Effects of land use at a landscape scale on bumblebee nest density and survival

Dave Goulson[1]*, **Olivier Lepais**[1], **Stephanie O'Connor**[1], **Juliet L. Osborne**[2], **Roy A. Sanderson**[3], **John Cussans**[2], **Louis Goffe**[3] **and Ben Darvill**[1]

[1]*School of Biological & Environmental Sciences, University of Stirling, Stirling, FK9 4LA, UK;* [2]*Department of Plant & Invertebrate Ecology, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; and* [3]*Institute for Research on Environment and Sustainability, Devonshire Building, University of Newcastle, Newcastle-upon-Tyne, NE1 7RU, UK*

Summary

1. We have little idea how landscape-scale factors influence the success of wild bumblebee nests over time. Here for the first time we use molecular markers to estimate within-season changes in the numbers of nests.

2. Workers of two bumblebee species were sampled in an arable landscape in late May–June and late July–August, and the numbers of nests represented in each sample were estimated. We compare the methods available to estimate nest number from such samples and conclude that methods which allow for heterogeneity in the probability of capture of nests provide the best fit to our data. Changes in numbers of nests at the two time points were used to infer nest survival.

3. The two bee species appeared to differ markedly in survival over time, with estimates of 45% of nests surviving for *Bombus lapidarius* and 91% for *B. pascuorum*. However, our data suggest that the foraging range of *B. pascuorum* may be greater in late season, which would lead us to overestimate nest survival in this species. Differential survival may also reflect differences in phenology between the two species.

4. The land use class which had the most consistent effects on nest number and survival was gardens; for *B. lapidarius*, the area of gardens within a 750 and 1000 m radius positively influenced nest survival, while for *B. pascuorum*, the number of nests in late samples was higher at sites with more gardens within a 500 and 750-m radius. For *B. pascuorum*, the area of grassland within a 250 and 500-m radius also positively influenced nest number in late samples, probably because this is the preferred nesting habitat for this species.

5. The importance of gardens is in accordance with previous studies which suggest that they now provide a stronghold for bumblebees in an otherwise impoverished agricultural environment; furthermore, our data suggest that the positive influence of gardens on bumblebee populations can spill over at least 1 km into surrounding farmland.

6. *Synthesis and applications*. The substantial effects that even small areas of local resources such as rough grassland or clover leys can have on bumblebee nest numbers and survival is of clear relevance for the design of pollinator management strategies.

Key-words: *Bombus,* density, gardens, kinship, microsatellite, mortality, pollination services, population structure, social insects

Introduction

There is mounting evidence that bumblebees and other key pollinators have declined in Western Europe, North America, and parts of Asia (Goulson, Lye & Darvill 2008a; Brown & Paxton 2009; Williams & Osborne 2009). These declines raise concerns about the provision of pollination services for both crops and wildflowers, and there is growing interest in managing the landscape to combine anthropogenic needs with provision of ecosystem services such as pollination (e.g. Isaacs *et al.* 2009; Lonsdorf *et al.* 2009).

Studies of the factors affecting the population size of social bees are difficult, in part because nests can be hard to locate

*Correspondence author. E-mail: dave.goulson@stir.ac.uk

(notably in bumblebees). Studies which have manipulated habitats to examine the effect on pollinators tend to focus on counts of workers (e.g. Kells, Holland & Goulson 2001; Carvell *et al.* 2007; Heard *et al.* 2007), but ideally we would like to know how these manipulations impact on nest density and survival. One approach by which it is possible to indirectly measure nest number is via DNA sampling of workers. If workers are typed at sufficient microsatellite loci, it is possible to identify groups of sisters each representing a nest. These data can then be further analysed to estimate how many nests were foraging at a site but by chance were not represented in the sampled bees; the approach used for this has been to fit the data to a Poisson distribution. This method has previously been used to quantify the numbers of nests visiting particular flower patches (Chapman, Wang & Bourke 2003), to quantify foraging range by examining the distribution of sisters along a transect (Darvill, Knight & Goulson 2004; Knight *et al.* 2005) and to estimate population size in isolated populations of a rare bumblebee (Ellis *et al.* 2006). These studies have provided valuable insights into aspects of bumblebee ecology that had previously proved to be intractable.

For social insects such as bumblebees to thrive, they require suitable forage throughout the period of colony development (spring and summer). Modern agricultural landscapes consist of large areas of monocultures separated by field margins and interspersed by occasional patches of non-cropped areas (e.g. woodland) and clusters of housing with gardens (Osborne *et al.* 2008a). Some crop monocultures such as oilseed rape and field beans provide massive but short-lived bursts of floral resources during the season. This spatial and temporal patchiness of floral resources is likely to mean that bumblebee nests fare differently depending on where they are located in the agricultural landscape. However, because of the difficulty in finding bumblebee nests, we have very little information on nest survival rates and how nest density changes through the season.

Here, we study changes in nest density over time in two bumblebee species, *B. pascuorum* and *B. lapidarius*. We use DNA sampling of workers to detect sisters and infer nest number early in the season (late May–June) and late in the season (late July–August). We compare different approaches to estimating the numbers of nests present using such genetic samples. Our estimates of nest density in early and late season, and of nest survivorship, are examined in relation to a detailed remote-sensed land use map of the study area. This enables us to examine which land use classes influence nest density and survival, and over what spatial scale these effects are detected on the two contrasting bee species. Our results have clear implications for the management of pollination services in arable landscapes.

Materials and methods

SAMPLE COLLECTION

The study was carried out in a 10×20 km rectangle centred on Rothamsted Research (Harpenden, Hertfordshire, UK) where several studies of bumblebee foraging range and population size have already been conducted (Knight *et al.* 2005, 2009; Osborne *et al.* 2008a). Samples of *B. lapidarius* and *B. pascuorum* workers were collected from along a 200×10 m strip of a field margin at each sample site. Worker bees were sampled at the same sites on two occasions in 2007: between the 22 May and the 22 June (= early samples) and between the 25 July and 9 August (= late samples). Each site was visited repeatedly during each sample period until at least 50 bees of each species had been sampled or until the sampling period ended. Fourteen sites were selected for sampling, on the basis that they were at least 1 km apart and 1 km from any substantial urban area (Fig. S1, Supporting Information).

A total of 1660 *B. pascuorum* workers and 1083 *B. lapidarius* workers were caught (Tables 2 and 3). A non-lethal tarsal sample from a mid-leg (Holehouse, Hammond & Bourke 2003) was taken from each worker during the early sample, and foraging workers were collected during the late sample. All samples were preserved in ethanol.

MOLECULAR METHODS

DNA was extracted using the HotShot protocol (Holehouse, Hammond & Bourke 2003). *B. lapidarius* individuals were genotyped at ten microsatellites (BL11, BL06, BT24, BT09, BT18, B126, B96, B10, B11 and B118) and *B. pascuorum* individuals at nine microsatellites (BL03, BT10, BT26, BT18, B124, B126, B96, B132 and B118) (Estoup *et al.* 1995; Funk, Schmid-Hempel & Schmid-Hempel 2006). Microsatellite loci were amplified with a multiplex protocol following Darvill *et al.* (2006) and described in detail elsewhere (Lepais *et al.* 2010). The genetic data are archived in the Dryad database (available at http://hdl.handle.net/10255/dryad.1113).

LANDSCAPE CHARACTERIZATION

A detailed composition of the landscape around each sample site was created. The process consisted of identifying the key land cover classes and utilizing remote sensed data, digital cartography or a combination of the two to identify the feature in the study area. The two input datasets used for the generation of the land cover classification were IKONOS satellite imagery (4 m multispectral and 1 m panchromatic) and Ordnance Survey MasterMap topographic layer. These were processed using a combination of remote sensing and GIS techniques using ERDAS IMAGINE 9·1 and ESRI ArcGIS desktop 9·2. The incorporation of MasterMap (vector) data into the procedure means that the boundaries of specific features could be accurately defined (Fig. S1). Where classes were found to overlap, priority in the final classified map was given according to physical structure, e.g. trees were given priority over grass. Table S1 in Supporting Information provides a summary of each land cover class plus the dataset from which it was derived.

STATISTICAL METHODS

Genetic comparison of early and late samples

We used the complete dataset, i.e., all workers regardless of the sibship reconstruction, including sites that had at least 10 sampled workers and excluding the outlier site E (see 'Results' section covering sibship reconstruction) to compare genetic parameters between early and late samples. We used FSTAT version 2.9.3.2 (Goudet 1995) to compute allelic richness obtained by rarefaction (Petit, El Mousadik & Pons 1998), observed heterozygosity, gene diversity (Nei 1987), heterozygosity deficit, population differentiation (Weir & Cockerham

1984) and average relatedness within groups (Queller & Goodnight 1989). We compared these parameters between early and late samples. The significance of each comparison was tested using 1000 permutations of individuals between samples. The *P*-value of the test was computed as the proportion of randomized data sets giving a larger parameter than that calculated from the observed data sets.

Sibship reconstruction

We used the maximum likelihood sibship reconstruction method implemented in COLONY software version 1.2 (Wang 2004) to identify workers that belonged to the same colony. This software was found to produce the most accurate sibship reconstruction, in particular in the presence of genotyping error (Lepais *et al.* 2010). We ran the software with the following options: haplo-diploid species; sex 1 set as diploid females; allele frequencies updated each 1000 iterations; 2% genotyping error for all loci (0·5% of allele dropout and 1·5% other errors).

Nest number and survival estimations

Truncated Poisson method. . Based on the sibship reconstruction obtained from COLONY, we counted the number of nests represented by 1, 2, 3, …, *k* workers. We estimated the number of unsampled nests by fitting a truncated Poisson distribution to the data and extrapolating this distribution to the zero class, an approach previously used on similar data sets (Chapman, Wang & Bourke 2003; Darvill, Knight & Goulson 2004; Knight *et al.* 2005). This statistical method assumes that there is an equal probability of sampling workers from all of the nests which forage at the site. In practice, due to heterogeneity in nest size and location, it is probable that some nests are more likely to be sampled than others.

Applying DNA mark–recapture methods. Recent developments in the field of DNA-based capture–recapture models allow for multiple sampling of an individual. The number of times an individual is recaptured can be used to estimate the population size (Miller, Joyce & Waits 2005). This method is often used on data obtained from non-invasive DNA sampling, such as number of scats or hairs sampled per individual. Our data are similar, albeit that instead of trying to estimate the number of individuals we are interested in estimating the number of nests represented in our sample of workers. Interestingly, Capwire software (Miller, Joyce & Waits 2005) implements two methods. The Event Capture Model (ECM) assumes that each individual has an equal probability of being sampled, an assumption similar to the truncated Poisson methods described above. The Two Innate Rate Model (TIRM) allows for heterogeneity in capture probability

among individuals. Furthermore, Capwire uses a likelihood ratio test to find the best model to estimate the population size. We used this likelihood ratio test to find out which model would give the better fit to our data and subsequently used both ECM and TIRM models to estimate the number of nests foraging at each sample site. We finally compared these estimations with the previously used truncated Poisson method.

The values for nest numbers estimated by Capwire software (using the TIRM model) were used in subsequent analyses. Nest detectability was calculated as the ratio of detected nests to the total number of nests estimated to be present at each time point. This is simplistic since it makes the assumption that capture probability is equal for all nests. However, it enables us to estimate nest survival across the two sample time points, taking into account changes in detectability (see Table 2).

Impact of surrounding land use on nest number and survival

We chose to consider all land cover types as predictor variables in order to avoid potential issues associated with biased variable selection (Whittingham *et al.* 2006). We used hierarchical partitioning (HP) (Chevan & Sutherland 1991) to estimate for each predictor variable its independent and conjoint contribution with all other variables in a multiple linear regression setting, using the hier.part package (MacNally & Walsh 2004) of the R software (R Development Core Team 2005). Generalized linear models were run using a Poisson distribution (for count data) for performing the analysis of nest number in early and late samples, and with a Gaussian distribution when nest survival was the response variable. To identify the most important predictor variables, the randomization procedure was used (1000 randomizations performed) to test the significance of the independent contribution of each predictor (MacNally 2002). We performed the analysis independently for each landscape radius (250, 500, 750 and 1000 m) and each species, excluding the outlier site E.

Results

COMPARISON OF POPULATION GENETIC PARAMETERS BETWEEN SAMPLING PERIODS

Most of the population genetic parameters showed a significant difference between early and late samples in *B. lapidarius* (Table 1). Genetic diversity (R_s and H_s) significantly decreased, probably due to a higher family structure in late samples (lower numbers of nests and higher numbers of workers from

Table 1. Comparison of genetic parameters among sites and sampling periods

| Species	Sample	N_{pop}	N_{ind}	R_s (*P-value*)	H_o (*P-value*)	H_s (*P-value*)	F_{is} (*P-value*)	F_{st} (*P-value*)	Rel (*P-value*)
Bombus lapidarius	Early	11	607	8·241	0·789	0·786	−0·004	0·017	0·034
	Late	7	394	7·419	0·771	0·762	−0·012	0·036	0·070
	P-value			*(0·002)*	*(0·215)*	*(0·005)*	*(0·737)*	*(0·016)*	*(0·016)*
Bombus Pascuorum	Early	13	751	8·624	0·705	0·719	0·020	0·015	0·030
	Late	13	774	8·151	0·729	0·716	−0·018	0·021	0·041
	P-value			*(0·159)*	*(0·083)*	*(0·698)*	*(0·039)*	*(0·449)*	*(0·394)*

N_{pop}: number of sites, N_{ind}: number of individuals, R_s: allelic richness (Petit, El Mousadik & Pons 1998) rarefied to 21 and 49 individuals for *B. lapidarius* and *B. pascuorum*, respectively, H_o: observed heterozygosity and H_s: gene diversity (Nei 1987), F_{is}: heterozygosity deficit and F_{st}: genetic differentiation (Weir & Cockerham 1984), Rel: average relatedness (Queller & Goodnight 1989). *P*-values are obtained by 1000 randomizations of individuals among groups.

common nests) as shown by the significant increase in genetic relatedness (Rel) and genetic differentiation (F_{st}). Although the same trends are observed in *B. pascuorum*, the differences between early and late samples were smaller and were not significant (Table 1), with the exception of the deficit of heterozygotes (F_{is}) which was lower for late samples.

NEST NUMBER ESTIMATIONS

We found that the Even Capture Model (ECM) gave very similar estimates of the total number of nests present in a site compared to the truncated Poisson method (Fig. 1). However, using the likelihood ratio test (LRT), the Two Innate Rates Model (TIRM) was the more likely model in 18 out of 22 samples (82%) for *B. lapidarius* and 21 out of 28 samples (75%) for *B. pascuorum*. Simulated data using a range of degrees of heterogeneity in capture probability showed that the LRT rejected the ECM model in favour of the correct TIRM model only in about 30% of the cases (Miller, Joyce & Waits 2005). Given that a high percentage of selected TIRM models were selected to fit our data (82% and 75%), it appears that heterogeneity of capture probability is a strong characteristic of the nests. Thus the Capwire's TIRM model probably gave more accurate estimates of the number of nests foraging at a site. These estimates were approximately 1·4 times higher than the estimations produced using the Poisson method (Fig. 1). For subsequent analyses of the effects of landscape variables on nest numbers and survival we therefore used nest number estimates produced using the TIRM method (Tables 2 and 3).

The estimates for the total number of nests for *B. lapidarius* ranged from 32 to 416 in early samples and from 15 to 127 in late samples (Table 2). Site E appeared to contain many more nests than other sites (excluding site E, nest number ranged
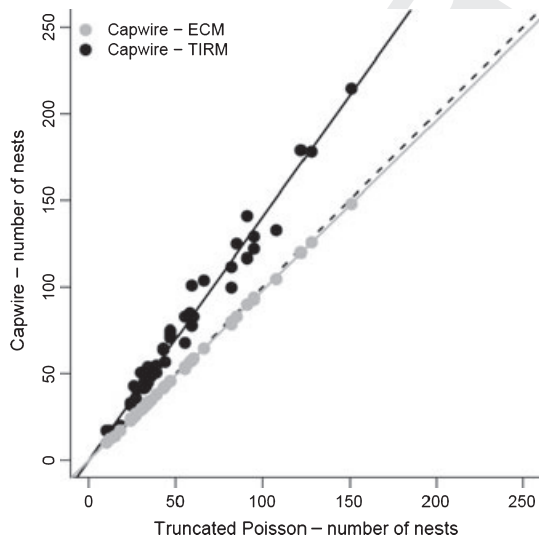
Fig. 1. Comparison of estimated total nest number for the two species combined by the extrapolation of a truncated Poisson distribution and DNA mark–recapture method (Capwire) implementing the even capture model (ECM) and the two innate rates model (heterogeneity of capture probability; TIRM). Dashed line indicates equality of estimation between models (regression slope of 1).

from 32 to 215 in early samples and from 15 to 45 in late samples). Estimated nest survival ranged from 0·25 to 0·87 (Table 2). The estimate of the total number of nest for *B. pascuorum* ranged from 33 to 179 in early samples and from 17 to 313 in late samples (Table 3). Again, site E appeared to be an outlier. Nest survival ranged from 0·38 to 2·15 including all sites or from 0·38 to 1·13 excluding site E (Table 3).

Site E was highly atypical for both bee species; these results are likely to be explained by the presence of a 5-ha clover ley in close proximity to the sample area, a land cover type that is rare in the study area. Hence we chose to exclude site E from all subsequent analyses.

Estimated survival varied significantly between species (paired *t*-test, $t_7 = 6·05$, $P = 0·001$), being consistently lower at all sites for *B. lapidarius* than for *B. pascuorum* (Tables 2 and 3).

Some nests were detected in both early and late samples. It is informative to compare this number to the number of nests which we would expect to observe in both samples. For *B. lapidarius*, we directly observed 414 nests across all sites in the first sample, but estimated that in total there were 1372 nests present. The proportion of 'marked' nests (i.e. those sampled and genotyped) was therefore 0·301. So long as detected nests were not more or less likely to die than undetected nests (unlikely, as we used non-lethal tarsal sampling), in the second sample in which we directly detected 206 nests, we would expect 62 of these to be nests also directly detected in the first sample. The actual figure for the number of nests detected in both samples is 80 (Fisher's exact test, two-tailed, $P = 0·078$).

For *B. pascuorum*, we directly observed 501 nests in the first sample, and estimated that 1226 nests were foraging at sample sites (i.e. the proportion directly detected was 0·409). Of the 489 nests directly detected in the second sample, we would expect 200 to have been previously detected in the first sample, but in fact only 106 nests were detected in both samples (Fisher's exact test, two-tailed, $P < 0·001$).

IMPACT OF SURROUNDING LAND USE ON NEST NUMBER AND SURVIVAL

For *B. lapidarius*, the HP analysis showed that the area of woodland within a radius of 1000 m had a positive impact on the number of nests visiting the site in the early sample, while the area of woodland within 250 m had a negative impact on the number of nests in late samples (Fig. 2; Fig. S2, Supporting Information). Nest survival was significantly and positively associated with the area of gardens within both 750 and 1000 m (Fig. 2). This occurred despite the deliberate selection of sample sites away from urban areas; the highest proportion of gardens within 1000 m of any site was 5·1%. Nest survival was negatively predicted by the area of woodland within 500 m.

For *B. pascuorum*, the area of woodland within a radius of 750 and 1000 m and the area of made-man surface within a radius of 1000 m had a positive impact on early nest number, while the number of nests in late samples was positively associated with the area of grassland within radii of 250 and 500 m

Table 2. Nest number estimations for *Bombus lapidarius*

| Site | Early | | | Detection probability (*d*1) | Late | | | Detection probability (*d*2) | Change in detectability (δ*d*) | Estimated survival | N_{both} |
	N_{Ind}	N_{Nobs}	N_{Ntot1}		N_{Ind}	N_{Nobs}	N_{Ntot2}				
A	27	23	100	0·23	0	–	–	–	–	–	–
B	38	21	46	0·46	4	3	–	–	–	–	–
C	68	36	75	0·48	60	17	20	0·85	0·56	0·47	11
D	34	22	49	0·45	5	5	-	-	–	–	–
E	50	47	416	0·11	55	41	127	0·32	0·35	0·87	9
F	53	34	83	0·41	50	23	42	0·55	0·75	0·68	7
G	68	36	73	0·49	55	27	45	0·60	0·82	0·75	12
H	81	60	178	0·34	51	25	42	0·60	0·57	0·42	10
I	48	21	32	0·66	7	6	21	0·29	2·30	0·29	9
J	3	2	–	–	50	14	15	0·93	–	–	1
K	71	30	54	0·56	21	9	17	0·53	1·05	0·30	7
L	49	24	51	0·47	7	6	21	0·29	1·65	0·25	4
M	2	2	–	–	5	4	–	–	–	–	–
N	70	56	215	0·26	52	26	43	0·60	0·43	0·46	10
Mean	47·29	29·57	114·33		30·14	15·85	39·3				8·00
Mean excl. site E	47·08	28·23	86·91		28·23	13·75	29·56				7·89

N_{Ind}: number of sampled workers at each time point, N_{Nobs}: number of observed nests (based on Colony fullshib reconstruction), N_{Ntot}: total nest number including the unsampled nests (based on Capwire TIRM model estimations). Detection probabilities (*d*) are the proportion of detected nests relative to the total estimated number of nests. The change in detectability (δ*d*) is *d*1/*d*2. Estimated survival, taking into account changes in detectability, is given by (*N*tot2/δ*d*)/*N*tot1. N_{both} = no. of nests detected in both early and late samples.

Table 3. Nest number estimations for *B. pascuorum*

| Site | Early | | | Detection probability (*d*1) | Late | | | Detection probability (*d*2) | Change in detectability (δ*d*) | Estimated survival | N_{both} |
	N_{Ind}	N_{Nobs}	N_{Ntot1}		N_{Ind}	N_{Nobs}	N_{Ntot2}				
A	52	21	33	0·64	50	22	43	0·51	1·24	1·05	6
B	54	32	71	0·45	53	12	17	0·71	0·64	0·38	6
C	63	46	129	0·36	66	47	117	0·40	0·89	1·02	12
D	53	23	36	0·64	49	26	53	0·49	1·30	1·13	5
E	52	26	51	0·51	64	56	313	0·18	2·85	2·15	8
F	56	41	125	0·33	56	35	68	0·51	0·64	0·85	8
G	70	47	112	0·42	50	40	133	0·30	1·40	0·85	9
H	59	44	122	0·36	62	45	141	0·32	1·13	1·02	6
I	68	40	85	0·47	68	34	64	0·53	0·89	0·85	6
J	64	39	78	0·50	67	40	101	0·40	1·26	1·03	10
K	51	30	65	0·46	66	28	42	0·67	0·69	0·93	9
L	50	30	57	0·53	58	30	51	0·59	0·89	1·00	8
M	50	34	83	0·41	50	28	55	0·51	0·80	0·82	6
N	61	48	179	0·27	79	46	104	0·44	0·61	0·96	7
Mean	57·36	35·79	87·57		59·86	34·93	93				7·57
Mean excl. site E	57·77	36·54	90·38		59·54	33·31	76·08				7·54

N_{Ind}: number of sampled workers at each time point, N_{Nobs}: number of observed nests (based on Colony fullshib reconstruction), N_{Ntot}: total nest number including the unsampled nests (based on Capwire TIRM model estimations). Detection probabilities (*d*) are the proportion of detected nests relative to the total estimated number of nests. The change in detectability (δ*d*) is *d*1/*d*2. Estimated survival, taking into account changes in detectability, is given by (*N*tot2/δ*d*)/*N*tot1. N_{both} = no. of nests detected in both early and late samples.

and also with the area of gardens within 500 and 750 m (Fig. 3). The area of man-made land cover within a radius of 750 m was found to be positively correlated with nest survival (Fig. 3).

Discussion

Previous studies using microsatellite data to estimate colony number from samples of worker bumblebees have assumed that the number of nests detected by one, two, three, etc. workers follow a Poisson distribution, allowing estimation of the number of nests not detected (the zero category) (Chapman, Wang & Bourke 2003; Darvill, Knight & Goulson 2004; Knight *et al.* 2005, 2009; Ellis *et al.* 2006). These studies acknowledged that this approach is probably inaccurate since it assumes that all nests are equally likely to be sampled, an assumption which is clearly not valid (nests are likely to vary both in size and in their distance from the sample site). Here we
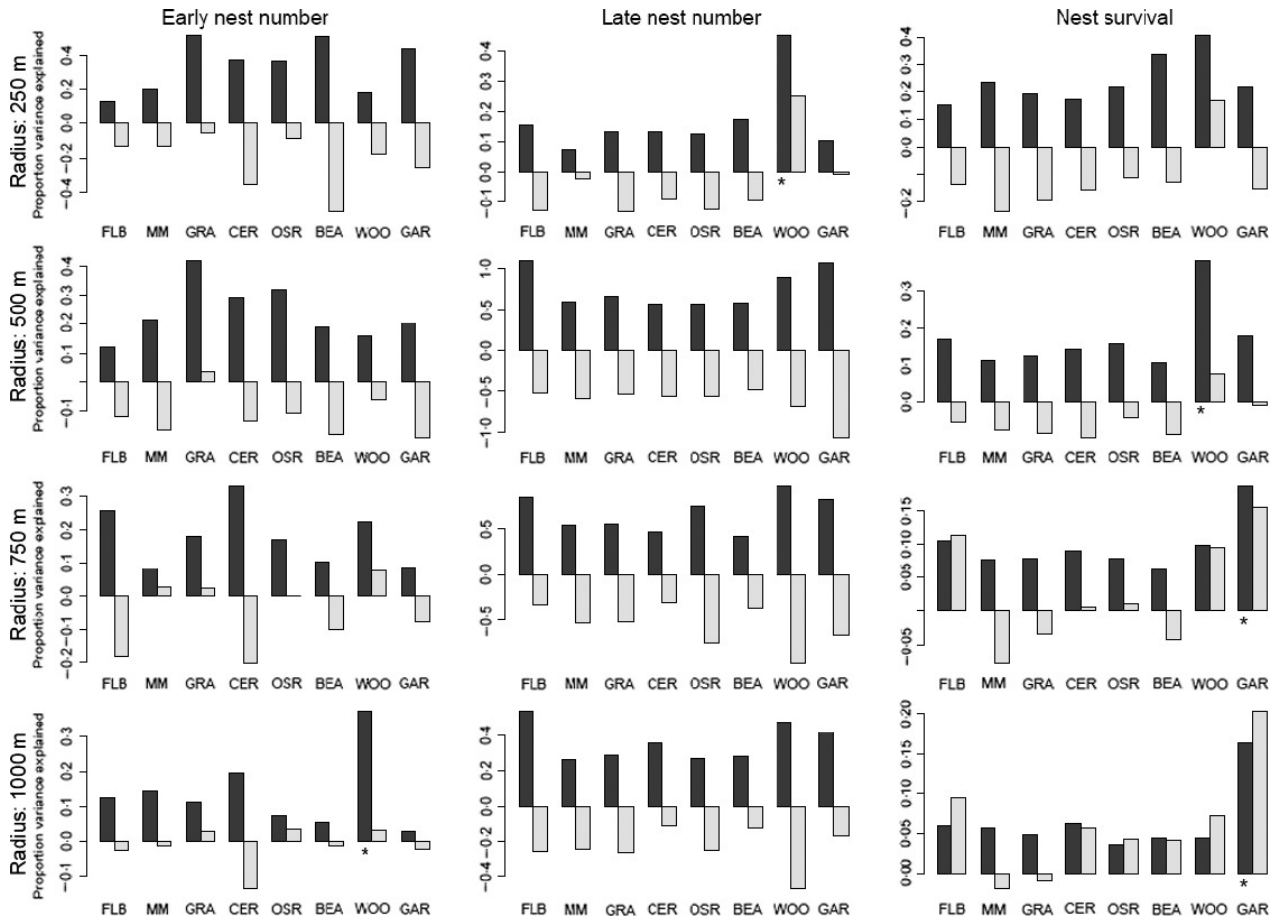
Fig. 2. Hierarchical partitioning showing independent (black) and conjoint (grey) effects of landscape variables expressed as the percentage of the total variance explained for *Bombus lapidarius*. FBL, field boundary length; GRA, grass; OSR, oilseed rape; WOO, wood; MM, man made; CER, cereal; BEA, bean; GAR, garden. *Significant independent contribution of a predictor variable at a 0·05 level.

explore the use of the program Capwire (Miller, Joyce & Waits 2005) to estimate the number of nests present. This software allows us to either assume that nests have an equal probability of capture (the Event Capture Model, ECM), or that the probability of capture varies (the Two Innate Rate Model, TIRM). We demonstrate that the former gives estimates of nest number that are nearly identical to those obtained from the use of a Poisson distribution. Likelihood ratio tests implemented in Capwire suggested that the TIRM provides a better fit to our data for both bee species. The TIRM method produced estimates of the number of nests present which were consistently higher by around 40%. It therefore seems likely that the nest density and colony number estimates derived in previous studies were underestimates.

The main purpose of our study was to derive estimates of nest number from multiple sites and at two points in time (late May–June and late July–August), and to use these data to examine how the surrounding land use influenced changes in nest number over time. There have been very few previous estimates of bumblebee nest survivorship, reflecting the difficulty in finding sufficient nests to obtain meaningful data. Our data allow us to indirectly estimate survivorship over an approximately 2-month period which roughly corresponds to the last 2 months of nest development.

Our estimates must be interpreted with care. We can account for changes in detection probability over time, which we might expect as nests grow larger. However, the number of nests detected at each time point will also be influenced by any seasonal changes in foraging range. Although there was no *a priori* reason to believe that foraging ranges systematically change through the season, our data provide evidence that they do in *B. pascuorum*.

For *B. lapidarius*, in the early sample, we directly detected 414 nests, and estimate that there were another 958 that we had not caught, so that we have recognizable genotypes for 30% of the population. In the second sample we directly detected 206 nests of which 80 (37%) were also in the first sample. If foraging range remained unchanged, we would expect the proportion to remain unchanged, regardless of mortality. In this instance the proportion is similar, suggesting that foraging range has indeed not changed substantially. We would not see this pattern if, for example, high nest mortality was being offset by increasing foraging range (this would give us an apparently high nest survivorship but an unexpectedly low recapture rate).

In contrast, for *B. pascuorum*, we directly detected 501 nests in the first sample, and estimate that there were a further 725 that we did not detect, so that we have recognizable genotypes
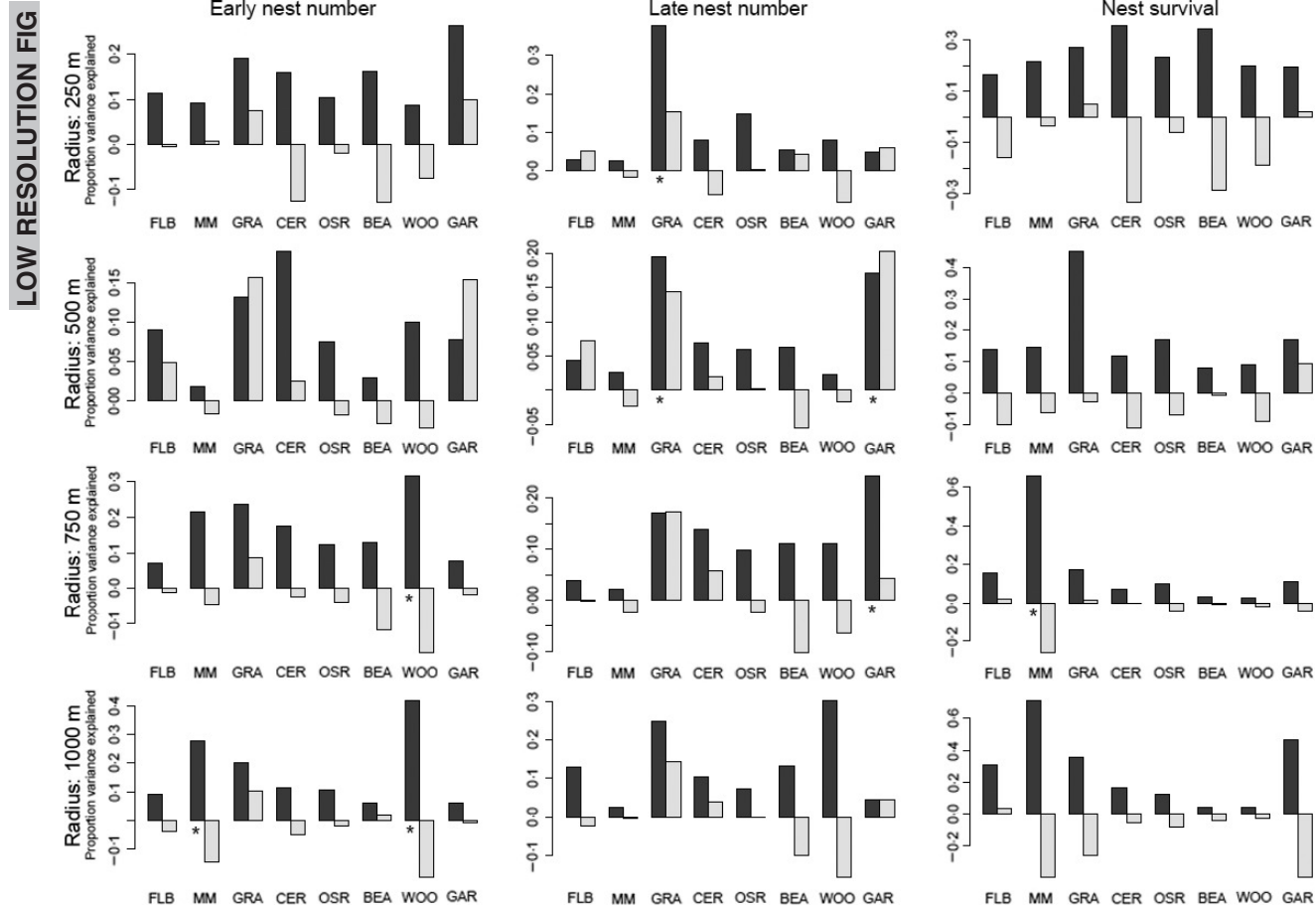
Fig. 3. Hierarchical partitioning showing independent (black) and conjoint (grey) effect of landscape variables expressed as the percentage of the total variance explained for *B. pascuorum*. FBL, field boundary length; GRA, grass; OSR, oilseed rape; WOO, wood; MM, man made; CER, cereal; BEA, bean; GAR, garden. *Significant independent contribution of a predictor variable at a 0·05 level.

for 41% of the population. In the second sample we directly detected 489 nests of which 106 (21%) were also in the first sample, a significant decrease. The numbers of nests detected at each time point suggest little mortality, but the unexpectedly low number of recaptures (21% compared to 41%) strongly suggests that we are sampling from a larger pool on the second occasion, which (given that nests are fixed and that there is no reproduction) can only be explained by increasing foraging range.

Our data suggest marked differences in the survival of the two species, with *B. lapidarius* having a mean survival rate of 0·45 over this ~2 month period compared to 0·94 in *B. pascuorum*. However, our estimates of survival taking into account nest detectability make the assumption of equal detection probability for all nests, something which our own analyses of methods for estimating the number on non-detected nests suggest is untrue. If workers of *B. pascuorum* are foraging further afield during the late sample period then we might expect this to increase heterogeneity in detectability among nests, inflating estimates of nest numbers at this time and therefore inflating estimates of nest survival. Conversely, there is some evidence that *B. lapidarius* may have suffered greater mortality than *B. pascuorum*. *B. lapidarius* has a southerly distribution compared to *B. pascuorum*, and 2007 was an exceptionally cool

and wet summer, so we might have expected *B. lapidarius* to fare poorly. The low abundance of *B. lapidarius* relative to *B. pascuorum* in this year is reflected in the sample sizes obtained for the two species; for *B. pascuorum* it was relatively easy to obtain the target figure of ~50 bees per site per sample period. In contrast, for *B. lapidarius*, sample sizes for two sample sites in the early period and six in the later period were too small to allow analysis (Table 2). An alternative explanation for differences in survival is that it is driven by phenology. *B. pascuorum* nests can last through to September while *B. lapidarius* nests tend to die off by late July/August, the time at which we took the second sample. This might explain why *B. lapidarius* apparently exhibited higher nest mortality, for nests were approaching the end of their natural life.

The particular landscape factors that were found to affect nest number and survivorship differed between species, but one factor was consistent for both. The area of gardens within 750 or 1000 m was found to positively influence nest survivorship in *B. lapidarius*, while the area of gardens within 500 and 750 m was found to positively influence the number of *B. pascuorum* nests in the late sample. Young nests of *B. terrestris* placed in suburban gardens have been found to grow more quickly when compared to nests placed in arable farmland (Goulson *et al.* 2002). Osborne *et al.* (2008b) used a public

survey to quantify bumblebee nest densities and found the highest nest densities in gardens. The present study was not specifically designed to examine the impacts of urban areas; the highest proportion of habitat classed as garden within 1 km of our study sites was 5·1%. Our data suggest that not only are gardens important for bumblebees even when they represent a small proportion of the landscape, but also that their positive influence on bumblebee populations can spill over onto neighbouring farmland that is 1 km distant. The positive relationships between the numbers of nests detected and the area of gardens nearby found for both bee species have two possible explanations. It may be that these nests are situated in the farmland, but benefiting from the floral resources obtained from gardens, or it may be that these nests are situated in gardens, in which case it may be that gardens are providing both floral resources and nest sites. Under either scenario, our results suggest that lack of resources in farmland is currently limiting pollinator populations.

Aside from gardens, the only significant land use to influence nest number of *B. lapidarius* was woodland; nest number in the early sample was higher at sites with more woodland within 1000 m, but nest number in the late sample period was lower at sites with more woodland within 250 m and nest survival was negatively correlated with area of woodland within 500 m. We have only speculative explanations for these results. Woodland in this area is typically deciduous, and deciduous woodland provides plentiful spring flowers such as bluebells. However, once the tree canopy closes in ~May there are few flowers. It may be that woodland spring flowers are important to *B. lapidarius* queens, and so boost the number of nests found in the early sample period. Conversely, woodland later in the season may provide an obstacle to foragers, so that woodland close to nests is particularly disadvantageous.

The factors influencing nest numbers for *B. pascuorum* (other than gardens) are more readily explained. *B. pascuorum* numbers in early samples were positively associated with the area of woodland within 750 and 100 m, and in late samples with the area of grassland within 250 and 500 m. *B. pascuorum* tends to nest above the ground in grass tussocks, leaflitter and thickets, so these land use categories are likely to be providing nest sites (Goulson 2003).

We found no significant effect of either oilseed rape or field beans on nest number at either time point, nor any effect on survival. Previous studies of the effects of mass-flowering crops on bumblebee populations have produced mixed results. Herrmann *et al.* (2007) found no effect of mass-flowering crops on the number of *B. pascuorum* nests detected using microsatellite markers. Westphal, Steffan-Dewenter & Tscharntke (2009) examined colony growth of *B. terrestris*, and found greater colony growth in early season when artificial nests were placed near oilseed rape field, but they found no effect on colony reproduction. Perhaps the most marked contrast between our findings and previous work is with Knight *et al.* (2009), who studied the same area as ourselves in 2004 using an essentially similar approach. Knight *et al.* (2009) sampled bees in late July (hence equivalent to our late sample period). They found that the area of field beans, oilseed rape and non-cropped area

(including gardens) within 1 km of each sample site was positively correlated with the number of *B. pascuorum* nests detected. Their landscape classification was much simpler than ours; they did not have separate garden and grassland categories, both of which were significant predictors of *B. pascuorum* nest abundance in our late samples. These differences in methodology may explain the differences in results obtained, but it is also possible that the relative importance of different landscape factors varies from year to year, according to the weather and bumblebee population density.

Our results have practical relevance for farmers wishing to maximize pollination services to their crops. Crops grown within 1 km of gardens are likely to receive more visits from bumblebees. Those growing deep-flowered crops such as field beans might consider ensuring that there are areas of rough grassland within 750 m of their bean fields to boost populations of *B. pascuorum*. Although unreplicated and hence anecdotal, the marked difference between site E and our other sample sites illustrates that on-farm management can have a striking effect on bee numbers. This site appeared to have approximately four times as many *B. lapidarius* nests in both early and late samples, and approximately five times as many *B. pascuorum* in late samples, compared to other sites. These large differences are almost certainly attributable to a ~5-ha clover ley adjacent to this site. Clover leys were once a common feature on arable farms since they boost soil fertility, but the advent of cheap artificial fertilizers led to their abandonment; it has been argued that this change in farming may have played a significant role in driving bumblebee declines in the twentieth century (Goulson *et al.* 2005, 2008a). It would appear that reinstatement of clover leys may provide a swift way to rapidly boost bumblebee numbers on farmland (Carvell *et al.* 2006, 2007).

Our study is one of the first to provide estimates of changes in nest density over time in bumblebees, and suggests that there may be differences between species in their patterns of seasonal mortality. Interpretation of our data is complicated by apparent changes in the foraging range of one of the two study species through the season. If, as suggested by the data, *B. pascuorum* are forced to forage further afield in late season due to a paucity of forage, then this would argue that conservation measures might better target forage provision in late July/August rather than late May/June.

In addition to the difficulties posed by varying foraging range, our approach is clearly not suitable for examining the early stages of colony development when only the queen is present, or when workers are very scarce. This is unfortunate as many authors have speculated that this is likely to be the time when most nest mortality probably occurs (e.g. Goulson, Lye & Darvill 2008a); a major challenge for future research is to develop means of examining survivorship in the early season, from queen emergence onwards.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Summary of main landscape classes and primary data sources; IKONOS – raster-based multispectral data derived from satellite data collected in late August 2007; MasterMap – vector-based information derived from published Ordnance Survey 1:1250 high resolution digital data.

Fig. S1. The sample sites marked on a classified map of the study area, created using IKONOS satellite imagery (4 m multispectral and 1 m panchromatic) and Ordnance Survey MasterMap topographic layer.

Fig. S2. Correlation coefficients of linear regressions between land cover and nest number in early samples (A: *B. lapidarius*; B: *B. pascuorum*), nest number in late samples (C: *B. lapidarius*; D: *B. pascuorum*) and nest survival (E: *B. lapidarius*; F: *B. pascuorum*) for different radii.

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