size should not affect the sister pairs detected. With roughly equal samples sizes for each species (see Table 1, with the possible exception of *B. lapidarius*, see Discussion), we would either detect a small number of large nests, or a large number of small nests but there is an equal probability of detecting sisters from either, both within and between sites.

Estimating nest density

Nest density for each species was estimated as the number of nests represented at an average site, calculated by establishing the number of nests represented by 1 bee, 2 bees, 3 bees, and so on, from the sisters found by KINSHIP for each site (see also Darvill *et al*. 2004). Since sample sizes were not completely uniform between sites, the number found within each category (nests represented by 1 bee, 2 bees, 3 bees, etc.) at each site was then divided by the sample size for each site and multiplied by the average number of bees caught per site to give a relative frequency for each category at each site. Since for our calculation we were interested in the number of nests represented per site, nests that were represented at more than one site were counted independently for each site. The mean number of bees representing 1 nest, 2 nests, etc. was then calculated across all sites with 95% confidence limits. Assuming that nests are randomly distributed, the number of nests represented by 0, 1, 2, 3, etc. bees should follow a Poisson distribution (see Chapman *et al*. 2003; also Heath 1995). The best fit of a Poisson distribution was found through an iterative process of estimating the number of nests that were not sampled (the 'zero bee' category) for each sampling point along the transect and therefore a best estimate of the number of nests that were not sampled. To obtain an estimate of error for the zero category, a Poisson distribution was also fitted to the extremes of the 95% confidence limits. An estimate for the mean total number of nests represented per site was then established by adding all categories. Nest density can then be calculated by taking the mean number of nests per site and dividing that number by the area sampled (of which the estimated foraging range of each species is the radius) to gain an estimate for the number of nests per km^2.

Results

One hundred and twenty-seven *Bombus pascuorum* sister pairs ($n = 184$, 8 loci, type II error where $P \leq 0.001 = 0.092$), 75 *Bombus terrestris* sister pairs ($n = 208$, 9 loci, type II error where $P \leq 0.001 = 0.0068$), 166 *Bombus lapidarius* sister pairs ($n = 379$, type II error where $P \leq 0.001 = 0.023$) and 197 *Bombus pratorum* sister pairs ($n = 203$, 8 loci, where type II error $P \leq 0.001 = 0.022$) were identified. At these levels of type II error, we would expect to have falsely rejected 11 *B. pascuorum* sister pairs, one *B. terrestris* pair, four *B. lapidarius*, and four *B. pratorum* from the overall results. Given that any falsely rejected pairs would be expected to be randomly distributed, this was not considered to have a significant effect on the overall patterns of sisters found along the transect. 'Noncircular nests' featured at low frequency in all species (cases where individual A is found to be a sister of individuals B and C but where individual B is not found to be a sister of individual C). This inevitably occurs when using stringent criteria for acceptance of sister relationships ($P \leq 0.001$). In these cases, data were re-examined and where individuals B and C would have been accepted as sisters at a less stringent significance level ($P \leq 0.01$), the group were accepted as true sisters. Where no such relationship was evident between individuals B and C, then the most parsimonious route was taken to gain circularity by omitting individuals from the family using a rule that where a choice existed between individuals, the one collected from the most distant site was eliminated, thus any introduced bias was conservative with regard to foraging range.

Hardy–Weinberg and linkage disequilibrium

Bombus pascuorum, B. terrestris *and* B. lapidarius. After correction for multiple tests, there was no indication of linkage for any locus pair for any of these three species. One locus for each of these three species was found to not meet Hardy–Weinberg assumptions (*B121* in *B. pascuorum*; *B100* in *B. lapidarius*; *B126* in *B. terrestris*). In all three cases this was due to a deficit in heterozygotes and it is suggested that null alleles may be present in these species at these loci. Allelic dropout is another potential source of apparent heterozygote deficiency although we do not expect this to cause major discrepancies where source tissue material is of high quality (see, e.g. Flagstad *et al*. 1999). Further, from rescoring 378 homozygous *B. terrestris* samples we found only three discrepancies of false homozygotes (0.8%), suggesting that allelic dropout and/or scoring errors are unlikely explanations. Without these loci included for the relevant species, all global tests for departure from Hardy–Weinberg equilibrium (HWE) were nonsignificant (*B. pascuorum* $\chi^2 = 21.50$, d.f. = 14, $P = 0.09$; *B. terrestris* $\chi^2 = 23.19$, d.f. = 16, $P = 0.11$; *B. lapidarius* $\chi^2 = 21.94$, d.f. = 16, $P = 0.14$). To ensure, however, that these loci had not biased the overall results, data for all three species were rerun through KINSHIP without the locus that failed to meet the HWE assumption. In no case did this significantly alter the foraging range or nest density estimates for any of the species (see succeeding section).

B. pratorum. Significant linkage disequilibrium was found between several locus pairs (*B131-B132*; *B132-B96*; *B10-B96*; *B132-B124*; *B96-B124*; *B131-B121*; *B100-B121*) after Bonferroni correction. Significant deviations from Hardy–Weinberg proportions were also detected at three of the eight loci screened (*B132*, *B11* and *B96*). Further tests revealed an excess of homozygotes at each of these loci. The presence of null alleles is one possible contributing explanation for these results. A pattern of consistent linkage disequilibrium and an excess of homozygotes might also imply either selection (either directly on particular

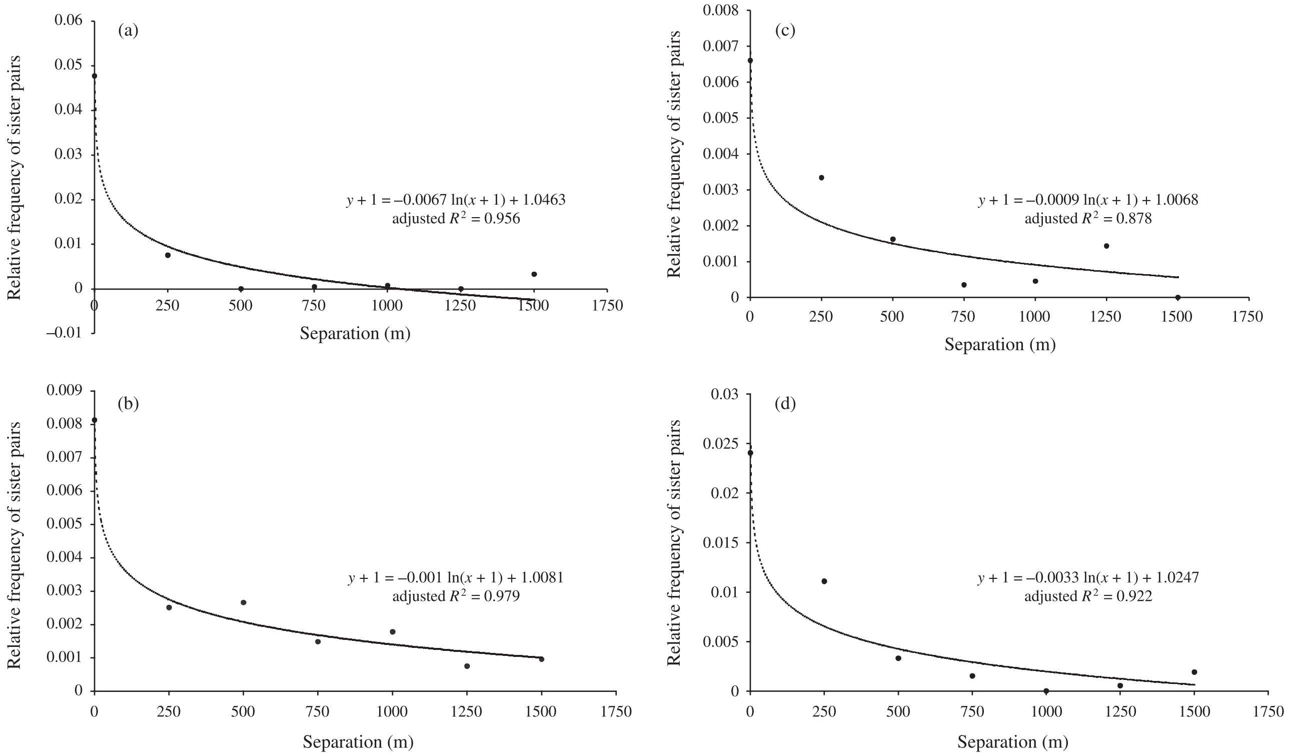

Fig. 2 Estimating foraging ranges for *Bombus pascuorum* (a), *B. terrestris* (b), *B. lapidarius* (c), and *B. pratorum* (d). Separation distance where $y = 0.001$ (at which frequency sister pairs become undetectable, see main text) was calculated for each species from the relevant equation as shown.

haplotypes/genotypes or through nonrandom mating) or the effects of drift in a finite population. The latter is expected to only have a significant impact on small populations. At this stage it is unclear which of these processes may be operating, although in the absence of further data the effects of null alleles and/or drift on small populations is the most plausible given that there is no evidence of selection in any of the other species under investigation either in the present or other published studies. Due to the number of loci involved, it was not possible to simply remove these from the data set and check that they were not biasing the analysis. Instead, two loci were removed and the data rerun through KINSHIP five further times (removing *B96* + *B10*, *B131* + *B132*, *B124* + *B10*, *B96* + *B100*, and *B131* + *B121*). As for the other species in no case did these removals significantly alter the foraging range or nest density estimates. Clearly, however, results for this species should be treated with more caution.

Foraging range estimates

All species closely matched a logarithmic curve (*B. pascuorum* adjusted $R^2 = 0.956$, $F_{1,5} = 132.344$, $P = 0.0001$; *B. terrestris* adjusted $R^2 = 0.979$, $F_{1,5} = 246.83$, $P = 0.0000$; *B. lapidarius* adjusted $R^2 = 0.878$, $F_{1,5} = 44.28$, $P = 0.0012$; *B. pratorum* adjusted $R^2 = 0.922$, $F_{1,5} = 71.84$, $P = 0.0004$; Fig. 2a–d). By calculating the separation distance where $y = 0.001$ (relative frequency of sister pairs), and assuming that this distance represents the diameter of the circle of foraging workers around the nest, minimum estimates for the maximum foraging distances are 449 m for *B. pascuorum*, 758 m for *B. terrestris*, 450 m for *B. lapidarius*, and 674 m for *B. pratorum*.

Nest density estimates

The frequency distribution of nests detected by 1, 2, 3, etc. sisters conformed to a Poisson distribution for all species (*B. pascuorum*: $\chi^2 = 0.11$, d.f. = 1, $P = 0.738$; *B. terrestris*: $\chi^2 = 0.12$, d.f. = 1, $P = 0.731$, *B. lapidarius*: $\chi^2 = 0.45$, d.f. = 2, $P = 0.797$, *B. pratorum*: $\chi^2 = 0.33$, d.f. = 1, $P = 0.569$, Fig. 3a–d). The mean number of nests not sampled at each site was estimated as 21 for *B. pascuorum*, 26 for *B. terrestris*, 27 for *B. lapidarius* and 14 for *B. pratorum*. Estimates for the total mean number of colonies per site were then calculated as: *B. pascuorum* 42.9 (range 33.2–48.7); *B. terrestris* 51.7 (range 48.0–55.4); *B. lapidarius* 74.5 (range 72.6–80.3); *B. pratorum* 37.3 (range 32.2–42.5). Assuming the foraging ranges as calculated above, these estimates translate to densities of 67.8/km² (range 52.5–76.9) for *B. pascuorum*, 28.7/km²

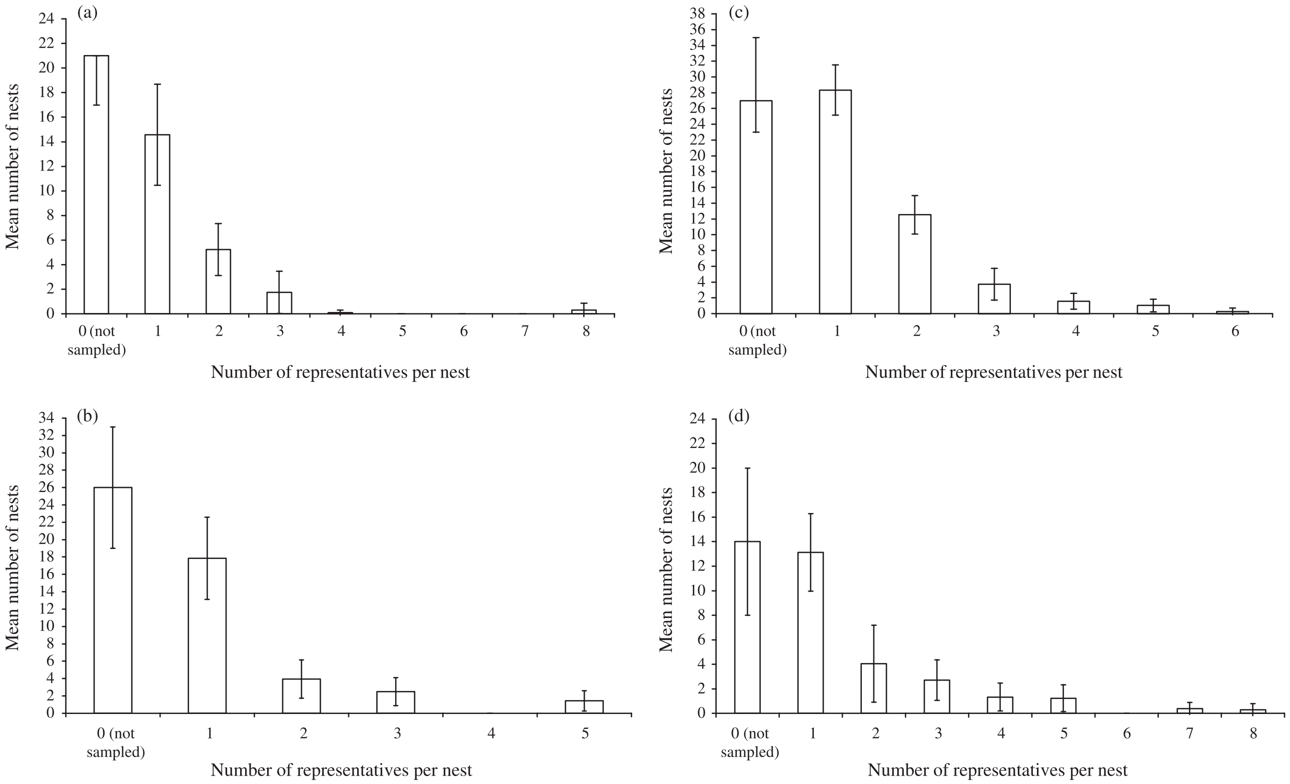

Fig. 3 Estimating nest densities for *Bombus pascuorum* (a), *B. terrestris* (b), *B. lapidarius* (c) and *B. pratorum* (d). Truncated Poisson distributions were fitted to data indicating the mean number of nests represented by 1, 2, 3, 4, etc. workers for each site to estimate the mean number of nests that were not sampled at all, with 95% confidence limits. The sum of all categories is then the estimated mean number of nests found per site.

(range 26.6–30.7) for *B. terrestris*, 117.2/km^2 (range 114.2–126.3) for *B. lapidarius* and 26.1/km^2 (range 22.6–29.9) for *B. pratorum*.

Discussion

How far do workers travel to forage?

Our estimates of foraging range for *Bombus pascuorum* (449 m) and *Bombus terrestris* (758 m) marry well with suggested ranges from previous studies. Although limited by sampling sites that were too distant, Darvill *et al*. (2004) found *B. pascuorum* workers to have a shorter range (rarely travelling more than 312 m) than *B. terrestris* workers, which were found to forage further than 312 m. Chapman *et al*. (2003) also provided an estimate of foraging distances for *B. pascuorum* (0.51–2.3 km) and *B. terrestris* (0.62–2.8 km). Although the method of calculation was quite different, and the study was carried out in an urban as opposed to rural landscape, our minimum estimates for the maximum distances that foragers of each species travel also concur with the lower ranges of Chapman *et al*.'s estimates, with *B. pascuorum* suggested to have a shorter range than *B. terrestris*. Our foraging range estimate for *Bombus lapidarius* was very similar to *B. pascuorum* at 450 m, and for *Bombus pratorum* more similar to the *B. terrestris* estimate at 674 m although, as noted earlier, results for the latter should be interpreted cautiously. While our estimate for the foraging range of *B. pascuorum* was from a year earlier than for the other three species, given the concordance with two earlier estimates along with the variability evident among species sampled the same year, we would suggest that foraging range would not be appreciably different between the 2 years for that species. We considered whether given that *B. lapidarius* was the most abundant species found on the transect we had sampled a substantially smaller percentage of the overall population and therefore may have underestimated foraging range. The likelihood of this is lessened by the larger sample size for this species (nearly twice that for the other three species) although it remains a possibility.

Why are there differences in foraging ranges?

Our results provide strong support for suggestions that there are important differences in foraging range between species (Walther-Hellwig & Frankl 2000; Darvill *et al*. 2004). Further, the similarity between estimates for *B. pascuorum* and *B. terrestris* from Darvill *et al*.'s study to that reported here implies that, at least within broadly similar habitat types, foraging ranges are consistent within species. Consequently there are substantial differences in the area of forage that species utilize; here, for example, the *B. pascuorum* foraging range of 449 m encompasses 62 ha, while that of *B. terrestris* encompasses 180 ha. Evidently, foraging range does not bear any simple relationship with body size. Although worker size is variable in all species, the order of average size from largest to smallest for these species is *B. terrestris*, *B. lapidarius*, *B. pascuorum*, *B. pratorum* (Peat *et al*. in press).

A key question arises from these data: Are foraging ranges fixed, or flexible according to resource availability? The latter would seem most probable, in which case, why do some species fly further than others within the same habitat? Why, in three independent studies in different habitats, does *B. terrestris* fly further than *B. pascuorum*?

Having a long foraging range may enable *B. terrestris* nests to survive in degraded habitats with a lower density of forage, compared to species with shorter foraging ranges such as *B. pascuorum* and *B. lapidarius*. Certainly, *B. terrestris* has proved to be an adaptable species, having successfully colonized remote parts of the globe such as New Zealand, Tasmania and Japan (although with the help of humans) (Goulson 2003b), and possibly a long forage range may be a key contributor to this adaptability.

While it is feasible to argue the advantages of long foraging ranges in degraded habitats, it is not immediately intuitive in an energetic context what the benefit is to *B. terrestris* workers in travelling further (than other species) to forage in the arable habitat studied here. If we assume that foraging range is a function of how far workers need to travel to gain enough forage to maintain nests, adequate explanations for the patterns found here are hard to pinpoint. One possible rationale is that *B. terrestris* individuals, for example, need to travel further as they have more specific foraging needs. This seems unlikely since *B. terrestris* are considered to be one of the most generalist foragers of all the British *Bombus* species (Goulson & Darvill 2004; Goulson *et al*. 2005). Another possibility connects foraging range to nest size. *Bombus terrestris* is thought to have much larger nests than *B. pascuorum* (Alford 1975). *Bombus terrestris* workers may thus simply need to forage more widely to be able to sustain these larger nests. A compounding feature of *B. terrestris* workers is that they are, on average, much larger than *B. pascuorum* (Peat *et al*. in press), adding to the greater foraging requirements placed on *B. terrestris* workers. Such an argument would not, however, stand for *B. pratorum* or *B. lapidarius*. Another potentially contributing factor is that foraging ranges may vary within species throughout the season depending on the specific needs of the colony. Clearly there is still much work to be done with regard to understanding the underlying factors that shape ecological parameters such as foraging range. It is quite possible that the explanation lies in complex interactions between nest density, nest size, nest longevity, foraging range and/or other unknown factors, and further studies would be required to uncover any such interactions. The extent of intraspecific variation either temporally or spatially or both depending on available forage, as well as insights into how this might relate to, for example, nest size would be a very promising area of future research.

Bumblebee species differ markedly in the degree with which they have declined in recent years as a result of habitat degradation and fragmentation (Goulson 2003a). Foraging range is an attribute which may in part explain these differences in sensitivity. However, all of the species in this study are ubiquitous in England. It would be informative to employ this approach to study some of the rarer species to test whether, as we might predict, they have shorter foraging ranges than common species.

How many nests do agricultural landscapes support?

The number of nests is the true measure of effective population size in bumblebees because each nest is effectively one breeding pair: the queen and her deceased mate (ignoring low levels of worker reproduction). To date nest density has proved extremely difficult to estimate since nests cannot be reliably found in large enough numbers. We attempted here to gather broadly comparable data for each species by sampling at the peak of their respective worker seasons. It should, however, be underlined that these estimates represent only the number of nests present in a subsample of the season — how many nests then went on to successfully produce reproductive individuals, or had already died out or been destroyed, and whether these factors vary significantly between species, remains unseen. It is also likely that there is variation between years depending on, for example winter temperatures, as to how large some, or all, bumblebee species populations are the following season (Cumber 1953).

Assuming that our estimates are broadly comparable between species, our estimates are in accordance with Darvill *et al*. (2004) and suggest that there are large differences in nest density even among the common bumblebee species. *Bombus lapidarius* was the most common species in the study area (117 nests/km^2), and certainly it was the most abundant along the transect, as reflected by the large sample size (Table 1). Should the foraging range for *B. lapidarius* be depressed due to a much higher abundance (see previous section), the density for this species would be overestimated. At an average site Darvill *et al*. (2004) estimated 54.7 (range 52.7–86.9) *B. pascuorum* and 20.4 (14.1–51.9) *B. terrestris* nests. Here we estimate 42.9 (range 33.2–48.7) *B. pascuorum*, 51.7 (range 48.0–55.4) *B. terrestris*, 74.5 (range 72.6–80.3)

B. lapidarius and 37.3 (range 32.2–42.5) *B. pratorum* nests sharing resources at an average site. Per site, we estimate fewer *B. pascuorum* nests and more *B. terrestris* nests than Darvill *et al*., a trend also evident in our nest density estimates (here, 67.8/km^2 for *B. pascuorum* and 28.7/km^2 for *B. terrestris*; Darvill *et al*., 193/km^2 for *B. pascuorum* and 13/km^2 for *B. terrestris*). We tentatively suggest that this reflects real differences between sites although acknowledge the problems associated with comparing across different sites, scales, and times. The transect in the present study, although crossing arable farmland, is close to large suburban areas. It has previously been shown that *B. terrestris* nests grow more quickly in suburban areas compared to farmland (Goulson *et al*. 2002). Chapman *et al*. (2003) estimated numbers of nests represented per site in urban London to be 96 for *B. terrestris*. It seems likely that *B. terrestris* nests are more abundant in suburbia than in the countryside (Goulson 2003a). Interspecific differences in nest site requirements may have a major influence on the number of nests of each species that habitats contain. Broad-scale differences do exist between species; *B. terrestris* and *B. lapidarius*, for example, nest in subterranean holes whereas *B. pascuorum* generally construct nests on or close to the soil surface in dense vegetation or leaf litter (Alford 1975). *Bombus pratorum* nests in a broad range of sites both above and below ground. Whether the availability of such sites is a limiting factor for populations of any species is currently unknown. Predation and parasitism may also differ between species and from site to site.

The use of microsatellite markers to identify sisters and from their distribution estimate the number of nests present in an area could prove to be invaluable in measuring the effect of habitat type and of varying conservation management strategies on the population density of both common and rare bumblebee species. Of course many of the nests detected here may not have successfully produced new queens or males. This study provides a snapshot of bumblebee nest densities in summer, but it is possible that many more nests were founded in the spring that did not survive. Workers can now be sampled in a nonlethal manner, using tarsal clips, following Holehouse *et al*. (2003) to avoid increasing nest mortality through worker sampling. Successive sampling of workers from the same site through the active season would enable estimation of nest mortality rates, something that has never previously been measured.

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