

Use of genetic markers to quantify bumblebee foraging range and nest density

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Bumblebees (Hymenoptera: Apidae) are important pollinators of crops and wildflowers, but many species have suffered dramatic declines in recent decades. Strategies for their conservation require knowledge of their foraging range and nesting density, both of which are poorly understood. Previous studies have mainly focussed on the cosmopolitan bumblebee species *Bombus terrestris*, and implicitly assume this to be representative of other species. Here we use a landscape-scale microsatellite study to estimate the foraging range and nesting density of two ecologically dissimilar species, *B. terrestris* and *B. pascuorum*. Workers were sampled along a 10 km linear transect and 8–9 polymorphic microsatellite markers used to identify putative sisters. We provide the first published estimates of the number of colonies using a circle of radius 50 m in an agricultural landscape: 20.4 for *B. terrestris* and 54.7 for *B. pascuorum*. Estimates of nest density differed significantly between the two species: 13 km⁻² for *B. terrestris* and 193 km⁻² for *B. pascuorum*. Foraging ranges also differed substantially, with *B. pascuorum* foraging over distances less than 312 m and *B. terrestris* less than 625 m. Clearly bumblebee species differ greatly in fundamental aspects of their ecology. This has significant implications for the development of conservation strategies for rare bumblebees and isolated plant populations, for the management of bumblebees as pollinators, and for predicting patterns of gene flow from genetically modified plants.

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Bumblebee (*Bombus*) populations have declined dramatically in recent decades, both in Europe and in North America (reviewed by Goulson 2003). Of the United Kingdom's 25 native species, three are now extinct, and several more could face extinction within the next few decades (Goulson 2003). As they are important crop and wildflower pollinators (Corbet et al. 1991) their declines may have serious consequences for agriculture and for wildflower populations.

Key ecological parameters, such as effective population size and foraging range, need to be understood if bumblebee populations are to be successfully managed (Kearns et al. 1998, Schulke and Waser 2001, Steffan-

Derwenter et al. 2002). Bumblebee nests are founded by a single queen, and queens of most species are monoandrous, therefore the number of nests in a given area determines the effective population size. Locating bumblebee nests of even the more common species is difficult in the field and only sparse, qualitative information exists concerning the spatial distribution of natural nest sites of different species (Alford 1975, Svensson and Lundberg 1977, Fussell and Corbet 1992). Current estimates used to inform conservation bodies wishing to conserve scarce bumblebee species are of the order of 2–10 nests km⁻², but these values are not substantiated by any empirical data (M. Edwards, in Goulson 2003).

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Our understanding of foraging behaviour is less incomplete, but still limited (Bronstein 1995, Dramstad 1996, Saville et al. 1997, Osborne et al. 1999). The maximum foraging distance of a particular species determines the area within which resources can be utilized (Bronstein 1995, Westrich 1996). Where bumblebees are used for crop pollination, foraging range will determine the area that workers from a nest may visit, and therefore the distances over which pollen may be carried (Corbet et al. 1991). Most studies to date have focussed on *Bombus terrestris* (L.), and have implicitly assumed that foraging range is similar in all species. However, a few authors have suggested that foraging ranges may differ quite dramatically among species (Free and Butler 1959, Witte et al. 1989, Hedtke, 1996, Walther-Hellwig and Frankl 2000). Most recently, Chapman et al. (2003) used molecular methods to study resource sharing in urban areas for *B. terrestris* and *B. pascuorum* (Scopoli). They found that a surprising number of colonies were sharing the resources in urban parks (96 and 66 colonies respectively). From this they inferred that the two species studied have large foraging ranges and that *B. terrestris* forages furthest from the nest (but see discussion). Species-specific differences in the nest densities and foraging ranges of bumblebees could potentially have major impacts on their management as crop pollinators. These differences could also be important when predicting potential gene flow from genetically modified crops (Raybould and Gray 1993, Scheffler et al. 1993, Rieger et al. 2002).

Here we use highly variable microsatellites to reconstruct sibships among *B. terrestris* and *B. pascuorum* individuals caught along a line transect in order to:

- 1) determine whether two ecologically dissimilar bumblebee species differ in foraging range;
- 2) establish the number of colonies sharing the resources at an average site;
- 3) estimate nest density for each species.

B. terrestris and *B. pascuorum* are both relatively common species, with long lifecycles. Workers typically appear in May, or May-June respectively. *B. terrestris* nests usually remain active until July-August, while those of *B. pascuorum* often survive well into the autumn. *B. terrestris* is a short-tongued species that produces large colonies in subterranean nests. By contrast, *B. pascuorum* has a medium-length tongue and produces smaller colonies at the ground surface (Alford 1975). In light of the findings of previous studies, we anticipate that measurable differences in foraging range will exist between these two species, with *B. terrestris* foraging furthest. However, as so little is known about the nesting density of bumblebees, it is difficult to predict whether the species will differ in this aspect of their ecology.

Material and methods

Collection of biological material

During July 2001, individuals of *Bombus terrestris* and *B. pascuorum* were collected from 17 sample sites spaced $625 \text{ m} \pm 20 \text{ m}$ apart along a straight 10 km transect (50:57:12N, 1:32:14W to 50:59:55N, 1:39:39W). The transect passed predominantly through mixed farmland, with some areas of woodland and gardens (Fig. 1). Ten kilometres represents the theoretical maximum range over which bumblebees can forage and return with a net profit (Cresswell et al. 2000), and also the maximum



Fig. 1. A map of the study area indicating the location of sample sites and major habitat areas.

distance from which they have returned in homing experiments (Goulson and Stout 2001). Bees were collected from patches of suitable forage within a radius of 50 m. A total of 2 hours was spent at each site, but forage quality, and hence number of bees caught, varied somewhat between sites. In total, 97 *B. terrestris* workers and 237 *B. pascuorum* workers were caught, representing an average of 5.7 and 13.9 bees per site respectively. Samples were preserved immediately in 100% ethanol.

Molecular methods

DNA was extracted from thoracic muscle tissue using a proteinase K/chloroform protocol (Rico et al. 1992). DNA was re-suspended in 200 µl Tris-HCl buffer (10 µM, pH 9.0), and diluted a further tenfold prior to PCR. Workers were genotyped at 9 microsatellite loci: B10, B11, B96, B100, B118, B121, B124, B126 and B132 (Estoup et al. 1995, 1996), although *B. pascuorum* was found to be monomorphic at B100. PCR products were visualised on an ABI PRISM™ 377 semi-automated sequencer using an internal size standard (GeneScan ROX 350, Applied Biosystems). Fragment sizes were scored using the Genotyper software package (Applied Biosystems). Repeat PCRs were carried out on any samples that had failed to amplify or were uncertainly scored.

Bumblebees are haplodiploid and full sisters have an average coefficient of genetic relatedness of 0.75 (Hamilton 1964, Estoup et al. 1995). In addition, both *B. terrestris* and *B. pascuorum* are monoandrous (Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000) so sisters can be reliably distinguished from unrelated individuals using sufficiently variable microsatellite loci (Queller et al. 1993). Sisters were identified using Kinship 1.3.1, with a target relatedness level of 0.75 and a null hypothesis of zero relatedness (Goodnight and Queller 1999). Likelihood tests were performed between all individuals of each species. The accuracy with which sister-pairs were identified was maximised by performing 900 000 calibration simulations. This figure was reached by running the program several times with increasing numbers of simulations, whilst checking the output for changes. Sisters were accepted at the $p < 0.001$ level, again to minimise type I errors. In performing simulations, Kinship assesses the power of the data set to resolve full sisters (i.e. the frequency of type II errors).

Hardy–Weinberg and linkage disequilibrium

Tests for genotypic linkage disequilibrium and departure from Hardy–Weinberg equilibrium were performed using GENEPOP version 3.1b (Raymond and Rousset

1995). Sequential Bonferroni corrections (Rice 1989) were applied to minimise type I errors.

Forager distributions

To examine forager distributions, the distribution of sister-pairs along the transect was compared to the distribution of potential sister pairs among the sampled workers within each site. The number of possible pairwise relationships within samples sites is given by $n(n-1)/2$ where n is the number of bees in the sample. This was summed for all 17 sites, and the number of detected within-site sister-pairs expressed as a proportion of this number.

The number of possible pairwise relationships between samples a and b is given by $n_a \times n_b$. The total number of possible pairwise relationships was summed for all adjacent sites separated by 625 m (sites 1 and 2, 2 and 3, etc.), and the number of identified sister-pairs found at adjacent sites expressed as a proportion of this number. This process was then repeated for sites 1250 m apart (sites 1 and 3, 2 and 4 etc.), and so on up to sites 10 km apart (sites 1 and 17). At sites near the extremes of the transect, we would expect to detect fewer sister-pairs, as nests located beyond the end of the transect would not have been sampled. However, by comparing the number of detected sister-pairs with the number of possible sister-pairs, based on our sample sizes, we account for this problem. When accepting sisters at the $p < 0.001$ level, false sister-pairs are expected at a frequency of 1/1000. The Binomial exact test was used to determine whether frequencies were significantly greater than this. Sequential Bonferroni corrections were applied to minimise type I errors (Rice 1989).

Resource sharing and nest density

The number of colonies sampled at a site is given by the total number of sister-groups detected (with many groups containing just one bee). However, due to limited sampling effort it was unlikely that, at any site, representatives of all nests were caught. To estimate how many nests were not detected, a frequency distribution of the number of workers caught per colony was constructed for each site. These values were averaged across all sites to give a frequency distribution of detected workforce sizes for an average site. In the absence of any data on the distribution of foraging bumblebees, a random distribution is the safest assumption to make, in which case a Poisson distribution would be expected (Heath 1995). For both species, truncated Poisson distributions closely conformed to our data. Following Chapman et al. (2003), best-fit Poisson distributions were fitted to our data by iteration, comparing our observed distribution with numerous

Poisson distributions until a best-fit was achieved (Heath 1995). This distribution was then used to estimate the number of nests within range of an average site that were not sampled (i.e. the magnitude of the zero category). In order to estimate the error in these values, Poisson distributions were fitted through the extremes of our confidence intervals, such that a maximum and minimum estimated value were produced.

Results

Hardy–Weinberg and linkage disequilibrium

No locus pair showed significant linkage disequilibrium after Bonferonni correction. Similarly, no loci deviated significantly from Hardy–Weinberg equilibrium (HWE) (Table 1). A global test across all loci found no deviation from HWE in *B. terrestris* (Fisher's global test, $\chi^2 = 26.6$, $df = 18$, $p = 0.09$) and slight deviation in *B. pascuorum* (Fisher's global test, $\chi^2 = 30.0$, $df = 16$, $p = 0.02$). This may suggest the presence of null alleles at low frequencies within our population. The two loci showing the highest F_{is} values were experimentally removed in order to determine their effect on the results. As only very minor changes were observed (which made no difference to the overall conclusions), it was decided to include them in the analysis.

Species-specific variations in forager distributions

Thirty of 97 *B. terrestris* workers (31%) were sisters to at least one other sampled bee. In total 80 colonies were detected. Seventeen sister pairs were found within sites and 7 at adjacent sites (Fig. 2a). For *B. pascuorum*, 84 of

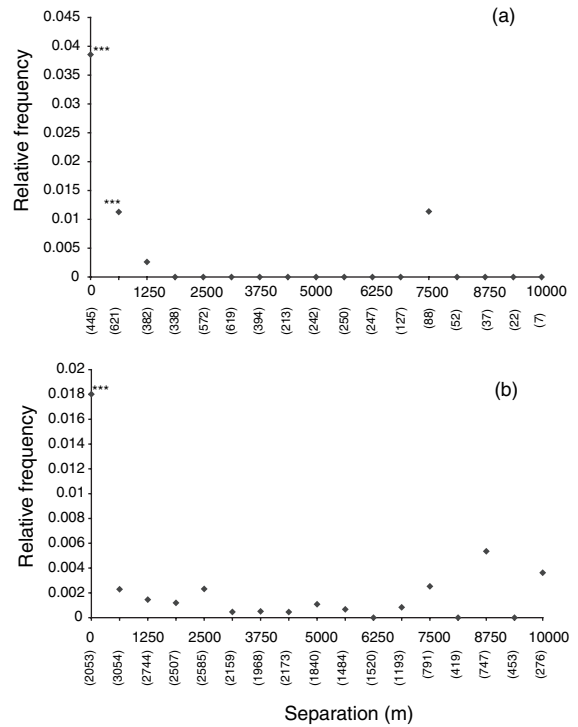


Fig. 2. The frequencies at which (a) *Bombus terrestris* and (b) *B. pascuorum* sister-pairs were detected at increasingly distant sample sites, expressed as a proportion of the number of pairwise comparisons made (shown in brackets). Within-site sister pairs are separated by zero metres, and between site sister pairs by 625 m and above. Sample sites were 625 m apart. *** $p < 0.001$, ** $p < 0.01$.

237 workers (35%) collected were sisters of at least one other sampled bee, and in total 190 colonies were detected. Thirty-seven sister pairs were found within

Table 1. Summary of genotypic data for the 9 loci used. Allele frequencies and F_{is} are based on a subsample with just one representative from each nest included. The sizes of the three most common alleles at each locus are shown in brackets (in base pairs).

Locus	Sample size	F_{is}	Allele number	Frequencies of the three most common alleles		
<i>B. terrestris</i>						
B132	97	-0.017	11	0.253 (165)	0.165 (163)	0.133 (161)
B100	97	-0.025	12	0.348 (154)	0.259 (162)	0.171 (164)
B118	97	+0.017	8	0.386 (219)	0.259 (213)	0.158 (221)
B96	97	+0.177	7	0.525 (238)	0.373 (244)	0.069 (242)
B10	97	+0.038	19	0.234 (196)	0.120 (182)	0.114 (198)
B11	97	-0.021	11	0.373 (168)	0.253 (160)	0.146 (172)
B126	97	+0.002	14	0.310 (174)	0.215 (180)	0.146 (176)
B124	97	-0.022	15	0.215 (254)	0.139 (250)	0.114 (244)
B121	97	+0.005	5	0.816 (166)	0.158 (160)	0.013 (168)
<i>B. pascuorum</i>						
B132	237	+0.055	14	0.377 (153)	0.144 (155)	0.144 (151)
B118	237	+0.068	17	0.223 (219)	0.192 (213)	0.182 (215)
B96	237	-0.007	11	0.421 (164)	0.272 (224)	0.164 (222)
B10	237	+0.039	2	0.943 (174)	0.056 (176)	-
B11	237	+0.020	6	0.859 (134)	0.053 (136)	0.046 (138)
B126	237	+0.013	6	0.766 (126)	0.100 (128)	0.072 (130)
B124	237	+0.160	8	0.405 (254)	0.195 (256)	0.133 (248)
B121	235	+0.018	24	0.167 (150)	0.131 (154)	0.113 (152)

sites and 7 at adjacent sites (Fig. 2b). For both species, sister-pairs were found more frequently within sites than between adjacent sites (Fisher's exact test, $p=0.009$, and $p<0.0001$ respectively for *B. terrestris* and *B. pascuorum*). *B. terrestris* sisters were present at adjacent sites significantly more frequently than can be explained by erroneous identification of sister-pairs (type I errors) (binomial exact test, $p < 0.001$). In contrast, the small number of apparent *B. pascuorum* sisters found at adjacent sites was not significantly more than would be expected from type I errors (binomial exact test, $p > 0.05$), suggesting that workers of this species seldom forage more than 300 m from the nest. Sisters were not found two or more sites apart more frequently than can be explained by type I errors for either *B. terrestris* or *B. pascuorum*. Type II error rates for *B. terrestris* and *B. pascuorum* were estimated by Kinship as 0.0051 and 0.0689 respectively. At these rates we would expect to mistakenly reject close to zero *B. terrestris* sister-pairs, and up to 4 *B. pascuorum* sister-pairs.

Resource sharing and nest density

The observed distributions of workforce sizes were well described by truncated Poisson distributions (*B. terrestris*: $\chi^2=1.135$, $df=2$, $p>0.05$; *B. pascuorum*: $\chi^2=3.007$, $df=2$, $p>0.05$). Fitted Poisson distributions gave estimates for the number of undetected nests that were within range of an average sample site (Fig. 3); 15.7 for *B. terrestris* and 43.5 for *B. pascuorum*. These values were added to the number of detected nests to estimate the total number of colonies within foraging range of an average site. It was thereby estimated that 20.4 *B. terrestris* colonies (range 14.1–51.9), and 54.7 *B. pascuorum* colonies (range 52.7–86.9) were sharing the resources at an average site.

The evidence suggests that *B. terrestris* occasionally travels more than 600 m in search of flowers (Osborne et al. 1999). Our data suggest that *B. pascuorum* rarely forages more than 300 m from the nest. Clearly, individuals found foraging at a site must have originated from a nest lying within the foraging range of that species. If the 20.4 *B. terrestris* colonies found at an average site were distributed at random within a circle of radius 700 m, then nest density would be approximately 13 nests per square kilometre. Similarly, if 54.7 *B. pascuorum* nests lay within 300 m of an average sample site, approximately 193 nests were present within an average square kilometre. Our analysis assumes that foraging bumblebees are located randomly within their foraging range, as little data are available to suggest an alternative distribution. If, however, bumblebee from nests located at the periphery of the species' foraging range seldom forage at long distances, then this method may slightly underestimate nest density.

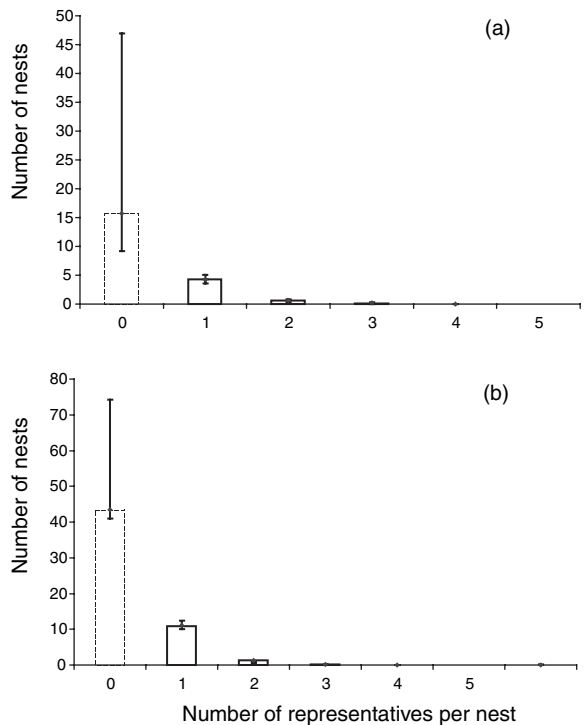


Fig. 3. The frequency distribution of workforce sizes from sampled nests at an average site (\pm SE), with the fitted Poisson distribution overlaid. The number of undetected colonies (dashed bar) is estimated for (a) *B. terrestris*; (b) *B. pascuorum*.

Discussion

Species-specific variations in forager distributions

One would expect bumblebee foragers to minimize their travel distances to maximise profits (Heinrich 1979). In this study, sisters of both species were found more frequently within sample sites than between adjacent sites (Fig. 2). This suggests that both bumblebee species do tend to forage relatively close to their nests. However, *B. terrestris* sister-pairs were also found with significant frequency at adjacent sites (625 m apart), but not at sites 1250 m apart. This species therefore must forage at least 312.5 m from its nest (further for nests that were not exactly halfway between sample sites and situated on the transect). By contrast, significant numbers of *B. pascuorum* sister-pairs were found only within sample sites, not at adjacent sites (Fig. 2). These data support the view of Osborne et al. (1999), that in an agricultural landscape, few *B. terrestris* foragers travel as far as 625 m from their nests. Previous data for *B. pascuorum* have suggested that it is a "doorstep forager", albeit based on meagre evidence (Witte et al. 1989). Our data concur, suggesting that few individuals forage further than 312.5 m from the nest in this species. However, it is important to recognise that resource availability is strongly linked to habitat type, and therefore that foraging range in

other habitats may differ from the estimates provided here. In particular, in urban areas where bumblebee resource densities are high, foraging ranges are likely to be somewhat lower.

The area of the annulus within which a bee may forage increases with the square of the distance from the nest. A small number of individuals foraging at large distances from their nests would be present at such low densities that distinguishing them from erroneously identified sister pairs (type 1 errors) would not be possible. Therefore our approach cannot place an upper limit on foraging range (as is true of all currently available techniques). It does however provide a rough indication of the rate at which forager density declines at increasing distances. Although the inter-specific differences in the relative frequencies of sister-pairs can in part be attributed to differences in nesting density or average worker number per nest, the contrast between the shapes of the two distributions cannot. The absence of a significant number of *B. pascuorum* sister-pairs found 625 m apart suggests that forager density declines more rapidly than it does for *B. terrestris*.

We might expect to find a similar trend, with many sister-pairs found within sites but few at adjacent sites, if high value forage was distributed in widely spaced clumps. Indeed there is evidence that when large patches of high value forage are available, bumblebees may travel over 1.5 km to utilise these resources (Walther-Hellwig and Frankl 2000). However, in our study sample sites were determined by their position on the transect line, and were not chosen on the basis of forage availability. Although patch quality did vary somewhat between sites, none of the sample sites were particularly rich in resources relative to those available in the hedgerows and field margins nearby. It is therefore highly unlikely that any sites acted as strong 'magnets'.

Resource sharing and nest density

To date it was not known whether the bumblebees visiting a small area of countryside were largely from a small number of nests. Our data clearly demonstrate that this is not the case. We estimate that 20.4 colonies of *B. terrestris* and 54.7 colonies of *B. pascuorum* were utilising the resources at an average site. Combined with estimates of foraging range, these values lead to estimated nesting densities of 13 km⁻² and 193 km⁻² respectively – a 15-fold disparity between species. This difference, although large, is supported by a comparative analysis of our data. Despite equal sampling effort for both species, 2.44 times more *B. pascuorum* workers were caught. On average, *B. terrestris* nests contain roughly 4.5 times as many workers than those of *B. pascuorum* (Alford 1975). This difference, combined with the observed difference in species abundance, suggests that

B. pascuorum nests were roughly 11 times more abundant. Clearly, if the species' foraging ranges differed more than is estimated here, as some authors suggest (Hedtke 1996, Walther-Hellwig and Frankl 2000), differences in nest density may have been even greater.

Producing a precise value for the number of undetected nests at an average site is not straightforward, and the errors in our estimates are large. However, considering the case where the true values lie at opposite extremes of the error bars is highly informative. Under these circumstances, although estimates of the number of undetected nests overlap (46.9 for *B. terrestris* and 43.5 for *B. pascuorum*), following through the same logic still leaves a 5.5 fold difference in nesting density between species.

A recent study of the same bumblebee species using similar methods, but in an urban area, demonstrated that a comparable but larger number of colonies utilised patches of flowers in urban parks (Chapman et al. 2003). They estimated that 96 colonies of *B. terrestris* and 66 colonies of *B. pascuorum* were sharing the resources at sample sites of similar size to ours. Chapman et al. (2003) then assumed that nest densities were in the range 2–40 nests km⁻² and so inferred maximum foraging distances of 0.87–3.9 km for *B. terrestris* and 0.72–3.2 km for *B. pascuorum*. These values are significantly higher than those produced by our method. In arriving at their values, Chapman et al. (2003) depend entirely on their assumed nesting densities, for which little empirical data is provided in support. Indeed the studies cited suggest nest densities of 200–700 nests km⁻² per species in suitable areas (Cumber 1953, Harder 1986). We suggest that the nest density values assumed by Chapman et al. (2003) are unrealistically low, and that as a result they have overestimated foraging range. Nevertheless, our findings are broadly complimentary, both demonstrating that an unexpectedly large number of colonies share patches of forage. However, it seems that urban parks may support higher densities of bumblebee nests than farmland areas. In contrast to our study, Chapman et al. (2003) found that *B. terrestris* was the most abundant species, suggesting that the distribution of resources in urban areas may better suit this species. Indeed, Goulson et al. (2002) demonstrated that *B. terrestris* nests in suburban areas grew faster than those in the countryside, and also that a specialist bumblebee parasite, the moth *Aphomia sociella* (L.), was more common in urban areas than elsewhere. Overall, it seems likely that the higher floral diversity of urban gardens compared to agricultural areas supports an increased density of generalist bumblebee species.

The observed differences in foraging range and nest density between bumblebee species could potentially have major impacts on their management as crop pollinators, and may also be important when predicting

potential gene flow from genetically modified crops (Raybould and Gray 1993, Scheffler et al. 1993, Rieger et al. 2002). Within agricultural landscapes, bumblebees are thought to nest mainly in hedgerows or field margins (Svensson et al. 2000, Kells and Goulson 2003). Both foraging range and nesting density are therefore crucial factors in predicting the likelihood of yield loss due to inadequate pollination in fields of differing sizes (Free and Williams 1976). Differences in foraging range have implications for wild flowers too. The maximum distance over which pollen is carried may depend on the species of bumblebee that a plant relies on for pollination. Thus detailed knowledge of plant–pollinator ecology is critical if we are to maintain genetic variability in isolated populations (Kwak et al. 1991, 1996, Young et al. 1996, Steffan-Derwenter and Tscharrntke 1999).

The large foraging range of *B. terrestris* may explain why it has proved to be so adaptable and remains ubiquitous throughout much of Europe despite widespread environmental change. Nevertheless, *B. pascuorum* was more abundant than *B. terrestris* in our study area (both in numbers of foragers caught and in our estimate of nest density), and remains widespread in Europe. Clearly foraging range alone does not predict species success. It would be highly informative to conduct a similar landscape-scale study with rare and declining species, to better understand their foraging ranges, resource requirements, and effective population sizes. If we are to effectively conserve and enhance populations of these species, it is clearly no longer acceptable to study *B. terrestris* and make assumptions about other species.

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