Predicting bumblebee density from landscape classification

Determining the scale on which forage availability affects bumblebee colony abundance in arable landscapes (?)

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Abstract

It is becoming increasingly recognised that combining the needs of agricultural production with enhancing biodiversity requires a landscape-scale approach. Bumblebees are a particularly important component of farmland biodiversity because they play an economic role as pollinators of various crops. Here we quantify the effects of land use at various spatial scales to find the best predictor of colony abundance for the bumblebee *Bombus pascuorum* in an arable landscape. Finding bumblebee nests is notoriously difficult, but here we use molecular markers to distinguish sisterhoods among foraging workers, and so infer colony number. Landscape use was measured using remote-sensed data combined with ground-truthing, and the number of bumblebee colonies found at 10 different sites within a 10 x 10 km study area was compared to availability of different types of forage in the surrounding area. Colony abundance was positively correlated with the area of three separate habitat categories (oilseed rape, field beans and non-cropped areas) within 1000 m of the sample site. No significant relationships were found for greater or lesser distances. This tallies well with earlier estimates of foraging range for this species (449m), for workers that were at the outer limit of their foraging range at the sample site will be likely to have sisters that are foraging in the opposite direction from their nest, giving a predicted radius of influence of forage availability on bee abundance of twice the foraging range (~900m). It has long been suspected that forage availability limits bee abundance in agricultural landscapes, and that declining availability of flowers has driven the declines of bumblebees, but there is little direct evidence for this. For the first time we demonstrate a direct link between floral abundance (provided by both mass flowering crops and non-crop areas) and the number of bumblebee nests in an area.

Comment [D1]: Newcastle please modify so it makes sense!

Introduction

Intensification of farming systems in the last 60 years has led to declines in farmland biodiversity (ref?). Of particular concern are declines in pollinator abundance, since pollinators provide an essential ecosystem service and direct economic benefit through pollination of crops. The area of entomophilous crops in the EC and USA is increasing, and some researchers have predicted that we will soon be facing a serious shortage of both wild and managed bees (Borneck and Merle 1989; Torchio 1990; recent ref??; see also Ghazoul 2005 for a contrasting view). Bumblebees (*Bombus* spp.) are a major group of pollinators in northern temperate climates, and many species have suffered large range contractions in recent decades (Williams 1995; Goulson 2003; Goulson et al. 2005). At least 25 major crops grown within the EC are visited and pollinated by bumblebees, including field beans, red clover, alfalfa, oilseed rape and various hard and soft fruits (Corbet *et al.* 1991). Some crops such as field beans are entirely dependent on pollination by long-tongued bumblebee species such as *B. pascuorum* and *B. hortorum* (Fussell and Corbet 1991). There is evidence that large crop monocultures may suffer from inadequate pollination since non-cropped areas are inadequate to support a sufficiently large bumblebee population to pollinate the crop when it flowers. For example in fields exceeding 12 ha in size the yield of field beans was reduced through inadequate pollination by long-tongued bumblebees (Free and Williams 1976). Similarly, Clifford and Anderson (1980) estimated that if field sizes exceeded 5 ha then yield of red clover in New Zealand declined through a shortage of bumblebees.

It is clear that appropriate management and conservation of bumblebee populations on arable farmlands is important both for ecological and economic reasons. However, as social insects with fairly large foraging ranges (Goulson & Stout

2001; Darvill et al. 2004; Knight et al 2005), their populations are presumably determined by patterns of resource availability at a landscape scale. Here we examine the scale over which availability of floral resources influences colony abundance of the long-tongued bumblebee species, *B. pascuorum*, in an arable landscape.

Intro needs padding out…

Methods

The 10km square transect – vegetation classification

Rotho & Newcastle people to fill in survey/ sensing methods, this is lifted straight from the report

This work focussed on bumblebee populations within a 10 x 10 km square centred on Rothamsted Research experimental farm, Hertfordshire, UK. Twenty 1 x 1 km squares within the area were surveyed using NCC Phase 1 survey methodology, modified to be bumblebee-specific by scoring forage availability and nesting suitability of each landscape feature. To account for temporal changes in forage availability, surveys were performed in spring (April/May), early summer (June/July) and late summer (August/September) 2002. These training areas were used to guide a supervised classification of the entire 10 x 10 km landscape into 25 cover types. Post-classification testing gave an accuracy of 83% for the satellite and 91% for the aerial data (Kappa). A separate 'garden' category was created, by using a moving-window search around urban areas for pixels of short or long grassland, ruderal or scrub, as an individual garden can contain a wide variety of small-scale habitats, of value as both forage and nesting sites for bees. Once the two thematic maps were created they were fused together so that the CASI digital data formed the 5 x 5km core, surrounded by the 10 x 10km satellite data, in order to maximise the precision of the map in the centre of the

study area (Fig. 1). Surveys were also performed in 2003 and 2004 to update the map with respect to locations of mass-flowering crops.

Site locations and sample collection

Individual *B. pascuorum* workers were caught at twelve locations within the 10km square transect (Table 1) over a three day period in late July 2004 (26th-28th), when *B. pascuorum* colonies are at their peak of activity. Sampling continued until approximately 50 individuals had been sampled at each site. Sampling effort at each site was approximated by timing searches. Sites were selected according to the following criteria:

1. A location as central within the 10km transect as possible while also being a minimum of 1km apart. This distance of 1km was chosen from a previous study that had estimated foraging range of this species to be approximately 450 meters. Thus, sample sites 1 km apart should not be sampling workers from the same nests.

2. To span as much variation as possible in terms of forage availability within the surrounding area (i.e. to include sites we would expect to be both 'good' and 'bad' for bees)

3. Having a suitable patch of forage to attract sufficient numbers to allow a sample size of 50 individual workers to be relatively easily caught. All sampling was within a small area at each site (<10m^2). At this time of year for this species suitable forage largely consisted of borage (*Borago officinalis*), white clover (*Trifolium repens*), comfrey (*Symphytum officinale*), bird's foot trefoil (*Lotus corniculatus*), teasel (*Dipsacus fullonum*), white dead nettle (*Lamium album*), red dead nettle (*Lamium purpureum*), and a variety of non-native ornamental plants in gardens, notably

Lavandula spp.. Samples were immediately preserved in 100% ethanol for later DNA extraction.

Microsatellite genotyping

DNA was extracted from thoracic muscle tissue using the HotSHOT protocol (Truett *et al.* 2000) and amplified at 9 variable microsatellite loci (B118, B131, B132, B11, B10, B96, B126, B124, B121, Estoup *et al*. 1995, 1996) using FAM-, HEX- or NED-labelled forward primers. PCR products were resolved on an ABI 377 automated sequencer (Applied Biosystems) with internal size standards (Genescan ROX 350, Applied Biosystems). Identical sample controls were used throughout. Alleles were sized using Genescan and Genotyper software (Applied Biosystems). Any cases of scoring ambiguity or non-amplification were re-processed for confirmation of allele sizes.

Data analysis

Sister identification

Genotypes were checked for typographic errors using MSAnalyser (Dieringer & Schlötterer, 2002; http://i122server.vu-wien.ac.at). Current evidence suggests that queens of all of the study species mate only once (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000). In the absence of polyandry any pair of bumblebee workers from the same nest has an expected relatedness of 0.75 (Hamilton 1964). Sister relationships among the individuals sampled were established within each species using the likelihood function of Kinship 1.3.1 (http://www.gsoftnet.us/GSoft.html; Goodnight & Queller 1999) where Rm= 0.5 and Rp=1.0. Confidence in sister pair assignment was calculated from 100,000 simulations, the number of iterations

determined by repeating analysis runs using variable numbers of simulations and establishing the point after which results reached a plateau. To minimise Type I errors, given the high number of pairwise comparisons within each data set, only sisters designated at $P \leq 0.001$ (the most stringent value that Kinship will return) were used in further analysis. Kinship assumes linkage equilibrium and no inbreeding. This particular population had previously been extensively sampled and was known to conform to these assumptions (Knight et al 2005).

Estimating numbers of colonies present

Since the sample sizes varied and this would have a direct effect on the number of colonies counted, sample sizes were standardised to n=48 (the smallest number of individuals collected at any of the sites) by randomly removing the appropriate number of individuals from each sampling site data set. Inevitably some colonies that were present would by chance not be represented in samples. To estimate the number of colonies that were not sampled at each site the number of colonies represented by lone individuals, two bees, three bees etc. were counted. Fitting a Poisson distribution to these data then allows the number of colonies not sampled at each site to be estimated. Summing all categories including the estimated 'not sampled' then gave an estimate of the total number of colonies within foraging range of each site sampled (for full details of this approach see Darvill et al. 2004, Knight et al. 2005).

Assessing relationship with available forage

The percentage of available forage was estimated from the classified map at the following radii from the sample sites: 250m, 500m, 750m, 1000m, 1250m, 1500m.

Forage within these radii was separated into the areas consisting of oilseed rape, field beans or non-farmed areas (hedges, margins, set-aside, gardens etc. combined). Whether the estimated number of colonies in an area bore any relationship to the area of forage available was assessed separately for each of the radii using multiple regression.

Results

A total of 12 sites were sampled, each a minimum of 1.05 km, maximum 6.90 km apart. From these sites 578 individual workers were sampled (Table 1). Two of the 12 original sample sites yielded considerably fewer than the targeted 50 individuals (sites 5, n=34 and 11, n=29) and so were excluded from the analysis. There was a low expected Type II error from Kinship (0.015 with 100,000 simulated iterations where $P \leq 0.001$), thus falsely rejected sister pairs are unlikely to have had any significant effect on data trends, particularly since this very low frequency of falsely rejected sisters are expected to be distributed randomly with respect to sampling site.

After standardising for a sample size of 48, 346 independent nests were identified in total from the 10 sites (Table 1), of which 68 were represented by more than one sampled individual. Twelve "non-circular nests" (cases where individual A is found to be a sister of individuals B and C but where individual B is not identified as a sister of individual C) were found. In these cases, data were re-examined and where individuals B and C would have been accepted as sisters at a less stringent significance level ($P \leq 0.01$), the group were accepted as true sisters (5/12 cases). Where no such relationship was evident between individuals B and C (7/12 cases) then the most parsimonious route was taken to gain circularity by omitting individuals from the family. While this may result in slightly overestimating the numbers of

colonies sampled, these cases were randomly distributed among sites and given their small number are not expected to have had any significant effect on any observed trends in the data set as a whole.

Distributions of the number of workers sampled from each colony closely conformed to a Poisson distribution in all cases (Table 1). Estimated total numbers of colonies within range of each site ranged from 62-162.

Significant relationships were found between estimated colony number and forage availability at 1000m only (Table 2). All three habitat categories (oilseed rape, field beans and non-cropped areas) contributed significantly to the model, suggesting that they all enhance colony survival. Availability of forage within lesser or greater distances poorly predicted the number of colonies present.

Discussion

It has long been suspected that forage availability limits bee abundance in agricultural landscapes, and that declining availability of flowers has driven the declines of bumblebees, but there is little direct evidence for this (reviewed in Goulson 2003). Here we demonstrate a direct link between floral abundance (provided by both mass flowering crops and non-crop areas) and the number of bumblebee colonies in an area. Whether this is because more queens choose to nest in the area, or because fewer nests survive until July (our sample period) in areas where there are fewer flowers, remains to be tested.

Previous studies conducted in the same area estimated the foraging range of *B. pascuorum* to be 449m (Knight et al. 2005). Workers that were at the outer limit of their foraging range at our sample sites will be likely to have sisters that are foraging in the opposite direction from their nest, giving a predicted radius of influence of forage

availability on bee abundance of twice the foraging range (~900m). This tallies closely with the results presented here, which suggest that the number of colonies present at any particular site is best predicted by the availability of forage within 1000m. Westphal et al. (2003) found that overall numbers of individual bumblebees (all species combined) were correlated most strongly with availability of oilseed rape within 3000m of their focal plots, a considerably greater range than that found here. The difference in scale is likely to be because they did not separate bumblebee species, and *B. pascuorum* has a shorter foraging range than the other common bumblebee species (Knight et al. 2005). Most of their records were probably of *B. terrestris* and *B. lapidarius*, the two most common species on rape (DG, pers. obs.), which have longer foraging ranges. Westphal et al.'s work also differs from ours in finding no significant effect of non-cropped areas. This difference may also be because they were largely looking at different species, and it seems likely that mass-flowering crops benefit most those species with long foraging ranges, while those with shorter foraging ranges will be more dependent on small patches of resources available near the nest. It may also be because they examined numbers of bees rather than numbers of nests; mass-flowering crops must provide a great boost to nests that have survived up until they flower, but in an intensive arable landscape with few non-cropped areas then colonies will not survive until then. Thus in areas with a high proportion of oilseed rape they may have detected lots of workers from a small number of large nests. Clearly further work is needed to resolve these differences, but overall it is probably safe to conclude that mass-flowering crops do enhance populations of the more common bumblebee species, but that non-cropped areas are also needed for colony survival through the season.

Our results suggest that quantifying available forage is a good predictor of relative bumblebee colony density at any particular site, but only if foraging range is

known for the species on question. Once this is known, appropriate strategies to conserve particular bumblebee species and/or to improve crop pollination could be developed. For example if a farmer wishes to conserve populations of the relatively long-tongued *B. pascuorum*, needed to pollinate crops with deep flowers such as field beans, then he must provide adequate resources to support nests through the season within 1000m of his crop. If the aim is to encourage nests of longer ranging species such as *B. terrestris* (an important pollinator of rape), then resources can be more widely dispersed. Forage ranges are not known for many bumblebee species, but have been estimated for four of the more common bumblebee species, *B. pascuorum, B. terrestris, B. lapidarius* and *B. pratorum* (Knight et al. 2005). In order to develop conservation strategies for rarer species (which may differ markedly from common species) it would be valuable to quantify their foraging range or repeat the approach used here, to estimate the scale of habitat management needed to maximise colony numbers.

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1 Table 1. Sampling summary and number of nests identified and estimated at each site using Kinship. Note that the number of nests identified is
2 based upon a standard sample size of 48 individuals (see main text). $\chi 2$ and P columns refer to the goodness of fit of colony representatives
3 frequency data to a Poisson distribution. Figures given in brackets were not included in final data analysis due to unequal sample sizes and /or
4 too few nets identified to fit a Poisson distribution.

Site number	**Site name**	**OS grid reference**	**n**	**Effort (minutes)**	**Number of nests**			
					identified	**$\chi 2$**	**P**	**estimated**
1	Cutting	TL115127	60	40	38	0.02	0.888	149
2	Leeside Walk	TL128172	53	34	41	0.01	0.905	147
3	Golf Course	TL107140	51	66	26	0.05	0.817	66
4	Oryx	TL123103	52	35	39	0.01	0.928	162
5	Great Cutts	TL128172	34	69	(19)			(38)
6	Turner's Hall	TL099157	51	39	28	0.19	0.661	62
7	Hillside	TL115178	54	25	34	0.03	0.851	109
8	M1	TL093147	56	52	31	0.27	0.606	62
9	Rothamsted	TL134135	59	28	40	0.03	0.862	140
10	Batford Mills	TL146151	48	62	36	0.02	0.875	105
11	John Fisher	TL104129	29	53	(20)			(47)
12	Cemetery	TL136157	50	52	33	0.03	0.864	95

Table 2. P values of regression analysis …OSR=oil seed rape, FB = field beans, MFC= mass flowering crops. Significant figures highlighted in bold.

Comment [D2]: Suggest include r2 values too?

radius(m)	*overall*	*OSR only*	*FB only*	*no MFC*
250	0.824	0.929	0.405	0.964
500	0.601	0.896	0.278	0.983
750	0.365	0.295	0.107	0.376
1000	**0.032**	**0.011**	**0.010**	**0.014**
1250	0.222	0.065	0.130	0.086
1500	0.492	0.151	0.315	0.214

Figure 1. Thematic map showing 25 final habitats for 10 x 10 km study area