

Molecular and spatial analyses reveal links between colony-specific foraging distance and landscape-level resource availability in two bumblebee species

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Foraging distance is a key determinant of colony survival and pollination potential in bumblebees *Bombus* spp. However this aspect of bumblebee ecology is poorly understood because of the difficulty in locating colonies of these central place foragers. Here, we used a combination of molecular microsatellite analyses, remote sensing and spatial analyses using kernel density estimates to estimate nest location and foraging distances for a large number of wild colonies of two species, and related these to the distribution of foraging habitats across an experimentally manipulated landscape. Mean foraging distances were 755 m for *Bombus lapidarius* and 775 m for *B. pascuorum* (using our most conservative estimation method). Colony-specific foraging distances of both species varied with landscape structure, decreasing as the proportion of foraging habitats increased. This is the first time that foraging distance in wild bumblebees has been shown to vary with resource availability. Our method offers a means of estimating foraging distances in social insects, and informs the scale of management required to conserve bumblebee populations and enhance their pollination services across different landscapes.

Bumblebees are important insect pollinators, contributing to a key ecosystem service that is vital to the maintenance of both wild plant communities (Aguilar et al. 2006) and agricultural productivity (Klein et al. 2007). However there is mounting evidence that bumblebees have declined across Europe and North America (Biesmeijer et al. 2006, Grixti et al. 2009, Williams and Osborne 2009), along with the plant species they rely on as foraging resources (Carvell et al. 2006). Habitat loss and fragmentation are considered to be the most important drivers of these declines (Winfree et al. 2009, Potts et al. 2010) hence there is growing interest in developing mitigation options such as agri-environment schemes to restore the value of agricultural landscapes for pollinators (Carvell et al. 2007, Heard et al. 2007).

The spatial and temporal distribution of resources is particularly important for central place foragers such as bumblebees (Dukas and Edelstein-Keshet 1998), since their colonies occupy single nest sites from which foragers access a restricted area. The distance over which workers from a colony are able to locate and forage on floral resources is a key determinant of colony survival, especially where resources are widely dispersed (Schmid-Hempel and Schmid-Hempel 1998). It is also critical for pollination because it determines how far and how often pollen is transported across landscapes (Schulke and Waser 2001). According to optimal foraging theory, foragers will maximise their net rate of energy intake

by foraging close to the colony, given a relatively homogeneous distribution of forage patches (Heinrich 1979a). Variation in foraging range with resource distribution has been demonstrated for honeybees by decoding the 'waggle dance' of foraging workers (Beekman and Ratnieks 2000). However, predicting or estimating foraging distances and worker distributions for bumblebees (Cresswell et al. 2000) are difficult due to the challenges of finding and studying colonies in the field (Suzuki et al. 2009). This is further complicated by the fact that, unlike honeybees, bumblebees are thought not to communicate foraging locations to their nest mates (Steffan-Dewenter and Kuhn 2003, Dornhaus and Chittka 2004). In order to manage landscapes at the appropriate scale to conserve bumblebees and enhance their pollination services, it is important to understand how far individuals are capable of foraging, how nests are likely to be distributed across landscapes and how their location might influence the observed foraging distances of bees from those colonies.

Estimates of the flight distances and space use of bumblebees have previously been obtained by one of three general approaches: 1) observation of foragers from known wild or experimentally positioned colonies using mark-recapture approaches (Dramstad 1996, Walther-Hellwig and Frankl 2000, Osborne et al. 2008), radar tracking (Osborne et al. 1999) or timed observations of trip duration (Westphal et al.

2006a); 2) statistical correlations between observations of forager density and nectar distribution (Suzuki et al. 2009) or landscape composition (Westphal et al. 2006b) and 3) genetic analysis (Chapman et al. 2003, Darvill et al. 2004, Knight et al. 2005). The first two approaches exhibit various limitations; studies are often labour-intensive, they cannot be applied to large samples of wild colonies in typical landscapes, do not control for differences in sampling effort with distance from colonies or make simplifying assumptions about resource distribution and constancy of foraging ranges. By contrast, genetic analyses of bee DNA using molecular microsatellite markers permit population-wide inferences about patterns of space use across large numbers of wild colonies to be made. Such studies typically involve sampling worker bees from spatially independent resource patches (separated by a minimum of the foraging range of the species under study), grouping these individuals into families or 'sibships' on the basis of their multilocus genotypes (Wang 2004) and relating the number of colonies represented to surrounding landscape quality (Herrmann et al. 2007, Knight et al. 2009, Goulson et al. 2010). A related approach has involved genotyping workers sampled at intervals along a linear transect and determining the relationship between the probability that any two workers are full sisters and the distance apart at which they were sampled. This relationship is then used to estimate average foraging distances for different species (Darvill et al. 2004, Knight et al. 2005, Charman et al. 2010). However, in all molecular studies it is often the case that the majority of sampled colonies are represented by only a single worker. Furthermore, these techniques have not been used to determine the fine-scale distribution of workers in relation to forage or to predict nest locations. In particular, we know of no other study that has used genetic analyses to assign colony-specific foraging distances across different bumblebee species.

In this study we used analyses of microsatellite markers to construct probable sibship relationships for workers of two bumblebee species collected from multiple patches across a common landscape. We coupled these data with spatial analyses of GIS maps of the landscape to: 1) estimate colony-specific foraging distances and 2) relate these to the fine-scale distribution of surrounding foraging habitats. The study focuses on *Bombus lapidarius* and *B. pascuorum*, species that are relatively common and widespread in the UK but differ in aspects of their ecology (Goulson 2009). Although average estimates of foraging distance have been made for these species, they are highly variable (Knight et al. 2005, Westphal et al. 2006b, Lepais et al. 2010). We tested the hypothesis that bumblebee colonies vary their foraging distance according to resource availability, predicting that colony-specific foraging distances decrease as the proportion of high quality foraging habitat surrounding a colony increases. Furthermore, we asked whether this variation in foraging distances resulted in a greater proportion of suitable habitat being available to each colony than if all colonies foraged at a fixed distance, by comparing our estimates of colony-specific distance with previously published estimates of average foraging distance for each species (Knight et al. 2005). We also compared our method of estimation to an alternative recently proposed by Lepais et al. (2010) which assigns dispersal distances to individuals using a four case rule based on the distribution of full

sisters across sample sites, but is independent of any knowledge of landscape structure. We discuss why our method is potentially the most powerful to date for estimating both colony locations and foraging distances, and highlight its potential to inform the conservation of bumblebees across different landscapes.

Methods

Study landscape

The study was conducted across an agricultural landscape covering 2100 ha on the Hillesden Estate, Buckinghamshire, UK (1°00'01"W, 51°57'16"N) (Supplementary material Appendix A1). A number of standardised agri-environment mitigation options targeted at pollinators, including grass and wildflower mixtures sown along field margins (Natural England 2010), were established in 2005 alongside blocks of conventionally farmed arable fields as part of an ongoing landscape experiment (see Woodcock et al. 2010 for more details). In August 2007 airborne remote sensed data were acquired for the entire study landscape (ca 25 km²) using light detection and ranging and hyperspectral sensors (400–970 nm and 970–2450 nm). All datasets were geo-referenced and pre-processed before being classified using ERDAS Imagine software (ver. 9.0), while subsequent analysis was performed in ArcGIS (ver. 9.3). Supervised classification of the hyperspectral dataset, combined with a digital canopy height model derived from LiDAR, produced a high resolution (0.5 × 0.5 m pixels) map containing 18 land cover types including arable crops, intensive grassland, trees and buildings. Areas of rough vegetation (including sown wildflower mixtures and other herbaceous species within field margins or gardens) were the land cover type providing most bumblebee forage resources during our study (C. Carvell unpubl.) and are hereafter referred to as foraging habitats.

Sample collection

Workers of *Bombus pascuorum* (n = 170) and *B. lapidarius* (n = 214) were collected over three days during late July 2009 from searches of 100 × 6 m areas centred within flowering patches across the landscape. These patches were located along field margins or corners and represented the highest quality foraging habitats for both species at the time of the study, having been sown with a diverse mixture of grasses and wildflowers including *Centaurea nigra* and *Lotus corniculatus*. A total of 12 patches were sampled (Supplementary material Appendix A1), encompassing a variety of spatial scales and inter-patch separation distances (144–3555 m) to increase the probability of sister workers occurring across multiple patches, and thus reduce the number of colonies represented by a single worker.

Molecular methods and sibship reconstruction

Sampled workers were cooled and preserved in 100% ethanol and a single tarsus was removed in the lab from which DNA was extracted using the HotShot protocol with a total extraction volume of 100 µl (Truett et al. 2000). *Bombus*

lapidarius individuals were genotyped at eight microsatellite loci (mean number of alleles per locus = 14.6, SD = 1.38) and *B. pascuorum* individuals at 10 loci (mean alleles per locus = 9.3, SD = 1.8) divided between two multiplexes in each case. Allele range (number of alleles in least polymorphic locus to most polymorphic locus) for *B. lapidarius* was 9–21 and for *B. pascuorum* was 3–20. Effective number of alleles for *B. lapidarius* was 4.95 (SD = 0.48) and *B. pascuorum* was 3.67 (SD = 0.67). Amplification took place in a 10 µl reaction volume containing 5 µl QIAGEN Multiplex PCR Master Mix, 0.2 µM of each primer, 2 µl ddH₂O and 2 µl undiluted HotSHOT DNA. PCR involved a denaturing step at 95°C for 15 min, followed by 25 cycles of denaturing at 94°C for 30 s, annealing at 57°C for 90 s and extension at 72°C for 60 s, with a final extension of 60°C for 30 mins. PCR products were resolved on a capillary sequencer with internal size standards. Genotypes were determined using the GENEMAPPER ver. 3.7 software.

Tests for deviation from Hardy-Weinberg equilibrium were performed in GenAlEx 6.3 (Peakall and Smouse 2006) while exact tests for genetic differentiation were implemented in Arlequin 3 (Excoffier et al. 2005). The degree of relatedness between sister pairs for each species was estimated using the maximum likelihood sibship reconstruction method in Colony ver. 2.0 (Wang 2004, Jones and Wang 2010). This allowed us to group workers into colonies and to show the distribution of individuals from each colony across the landscape. We assumed that all workers within a colony were offspring of one singly-mated queen (Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000) and assumed genotyping error rates of between 0–1.2% per locus for *B. pascuorum* and 0–2.5% for *B. lapidarius* based on results of re-genotyping 10% of all samples. Colony was run ten times for each species, using a different random number seed each time, to give a maximum likelihood reconstruction of full sibships over all runs which was used for all further analyses.

Estimation of colony-specific foraging distances using ArcGIS

The location of each sampled individual was mapped onto the classified map of the study landscape in ArcGIS. All individuals from a patch were assigned to the same central location of the 100 × 6 m search area within that patch. The minimum convex polygon (MCP) encompassing all individuals is often used to delimit home range (Williams et al. 2004, Meyer et al. 2005) and in this case could be ‘drawn’ around the locations of full sisters to describe the observed foraging area of a colony. However, the MCP is unlikely to provide an accurate estimate of the colony location in situations where sister workers were not equally distributed across sample patches. We therefore used a fixed Kernel density estimate (KDE) (Worton 1989) to weight the estimated colony location by worker abundance. KDEs were created for all colonies, using a smoothing parameter of 750 m which was chosen based on the extent and spatial distribution of sampled patches, the known foraging distances of the species (Knight et al. 2005) and after exploring estimates derived from least squares cross validation (Hemson et al. 2005) and estimates of h_{ref} (Worton 1989). Multiple percent contours were generated for each KDE, representing

the areas encompassing a set probability of worker occurrence. The 75% contour was selected as giving largely continuous polygons covering the entire spatial distribution of sampled workers from each colony, whilst giving an appropriate weighting to patches with multiple sister workers (Fig. 1). The centroid of the 75% contour was then calculated and finally ‘snapped’ (i.e. moved to coincide exactly with the coordinates of another feature) to the nearest non-crop habitat excluding roads, buildings and water, as bumblebees are unlikely to nest within these features or within annually cropped fields which covered the majority of the landscape. The maximum ‘snapped’ distance for any colony was 134.9 m for *B. lapidarius* and 133.1 m for *B. pascuorum*. This snapped, weighted centroid represents an estimate of the approximate nest location for each colony (Fig. 1).

The foraging distance per bee from each colony was calculated as the distance from the weighted centroid to the centre of the patch at which a bee was sampled. Mean, maximum and minimum foraging distances from nest to sample patches were calculated for each colony. In cases where a colony was represented by a single worker, or more than one worker but all on the same patch, the weighted centroid was located on the sample patch giving an estimated foraging distance of 0 m. This approach was used initially to assign foraging distances for the full dataset (i.e. all sampled colonies). However, we considered it unlikely that a nest represented by one individual or several sister workers on a single sample patch would be located within that patch. There is evidence that bumblebees rarely forage close to their nests (e.g. within 50 m, Dramstad 1996). A single individual or several sister workers foraging in isolation may have been temporally ‘majoring’ on our flower-rich sample patches during the three day sampling period (Heinrich 1979b, Osborne and Williams 2001) or foraging away from the colony to reduce competition or predation (Dukas and Edelman-Keshet 1998). We therefore excluded colonies meeting these criteria due to the uncertainty in their nest location, to produce a more conservative reduced dataset for further analyses (Table 1). We compared our estimates of foraging distance with those of another study on the same two *Bombus* species (Lepais et al. 2010) conducted across a similar agricultural landscape in southern England to ours (approximately 50 km from our site). The ‘four case rule’ applied by Lepais et al. (2010) to assign a dispersal (foraging) distance to each individual in their sample was applied to our full dataset for each species (see Table 1 for further explanation of the four case rule). Mean, maximum and minimum foraging distances were then calculated for each colony from these data. In order to verify that our estimates of approximate colony location and hence foraging distance did not deviate significantly from the observed foraging area encompassed by each colony, we examined the relationship between the area within the 75% contour of the KDE and mean foraging distance for each colony.

Using the reduced dataset, a buffer was generated around each colony location with a radius of the mean foraging distance for that colony (hereafter the colony-specific foraging distance), and the proportions (as a percentage) of each land cover type within this buffer calculated from the GIS map (Fig. 1). The equivalent land cover proportions were also calculated using a buffer with a fixed radius for each colony, taken from

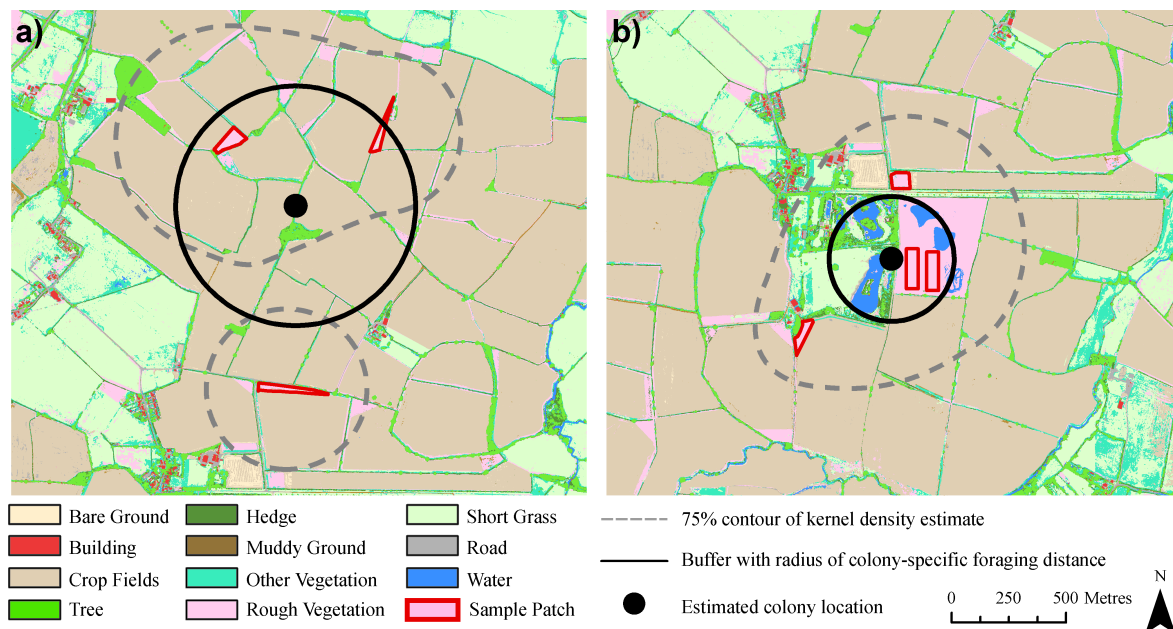


Figure 1. Illustration of the method used to estimate colony locations and foraging distances with examples for each species against the classified map of the study landscape: (a) *B. lapidarius* colony from which four workers were sampled across three patches and (b) *B. pascuorum* colony from which four workers were sampled across four patches. Colony-specific foraging distance was calculated as the mean of the foraging distances of all sister workers from estimated colony location to sample patches.

minimum estimated maximum foraging distances for each species in the published literature (450 m for *B. lapidarius* and 449 m for *B. pascuorum*; Knight et al. 2005).

Statistical analysis

We compared the mean, maximum and minimum foraging distances per colony between the two study species using two sample *t*-tests on both the full and reduced datasets. We tested for differences in foraging distance between the estimates derived from our method (referred to as the GIS method) and the Lepais et al. (2010) method (four case rule) using paired *t*-tests. For both analyses, foraging distances were log + 1 transformed to improve normality.

We tested for effects of surrounding land cover on foraging distances by fitting linear regression models for each species with log transformed colony-specific foraging distance as the response variable and the proportion of foraging habitats within this radius of the colony location as the explanatory variable. For both species we tested the effect of outliers on this overall relationship by excluding them from the analysis. In both cases the relationship remained highly significant ($p = 0.001$) with similar slopes and so the full data is presented. Differences in the proportion of foraging habitats available to colonies within our colony-specific and published minimum estimated maximum foraging ranges were tested for each species using paired *t*-tests. Habitat percentages were log transformed to improve normality.

Table 1. Classification of datasets according to the four case rule applied by Lepais et al. (2010) to assign a dispersal (foraging) distance to each individual in their sample (which included workers and some queens). $N_{colonies}$ = number of colonies in our sample.

	Outcome			<i>B. lapidarius</i>	<i>B. pascuorum</i>
	four case rule	GIS full dataset	GIS reduced dataset	$N_{colonies}$	$N_{colonies}$
Four case rule (Lepais et al. 2010)					
CASE 1 (nests represented by a single individual)	rejected	included	rejected	3	11
CASE 2a (nests with two or more workers in which all originated from a single site and were assigned a dispersal distance of 0 m)	included	included	rejected	10	12
CASE 2b (nests with two or more workers in which the majority originated from a single site. These were assigned a dispersal distance of 0m while any individuals from the same nest but sampled at a second site were considered dispersers and assigned a distance equal to the distance between the sample site and the main assumed 'nest' site.)	included	included	included	17	13
CASE 3 (nests with two or more workers which were sampled in equal number across two different sites. All individuals were considered dispersers with a distance equal to half the distance between the sites.)	included	included	included	23	17
CASE 4 (nests represented by individuals sampled from more than two sites)	rejected	included	included	26	15

Results

We sampled a total of 214 *B. lapidarius* individuals which were grouped into 79 colonies (an average of 2.7 workers per colony), and a total of 170 *B. pascuorum* individuals which were grouped into 68 colonies (2.5 workers per colony). The number of colonies represented by a single worker was low for both species: 4% of colonies in the full dataset for *B. lapidarius* and 16% for *B. pascuorum* (Table 1). The reduced dataset, in which these cases and colonies in which all sister workers were sampled on the same patch (hereafter 'single patch colonies') were removed, consisted of 66 *B. lapidarius* colonies and 45 *B. pascuorum* colonies.

There was no significant genetic differentiation among samples at a global level for either species (*B. pascuorum* $p = 1.000$; *B. lapidarius* $p = 0.933$) or in any pairwise test between samples. Out of 120 and 96 tests of deviation from Hardy-Weinberg equilibrium for *B. pascuorum* and *B. lapidarius*, respectively, after Bonferroni correction only one remained significant for *B. pascuorum* and five for *B. lapidarius*. No single locus showed exceptionally high levels of deviation from Hardy-Weinberg equilibrium, suggesting no systematic problems with PCR amplification.

The estimated foraging distances calculated from our GIS method with the full dataset were significantly higher for *B. lapidarius* (mean 630.9 m, range 0–1579 m) than *B. pascuorum* (mean 512.8 m, range 0–1597 m; Table 2: t -tests on differences in 1) mean, $t = 2.43$, $DF = 127$, $p = 0.016$; 2) min, $t = 2.22$, $DF = 127$, $p = 0.028$ and 3) max, $t = 2.49$, $DF = 127$, $p = 0.014$). The maximum distances estimated for an individual forager were 2213 m and 2317 m for *B. lapidarius* and *B. pascuorum*, respectively. Neither the estimated foraging distances calculated using the Lepais et al. (2010) four case rule, nor the estimated foraging distances calculated from our GIS method with the reduced dataset, differed significantly between species (all $p > 0.05$). However in the latter analysis there was a tendency for *B. pascuorum* to exhibit higher estimated foraging distances (mean 774.9 m, range 161–1597 m) than *B. lapidarius* (mean 755.2 m, range 64–1579 m) (Table 2).

The GIS method with the full dataset assigned significantly higher mean and minimum foraging distances per colony than the four case rule for both species (paired t -tests on differences in 1) mean for *B. lapidarius*, $t = 4.69$, $DF = 49$,

$p < 0.001$ and *B. pascuorum*, $t = 4.23$, $DF = 41$, $p < 0.001$; 2) minimum for *B. lapidarius*, $t = 4.92$, $DF = 49$, $p < 0.001$ and *B. pascuorum*, $t = 4.18$, $DF = 41$, $p < 0.001$). However, the increase in distances was only on average 45.6 m for *B. lapidarius* and 48.1 m for *B. pascuorum*. Maximum foraging distances were significantly higher using the four case rule for both species (paired t -tests on differences in maximum for *B. lapidarius*, $t = -3.97$, $DF = 49$, $p < 0.001$ and *B. pascuorum*, $t = -3.74$, $DF = 41$, $p < 0.001$).

The distribution of colonies from our dataset across the sampled patches varied between species (Table 1). *Bombus lapidarius* workers appeared to distribute themselves more widely across the landscape than *B. pascuorum*, with a higher proportion of 'Case 4' colonies (Table 1) represented by workers sampled across three or more patches. Correspondingly, the foraging area encompassed by each colony, represented by the area within the 75% contour of the KDE, was significantly greater for *B. lapidarius* (mean 1.25 km²) than *B. pascuorum* (mean 1.08 km²) when tested using the full dataset ($t = 2.62$, $DF = 143$, $p = 0.009$). This difference in foraging area between species was not significant when tested using the reduced dataset ($p > 0.05$). The foraging area and colony-specific foraging distance estimated using the GIS method were positively correlated for both species (*B. lapidarius*, Pearson's coefficient = 5.97, $DF = 64$, $p < 0.001$; *B. pascuorum*, Pearson's coefficient = 4.11, $DF = 43$, $p < 0.001$ on reduced dataset), verifying that our estimates did not deviate significantly from the observed foraging area encompassed by each colony.

Overall there was a significant negative relationship between mean foraging distance per colony and the proportion of foraging habitats within a buffer of that radius for both species (Fig. 2). Although this was characterised by large variation in colony-specific foraging distances these data suggest that in general workers foraged shorter distances when their colonies were surrounded by larger proportions of foraging habitat. Furthermore, our colony-specific measure of foraging distance resulted in a greater proportion of foraging habitats being available to each colony than would have resulted from previously published minimum estimated maximum foraging ranges that were fixed in our analysis (paired t -test: *B. lapidarius* $t = -6.37$, $DF = 65$, $p < 0.001$; *B. pascuorum* $t = -6.87$, $DF = 44$, $p < 0.001$) (Fig. 3). This occurred whether the colony-specific distance was greater

Table 2. Mean, minimum and maximum estimated foraging distances (metres) per colony for *B. lapidarius* and *B. pascuorum*, as derived from the GIS method proposed in this study and the four case rule proposed by Lepais et al. (2010). $N_{colonies}$ = number of colonies included in the sample. Asterisks indicate the p -value of t -tests on differences between species: * $p < 0.05$; ns, no significant difference.

Distance estimation method	Variable	<i>B. lapidarius</i>			<i>B. pascuorum</i>			Difference
		mean	SE	$N_{colonies}$	mean	SE	$N_{colonies}$	
GIS full dataset	mean	630.9	48.6	79	512.8	62.3	68	*
	minimum	458.1	43.5	79	407.5	55.4	68	*
	maximum	861.8	67.0	79	654.8	77.3	68	*
Four case rule full dataset	mean	517.9	60.4	50	514.1	77.1	42	ns
	minimum	389.1	68.8	50	372.3	80.2	42	ns
	maximum	806.1	91.9	50	803.6	132.3	42	ns
GIS reduced dataset	mean	755.2	44.2	66	774.9	65.6	45	ns
	minimum	548.4	44.3	66	615.8	64.4	45	ns
	maximum	1031.6	61.2	66	989.5	79.0	45	ns

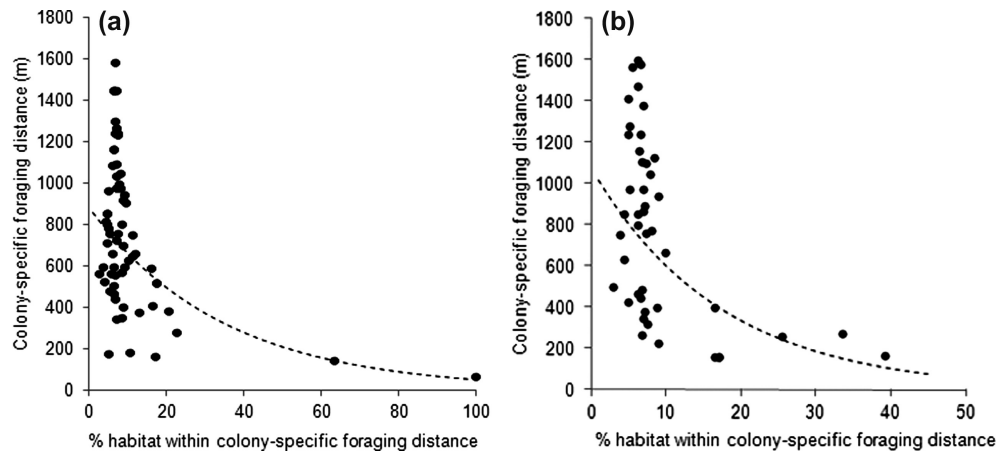


Figure 2. Relationships between colony-specific foraging distance and the percentage of foraging habitats for (a) *B. lapidarius* ($n = 66$; $R^2 = 0.41$, slope = -0.029 , $p < 0.001$) and (b) *B. pascuorum* ($n = 45$; $R^2 = 0.37$, slope = -0.058 , $p < 0.001$). Each point represents a single colony. Dashed lines represent fitted line equations based on the significant regression model of $\log(\text{foraging distance})$ vs % habitat.

or less than the minimum estimated maximum foraging distance for both species. Colonies of *B. lapidarius* and *B. pascuorum* had on average 10.5% and 9.2% foraging habitats respectively within a radius equal to their colony-specific foraging distance, compared with 7.1% and 6.2% respectively within fixed foraging radii.

Discussion

In this study we combined genetic analyses of relatedness in worker bumblebees with classified landscape models to provide estimates of foraging distance for a large number of wild bumblebee colonies across a landscape. We found a significant relationship between estimated foraging distance and the proportion of foraging habitats within these distances for *Bombus lapidarius* and *B. pascuorum*, providing the first indirect evidence that colonies of both species vary their foraging distance according to resource availability. The proportion of foraging habitats within foraging range of each colony varied from between 2.6–99.9% for *B. lapidarius* and 2.9–39.2% for *B. pascuorum*. Thus, as predicted,

colony-specific foraging distances decreased as the proportion of available foraging habitat increased. This finding supports the suggestion that higher bumblebee densities on flower patches in more intensively farmed landscapes are due to changes in foraging behaviour (Heard et al. 2007, Carvell et al. in press). Indeed our estimates of foraging distance from the experimentally manipulated study landscape (which included more patches of bumblebee foraging habitat than the average UK arable landscape) may be considered conservative since they are likely to be greater in more simply structured landscapes with fewer forage resources (Westphal et al. 2006b).

The location of bumblebee colonies may be determined by a combination of floral resources, vegetation structure and composition, microclimate and predation risk (Kells et al. 2003, Suzuki et al. 2009). While our predictions of approximate nest location are difficult to verify retrospectively in the field, because of the time taken for genetic analyses and limited period of bumblebee activity, we believe our method is potentially the most powerful to date for estimating both nest locations and foraging distances. First, because it is based on known distributions of sister workers, the assigned

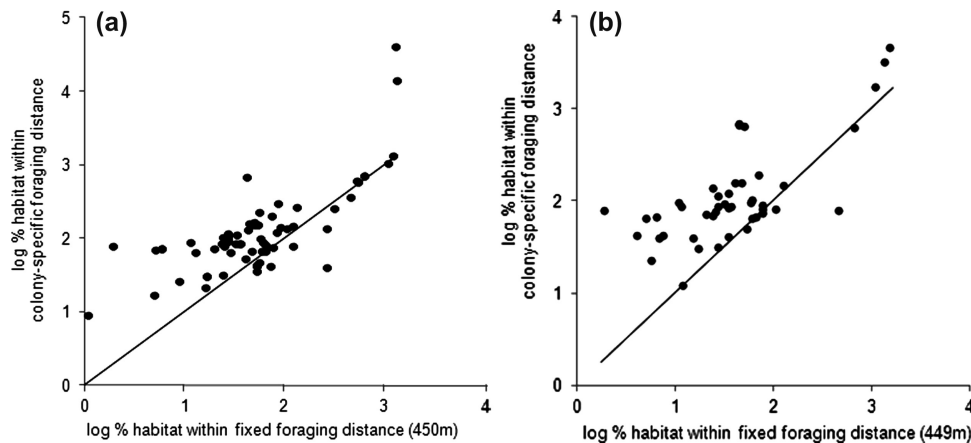


Figure 3. Relationships between the percentage of foraging habitats within colony-specific and fixed foraging distances of colony locations for (a) *B. lapidarius* and (b) *B. pascuorum*. Each point represents a single colony. The solid line represents the 1:1 relationship that would result if each sampled colony was foraging at the fixed distance.

colony location is unbiased by density estimates of both workers (from unknown colonies) and flowers (Nakamura and Toquenaga 2002). Second, by sampling across a variety of spatial scales in the landscape, it allows a large number of colonies to be detected with an average of more than two workers per colony. This improves on previous approaches which sample either from spatially independent resource patches separated by a minimum of 1 km, thus obtaining a high proportion of colonies represented by a single worker (Knight et al. 2009, Goulson et al. 2010, Lepais et al. 2010), or from linear transects along which the accuracy of foraging distance estimates depends heavily on distance between sample points (Darvill et al. 2004, Charman et al. 2010). Interestingly our estimates of mean foraging distance per colony (the most conservative being 755 m for *B. lapidarius* and 775 m for *B. pascuorum*) are higher than other published estimates from UK studies of 450 m for *B. lapidarius* (Knight et al. 2005) and less than 312 m (Darvill et al. 2004) to 449 m (Knight et al. 2005) for *B. pascuorum*. This is probably due to the improved spatial resolution of our sampling approach which was not restricted to points along a linear transect, although we cannot discount the effect of fundamental differences between the study landscapes. Interestingly our study landscape was only about 50 km away from the site where Knight et al. (2005) made their estimates and very similar in cropping intensity and landscape structure. Third, our method uses data on the spatial arrangement of habitats across the underlying landscape to assign colony locations based on the likely nest site preferences of bumblebees. The 'four case rule' applied by Lepais et al. (2010) that we tested on our dataset makes no link between observed worker distributions and landscape structure. It thus assigns equal foraging distances to bees from the same colony when they are distributed evenly between two sites (Case 3), and ignores colonies dispersed across more than two sites altogether (Case 4). At the same time, it assumes that two or more sister workers sampled on one patch ('single patch colonies') are nesting on that patch. This seems unlikely given the tendency for bumblebees to 'major' on different patches throughout the colony's lifespan (Heinrich 1979b, Osborne and Williams 2001) and forage away from the colony to reduce competition or predation (Dukas and Edelstein-Keshet 1998). By relating worker distributions to underlying landscape structure, our approach allows for variation in foraging ranges to be assigned to bees from the same colony and importantly uses the information from colonies dispersed across three or more sites to improve the accuracy of the nest location estimate.

Despite these advantages our estimates of foraging distance varied markedly depending on the assumptions behind the estimation method and proportion of the dataset used. Paired *t*-tests showed higher mean and minimum distances estimated using our GIS method than the four case rule of Lepais et al. (2010), but the opposite pattern for maximum distances per colony. This was mainly due to the assumptions made for 'Case 2b' colonies (Table 1) because using the 'snapped', weighted centroid to estimate nest location will always assign higher distances from nest to sample patch than 0 m (as in the four case rule) for multiple sister workers sampled on that same patch, but lower maximum distances for sisters sampled at a second patch. Foraging distances generated using our full dataset were lower for both species than those

from the reduced dataset (Table 2) because of the inclusion of single workers and single patch colonies assigned a distance of 0 m. Similarly, the lower mean foraging distance calculated from the full dataset for *B. pascuorum* was driven by the higher proportion of single workers and single patch colonies of this species than for *B. lapidarius*. Removing these individuals in the reduced dataset led to higher estimated foraging distances for both species but no difference between them. Nevertheless, in agreement with previous indirect estimates of foraging distance for these species (Westphal et al. 2006b) we found that the observed foraging area encompassed by each colony (and independent from any estimate of nest location) was generally greater for *B. lapidarius* than *B. pascuorum*.

Our model could be further refined as follows. The sampling was conducted during a short period in July that probably represented the peak of colony activity for each species, but only provided a snapshot of worker foraging distances. Although our approach allowed for the differential foraging behaviour of individuals to be assessed it is clear that there was considerable variation in colony-specific foraging distances where the percentage of foraging habitats was low. This might result from variation in the state and energy requirements of the colony (Cartar 1992), intraspecific variation in forager size (Peat et al. 2005) or seasonal changes in the resource environment (Goulson et al. 2010). For example, Suzuki et al. (2009) proposed a model suggesting that bumblebee queens choose nest locations in the spring that maximise their net rate of energy intake from nectar in the surrounding landscape. To support this hypothesis we would need to sample individuals and estimate colony locations at a number of stages throughout the season, and to relate these to the changing distribution of foraging resources. Furthermore, by using more detailed measures of habitat quality such as floral abundance (Osborne et al. 2008, Carvell et al. 2011) or nectar and pollen quality, our measures of resource availability could be improved, potentially allowing a formal model of optimal foraging theory to be tested (Heinrich 1979a).

The degree to which bumblebees have declined in recent years differs markedly between species (Goulson 2009). This may be partly explained by differences in foraging range and dispersal ability (Darvill et al. 2010) as species with larger foraging ranges should be better able to cope with the fragmentation of their forage resources. Our study suggests that even within a relatively small landscape area, bumblebees vary their foraging distances according to resource availability. Conservation efforts, such as targeted agri-environment schemes that encourage farmers to sow wildflower mixtures alongside their crops (Carvell et al. 2007), are therefore likely to reduce net energy expenditure and enhance the survival of colonies located within overall foraging distance. Our approach could also usefully be applied to test whether rarer *Bombus* species show similar variation in foraging distances despite reduced species distributions. In applying the technique care must be taken with species where foraging distances are short (hence greater spatial resolution is required) or colony sizes small (ensure adequate sample sizes can be achieved). However, in general it provides a powerful tool for informing the scale of management required for bumblebees in both agricultural and urban landscapes. By offering a

novel means of locating and studying colonies, it could also inform research on additional aspects of bumblebee ecology or on other social insects and taxa which exhibit central place foraging behaviour.

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Supplementary material (available as Appendix o19832 at < www.oikosoffice.lu.se/appendix >). Appendix A1.