

A comparison of techniques for assessing farmland bumblebee populations

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Abstract Agri-environment schemes have been implemented across the European Union in order to reverse declines in farmland biodiversity. To assess the impact of these schemes for bumblebees, accurate measures of their populations are required. Here, we compared bumblebee population estimates on 16 farms using three commonly used techniques: standardised line transects, coloured pan traps and molecular estimates of nest abundance. There was no significant correlation between the estimates obtained by the three techniques, suggesting that each technique captured a different aspect of local bumblebee population size and distribution in the landscape. Bumblebee abundance as observed on the transects was positively influenced by the number of flowers present on the transect. The number of bumblebees caught in pan traps was positively influenced by the density of flowers surrounding the trapping location and negatively influenced by wider landscape heterogeneity. Molecular estimates of the number of nests of *Bombus terrestris* and *B. hortorum* were positively associated with the proportion of the landscape covered in oilseed rape and field beans. Both direct survey techniques are strongly affected by floral abundance immediately

around the survey site, potentially leading to misleading results if attempting to infer overall abundance in an area or on a farm. In contrast, whilst the molecular method suffers from an inability to detect sister pairs at low sample sizes, it appears to be unaffected by the abundance of forage and thus is the preferred survey technique.

Keywords *Bombus* · Agri-environment · Nesting density · Pan traps · Pollinators

Introduction

In addition to facilitating the reproduction of wild plants, pollinating animals provide a valuable ecosystem service to food producers with approximately 35 % of the world's plant-based food supply dependent to a greater or lesser extent on animal pollination (Klein et al. 2007). The importance of wild pollinators for agricultural pollination has received more recognition in recent years, with wild populations providing an important service independent of the size of honey bee populations (Garibaldi et al. 2011, 2013). Given the well-documented declines in honey bee stocks in both the US and Europe in recent years (Natural Research Council 2006; Potts et al. 2010), it is even more important to maintain wild bee populations to provide the continuation of pollination services (Winfree et al. 2007) through their synergies with managed honey bees (Brittain et al. 2013), as well as for their contributions in areas where they provide the majority of crop pollination (Klein et al. 2003).

Bumblebees (*Bombus* spp.) are probably the most important wild pollinators in the UK for both crops and wild plants (Osborne and Williams 1996; Goulson 2010). Along with many other pollinating insects (Biesmeijer et al. 2006), the abundance and distribution of bumblebee

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species has declined substantially across Europe and North America since the Second World War (Kosior et al. 2007; Grixti et al. 2009; Williams and Osborne 2009). In common with many other taxa, declines in wild bumblebees have been particularly acute in intensively farmed areas, and consequently the UK and the European Union have introduced agri-environment schemes to counteract and reverse declines in biodiversity. These schemes offer the opportunity to provide resources for wild bumblebee populations. Previous work has highlighted the importance of robust and accurate population measurements of target taxa in order to determine the impact of agri-environment schemes (Kleijn and Sutherland 2003; Kleijn et al. 2006). Whilst there have been comparisons of the relative ability of different sampling techniques to measure bee diversity (e.g. Roulston et al. 2007; Westphal et al. 2008), there has been little assessment of the techniques used to measure the size of bumblebee populations.

The most commonly used technique to assess bumblebee abundance has been direct observations on standardised transect walks, based on the method for surveying butterflies (Pollard and Yates 1993) and modified for bees (e.g. Steffan-Dewenter et al. 2002; Westphal et al. 2006; Carvell et al. 2007). Transect walks are relatively time consuming and require trained surveyors, and consequently low-effort passive sampling techniques such as pan traps have been used to sample bee populations (e.g. Leong and Thorp 1999; Cane et al. 2000). Passive blue vane traps designed for sampling beetles have also been shown to capture a high proportion of bumblebees (Stephen and Rao 2005), but this technique is not in common usage. The use of pan traps in sampling bumblebee populations has been limited, in part because it is thought that they under-sample large-bodied bees (Toler et al. 2005). Nevertheless, because other passive sampling techniques such as trap nests are limited to cavity nesting bees (Tscharntke et al. 1998), pan traps are one of the few passive sampling techniques that can be used to survey bumblebees.

Bumblebee population estimates have also been obtained through the use of microsatellite markers. As eusocial insects, bumblebees form colonies, and because the queen is the dominant reproductive unit, the effective population size can be estimated from the number of colonies in an area. Nesting densities of wild bumblebees are poorly understood and direct surveys are infrequent and labour-intensive (Osborne et al. 2008; O'Connor et al. 2012). Because bumblebee colonies are founded by a single queen, and most species are monoandrous (Estoup et al. 1995; Schmid-Hempel and Schmid-Hempel 2000), the resulting high relatedness between workers makes social bumblebees amenable to identification of sisters using molecular techniques, such as microsatellite sequencing,

enabling an estimation of the number of nests present in an area (e.g. Knight et al. 2005; Goulson et al. 2010).

Bumblebees are known to be highly mobile insects, sometimes travelling up to several kilometres to forage (Walther-Hellwig and Frankl 2000; Knight et al. 2005). Where foraging resources are scarce, such as in intensively farmed landscapes, bumblebees may respond more strongly to the presence of sown forage patches (Heard et al. 2007; Scheper et al. 2013), and hence a direct survey of these areas may over-represent the total population present in the area. Here, we compare bumblebee population estimates using all three techniques on 16 farms in southern England. Using transects and pan traps, we examine abundance of all bumblebee species present, and we also estimate nest density via genetic sampling for two species, *Bombus terrestris*, a short-tongued bumblebee and *B. hortorum*, a long-tongued bumblebee. We then investigate local and landscape factors that may affect the bumblebee population estimates provided by these survey techniques. Our results have clear implications for future effective assessment and interpretation of bumblebee survey data.

Materials and methods

Study area

Sixteen farms were selected in north Hampshire and West Sussex, UK, representing a range of farming intensities and landscape gradients. The minimum distance between a pair of farms was 5 km. Farms were predominantly arable or mixed arable/dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops.

Sampling methods

A standardised 3-km line transect was designed for each farm passing through semi-natural habitats, primarily grass or floristically enhanced margins alongside agricultural fields, hedgerows and woodlands. Regularly grazed and mown grassland and cropped areas were not surveyed. Transects preferentially passed through areas of flower-rich habitat that were present. Moving at a standard pace, all bumblebees within 2 m of the surveyor were recorded to species level. Transects were walked three times throughout the summer of 2013, between 25 May and 5 June, 26 June and 15 July, and 3 and 11 August (henceforth described as rounds one, two and three). Transects were subdivided into habitat sections, and for each section, the number of flowering units of each plant species was recorded during each of the sampling periods. This assessment followed Carvell et al. (2007) with one flower cluster (e.g. an umbel, a capitulum) counted as a single unit. All sampling was conducted

between 0930 and 1700 hours and conformed to climatic guidelines from the UK butterfly monitoring scheme (Pollard and Yates 1993).

Twelve pan traps were placed on each farm. Pan traps consisted of 500-ml plastic bowls and were spray-painted fluorescent blue, yellow or white (Sparvar Leuchtfarbe; Spray-Color). One of each colour, adjacent to each other, were attached to a wooden post at an approximate height of 60 cm. On farms where flower-rich margins had been sown (8 farms), two such posts were placed in a flower-rich margin and two were placed in a general grass margin. On farms lacking any sown flower-rich margins (8 farms), two posts were placed in each of two separate grass margins. Of the 32 sampled margins, 27 were also surveyed as part of the transect, allowing for calculation of the floral density at the trapping location for each sampling round. Posts in the same margin were separated by a distance of 25 m. Sampled margins were not closer than 200 m. Traps were filled with approximately 400 ml of water with a few drops of odourless liquid detergent (Surcare Sensitive). Traps were left out for 96 h before being collected. All farms were sampled at the same time so they were all subject to the same weather conditions. Traps were set three times on the 10–15 June, 8–12 July, and 1–5 August (henceforth described as rounds one, two and three). Invertebrate samples were stored in 70 % ethanol and pinned prior to identification.

Genetic samples of *B. hortorum* and *B. terrestris* workers were collected between 26 June and 15 July. Each farm was divided into four sections and each quarter was searched for approximately 90 min, giving a total collection period of 6 h. This sampling area conformed to a circle with radius 800 m and covered the majority of each farm. Non-lethal tarsal samples (Holehouse et al. 2003) were taken from the mid-leg of collected workers. Tarsi were stored immediately in 95 % ethanol for later DNA extraction.

Molecular methods

DNA was extracted from the tarsal sample using the HOT-Shot protocol (Truett et al. 2000) and amplified at nine polymorphic microsatellite loci (B100, B118, B132, B10, B11, B96, B119, B124, B126; Estoup et al. 1995, 1996). Polymerase chain reactions (PCR) were carried out on samples using the QIAGEN Multiplex PCR kit. Multiplex PCRs were run for combinations of the loci B100(VIC)-B118(NED)-B132(FAM), B10(VIC)-B11(NED)-B96(FAM) and B119(FAM)-B124(NED)-B126(PET) (fluorescent markers indicated in parentheses). B119 amplified weakly in *B. hortorum* and was removed from further analysis. PCR reactions were 10 µl in volume and contained 1 µl of Q-solution, 5 µl of PCR MasterMix, 1.8 µl of RNase

free water, 1.2 µl of primer solution (6 × 0.2 µl of each primer, forward primers labelled with VIC, NED, FAM and PET dyes; Applied Biosystems) and 1 µl of template DNA. Samples were denatured at 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 54 °C annealing for 90 s and 72 °C for 90 s. This was followed by a final step at 72 °C for 10 min. PCR products were visualised on an ABI 3730 capillary DNA sequencer with a 1:125 dilution before the run and using a GeneScan LIZ 500 internal size standard. Fragments were sized using GeneMapper software (Applied Biosystems).

Colony v.2.0.5.0 (Jones and Wang 2009) was used to assign workers to unique colonies within each farm. This program uses maximum likelihood methods to assign sib-lingship or parent–offspring relationships, and has been found to be the most reliable method available for assigning sib-lingship in bumblebees (Lepais et al. 2010). Genotyping error was set at 2 % (allele dropout 0.5 % and other errors 1.5 %). DNA-based capture–recapture models allow for multiple sampling of an individual, and the frequency distribution of the number of times each individual is recaptured can be used to estimate the population size. Instead of trying to estimate the number of individuals, we were interested in estimating the number of nests represented by our sample of workers. Following Goulson et al. (2010), the program Capwire (Miller et al. 2005) was used to estimate the number of colonies present on each farm using the Two Innate Rate Model.

Landscape analysis

A buffer 1 km in radius was drawn around the centre point of each transect covering the majority of each farm and some of the surrounding area. The buffer covered the entirety of the 3-km transect. Using satellite imagery, ordi-nance survey maps and extensive ground truthing during the survey period, detailed land cover maps to a resolution of 2 m² were created in Google Earth (Google Inc). Habitat types were binned into nine discrete biotope classes: (1) crops non-attractive to bumblebees (wheat, barley, maize); (2) crops attractive to bumblebees (oilseed rape, field beans); (3) permanent grassland (grassland that is continuously grazed through the year or regularly cut for silage); (4) general grass (agri-environment grass margins, road verges, grassed access tracks, flower-poor grassland which is not regularly cut or grazed); (5) woodland (broadleaf, coniferous and mixed); (6) hedgerow (the woody centre, not including associated hedge bottom grass strips, maximum width 4 m); (7) flower-rich (florally enhanced margins, species-rich grassland and wild bird seed margins containing a high density of bumblebee attractive plants); (8) water (including drainage ditches, rivers and ponds); and (9) urban (farm buildings, paved roads, private houses

and gardens). These habitat maps were exported to ArcGIS 10.1 (ESRI) where the total area covered by each biotope was calculated. Following Fuentes-Montemayor et al. (2012), a Shannon diversity index was calculated from the number of different habitat types and their proportional abundance as a measure of landscape heterogeneity. Each of these factors was selected for inclusion in the initial model on the basis that they are likely to impact the size and distribution of bumblebee populations across the landscape.

Data analysis

Bumblebee population results obtained by the different sampling techniques were summed across all sampling rounds and tested for significant correlation using Spearman's rank correlation test.

Generalised linear models (GLMs) were run to investigate the impact of landscape variables on the number of bumblebees recorded along transects, caught in the pan traps and the estimated number of bumblebee colonies present on each farm. Models were run for the abundance of all bumblebee species and the abundance of the four most common bumblebee species, *B. hortorum*, *B. lapidarius*, *B. pascuorum* and *B. terrestris*, with the transect and pan trap data summed across all sampling rounds. Before performing the main analysis, explanatory factors were tested for collinearity revealing a group of significantly intercorrelated variables. This group represented farming intensity, with the cover of non-attractive crops and hedgerow positively correlated with each other and negatively correlated with the landscape Shannon diversity index, the cover of woodland and the cover of permanent grassland. Following Fuentes-Montemayor et al. (2012), these explanatory factors were correlated with the response variable for each model, and the most strongly correlated factor was selected for inclusion in the initial model. The following explanatory factors were not significantly correlated with any other factor and were always included in the initial model; cover of attractive crops, cover of general grass, cover of flower-rich grass, cover of water and cover of urban. Models were simplified using a backward-stepwise approach until only significant factors ($P = 0.05$) remained. At each step, models were assessed using an ANOVA for a significant increase in residual deviance. If removing a non-significant term led to a significant increase in residual deviance, the factor was returned to the model.

Generalised linear mixed effect models (GLMMs) were run to investigate the impact of floral abundance on the number of bumblebees recorded on the transects and caught in pan traps. For the transect abundance model, floral abundance was measured by the number of floral units counted on each transect for each sampling round. Data

were not summed across rounds; instead sampling round was included as a random factor to account for temporal variation. Floral abundance was included as a fixed factor. For the pan trap model, floral density measurements were available for 27 of the 32 margins in which pan traps were placed, so data without density measurements were excluded from this analysis. The number of bumblebees caught in the six traps placed in each margin were summed, to give two measurements per farm per sampling round. The farm nested within sampling round were included as a random factors to account for spatial and temporal variation. Floral density was included as a fixed factor.

The strength of the impact of floral density on both survey techniques was investigated further. The pan trap data were recalculated to provide a single bumblebee total and floral density measurement for each farm in each sampling round. A floral density score was calculated for each transect in each sampling round. A GLMM was run with floral density, survey type and their interaction as fixed factors, with sampling round as a random factor.

All GLMMs were compared by ANOVA with a null model to test for significance. GLMs and GLMMs were run with a Gaussian, negative binomial or Poisson/quasi-poisson error distribution for the response variable where appropriate and were tested for overdispersion. All data analysis was conducted in R v.3.0.2 using the lme4 package for GLMMs (R Development Core Team).

Results

Bumblebee abundance

A total of 6,014 bumblebees of 14 species were recorded on the transects. The 14 bumblebee species comprised the ubiquitous social species *B. terrestris*, *B. lucorum*, *B. pratorum*, *B. pascuorum*, *B. hortorum* and *B. lapidarius* as well as *B. jonellus*, *B. hypnorum* and *B. ruderatus*. Five cuckoo bumblebee species (formerly *Psithyrus*) were also recorded, comprising *B. vestalis*, *B. barbutellus*, *B. rupestris*, *B. sylvestris* and *B. campestris*. A total of 1980 bumblebees of 12 species were recorded in the pan traps. The 12 bumblebee species were the same as those recorded on the transects, less *B. barbutellus* and *B. ruderatus*. A total of approximately 3.5 million flowering units of 209 species of insect-pollinated flowering plants were recorded on the transects.

A total of 386 *B. hortorum* and 593 *B. terrestris* workers were genotyped for eight and nine microsatellite loci, respectively. Totals of 85 *B. hortorum* sister pairs and 58 *B. terrestris* sister pairs were identified. No 'noncircular' nests were identified. Of the 16 farms studied, no sister pairs were found for *B. hortorum* on four farms and no sister pairs for *B. terrestris* on four farms (i.e. all bees sampled

were from unique nests). Consequently, no estimate of the number of undetected nests could be made for these farms, leaving a total of 12 farms for each species. Estimated numbers of colonies per farm from Capwire ranged from 107 to 412 for *B. hortorum* and from 92 to 1780 for *B. terrestris*.

Relationship between different survey techniques

There were few similarities between the different sampling techniques. The abundance of bumblebees observed on the transects was not correlated with the number of bumblebees caught in the pan traps (Spearman’s rho = 0.18, *n* = 16, *P* = 0.50). The Shannon diversity index of bumblebees observed on the transects was not correlated with the diversity of bumblebees caught in the pan traps (Spearman’s rho = 0.19, *n* = 16, *P* = 0.47). The molecular estimate for

the number of *B. terrestris* nests in an area was not correlated with either the abundance of *B. terrestris* seen on the transects (Spearman’s rho = 0.26, *n* = 12, *P* = 0.42) or caught in the pan traps (Spearman’s rho = 0.16, *n* = 12, *P* = 0.63). The molecular estimate of the number of *B. hortorum* nests in an area was not correlated with either the abundance of *B. hortorum* seen on the transects (Spearman’s rho = 0.11, *n* = 12, *P* = 0.73) or caught in the pan traps (Spearman’s rho = 0.06, *n* = 12, *P* = 0.85).

Effects of the surrounding landscape

Sampled farms varied considerably in land use types (Table 1) with an average of 72.1 ± 2.7 % of land covered in crop or permanent grassland and 21.8 ± 2.8 % covered by semi-natural habitat. Few landscape variables were significantly associated with bumblebee abundance across the sampling techniques. Final model results are summarised in Table 2. There were no significant interactions between any of the final explanatory variables.

Landscape heterogeneity was significantly negatively associated with both the total number of bumblebees caught in pan traps (GLM, *t*_{1,15} = -2.382, *P* = 0.032) and the number of *B. terrestris* (GLM, *t*_{1,15} = -2.681, *P* = 0.018), the most commonly caught bumblebee (*n* = 925). The number of *B. hortorum* caught in pan traps was marginally non-significantly negatively associated with the cover of general grass habitats (GLM, *t*_{1,15} = -2.143, *P* = 0.0502; Fig. 1b). For the transect data, only the abundance of *B. hortorum* was associated with landscape variables, being positively associated with the area of flower-rich habitat (GLM, *t*_{1,15} = -3.259,

Table 1 Land use types for surveyed farms within a 1-km radius

Land use type	Area (%)	Minimum (%)	Maximum (%)
Attractive crops	10.99 ± 1.81	3.13	32.58
Non-attractive crops	46.52 ± 4.23	16.68	70.59
Permanent grassland	14.62 ± 2.52	0.68	34.32
Woodland	11.49 ± 3.01	0.85	35.18
Hedgerow	2.10 ± 0.24	0.39	3.34
Flower-rich	0.99 ± 0.32	0.00	4.36
General grass	7.23 ± 0.99	2.88	16.17
Water	1.05 ± 0.84	0.00	13.44
Urban	5.00 ± 0.52	1.83	7.44

Means ± 1SE are given for the 16 selected farms

Table 2 Summary table showing significant effects of landscape variables on bumblebee population metrics

	Variable	<i>df</i>	<i>t</i>	<i>P</i>	Signif.
Transect abundance all species	No significant factor				
Pan trap abundance all species	Landscape heterogeneity	1,15	-2.382	0.032	*
<i>Bombus terrestris</i>					
Molecular nest estimate	Attractive crop cover	1,11	2.237	0.0493	*
Transect abundance	No significant factor				
Pan trap abundance	Landscape heterogeneity	1,15	-2.681	0.018	*
<i>Bombus hortorum</i>					
Molecular nest estimate	Attractive crop cover	1,11	2.948	0.016	*
	General grass cover	1,11	-2.316	0.046	*
Transect abundance	Flower-rich cover	1,15	3.259	0.0062	**
	General grass cover	1,15	-2.524	0.025	*
Pan trap abundance	General grass cover	1,15	-2.143	0.0502	.
<i>Bombus lapidarius</i>					
Transect abundance	No significant factor				
Pan trap abundance	No significant factor				
<i>Bombus pascuorum</i>					
Transect abundance	No significant factors				
Pan trap abundance	No significant factors				

* *P* < 0.05, ** *P* < 0.01

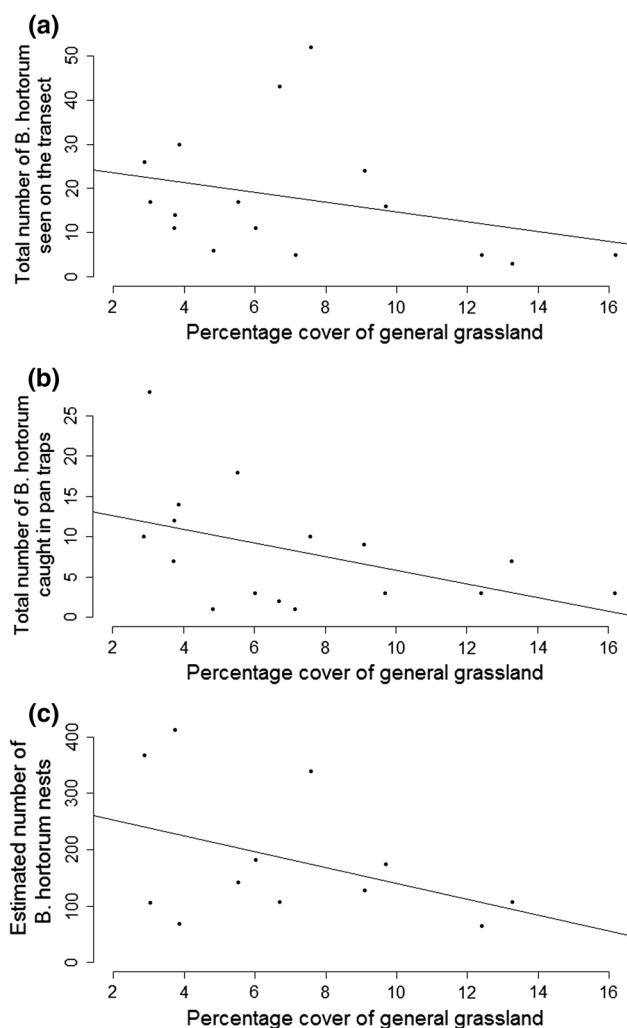


Fig. 1 Effect of the proportion of general grass habitats on *Bombus hortorum* as measured by **a** the total number of individuals counted on the transect, GLM $t_{1,15} = -2.524$ $P = 0.025$, **b** the total number of individuals captured in pan traps, GLM $t_{1,15} = -2.143$ $P = 0.0502$, and **c** the molecular estimate of number of nests, GLM $t_{1,11} = -2.316$ $P = 0.046$

$P = 0.0062$) and negatively associated with the cover of general grass habitats (GLM, $t_{1,15} = -2.524$, $P = 0.025$; Fig. 1a).

For the molecular estimates of the number of colonies present, *B. hortorum* (GLM, $t_{1,11} = 2.948$, $P = 0.016$) and *B. terrestris* ($t_{1,11} = 2.237$, $P = 0.0493$) were significantly associated with the cover of attractive crops. In addition, the abundance of *B. hortorum* colonies was negatively associated with the cover of general grass habitats (GLM, $t_{1,11} = -2.316$, $P = 0.046$; Fig. 1c).

Effects of local floral abundance

Bumblebee abundance on the transects was strongly associated with recorded floral abundance on the transects

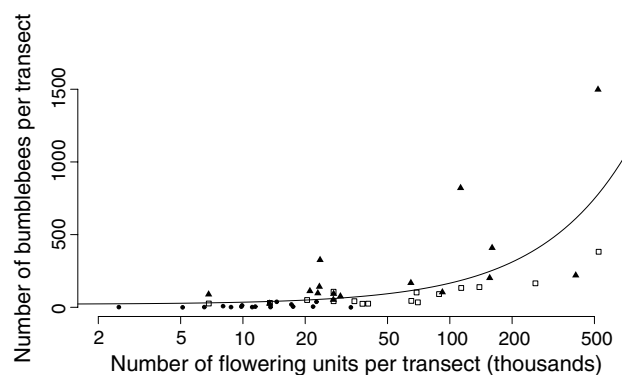


Fig. 2 Relationship between floral abundance and number of observed foraging bumblebees on 3-km transects during the May–August sampling period. Sampling round one, circles; round two, open squares; round three, triangles. GLMM, $\chi^2 = 30.9$, $P < 0.001$. Note log scale used on x-axis

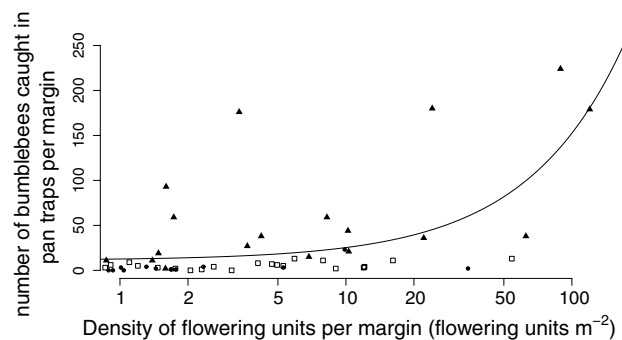


Fig. 3 Relationship between floral density and the number of bumblebees caught in pan traps placed out in agricultural margins during the May–August sampling period. Sampling round one, circles; round two, open squares; round three, triangles. GLMM, $\chi^2 = 8.1$, $P = 0.004$. Note log scale used on x-axis

($\chi^2 = 30.9$, $P < 0.001$; Fig. 2). The number of bumblebees caught in pan traps was also strongly associated with the density of flowers in the margin in which they were placed ($\chi^2 = 8.1$, $P = 0.004$; Fig. 3). This effect was similar for both methods with the inclusion of the interaction between floral density and survey technique not significantly improving the model ($\chi^2 = 0.8$, $P = 0.364$).

Discussion

If we are to devise sensible management strategies for bumblebees or other pollinators, to conserve rare species or maintain adequate populations of common species to deliver pollination services, then it is important that we have accurate means of measuring their populations. Without such measures, we are unable to detect population change, compare populations in regions, or detect the

effectiveness of different management techniques. It is thus of considerable concern that the three techniques here, all of which are in common use, appear to produce population measurements that do not correlate with each other. Rather than accurately measuring the bumblebee population at the farm level, each technique seems to capture a different aspect of local bumblebee abundance and distribution throughout the landscape. Such parallels can also be found with other invertebrate sampling techniques: for example, pitfall traps have long been regarded as measuring invertebrate activity rather than just abundance, where activity is governed by factors such as the availability of food resources and vegetation density (Sunderland et al. 1995).

Semi-natural environments on agricultural land can provide suitable foraging resources and nesting sites for bumblebees and are expected to be beneficial to bumblebee populations (Kells and Goulson 2003; Pywell et al. 2006). However, in our study, only one positive association with semi-natural habitats or factors correlated with semi-natural habitats was found, that of the abundance of *B. hortorum* to the area covered in flower-rich habitat. Instead, in common with other studies, the number of bumblebees recorded on the transects was strongly associated with floral abundance on the transect itself (Pywell et al. 2006; Carvell et al. 2007).

Whilst previous pan trap studies have caught few large-bodied bees (Toler et al. 2005; Westphal et al. 2008), pan traps in this study caught large numbers of bumblebees (1980 individuals representing 70 % of the total number of bees caught in pan traps). Whilst this represents a smaller proportion than the transects, where the 6014 bumblebees represented 93 % of the total bees recorded, our data show that bumblebees can be attracted to pan traps in large numbers. The total number of bumblebees caught in pan traps over the survey period on each farm depends on the wider landscape structure, with a greater number caught in more intensively farmed, less heterogeneous environments. It has been argued that landscape heterogeneity is important for farmland biodiversity (Benton et al. 2003), and it seems unlikely that less heterogeneous landscapes genuinely support more bumblebees, as the process of agricultural intensification that reduces landscape heterogeneity is the primary reason behind the decline in agricultural bumblebee populations (Goulson et al. 2005; Williams et al. 2005; Carvell et al. 2006). It is well known that bumblebees forage over large areas, in particular *B. terrestris* (Walther-Hellwig and Frankl 2000; Knight et al. 2005; Westphal et al. 2006), which was the most common bee caught in the pan traps. In landscapes with lower heterogeneity, bumblebees may have to travel greater distances to find suitable forage, and are more likely to encounter pan traps than bees in a more heterogeneous landscape in which resources are more widely distributed. This may result in

an overestimation of the total population, with the largest effect found in those species with the greatest dispersal ability. Previous studies have found that pan traps catch more bees in flower-poor areas, arguing that the relative lack of resources may make the pan traps more attractive (Baum and Wallen 2011; Morandin and Kremen 2013). It is consequently surprising that, as with transect surveys, our results suggest that pan traps catch more bumblebees in areas with a greater density of flowers. Despite the differences in this relationship, pan traps are clearly strongly influenced by both landscape structure and floral availability and are not suitable for measuring bumblebee population sizes.

Previous studies have highlighted the lack of an apparent relationship between observed bumblebee abundance and the presence of varying areas of semi-natural habitat (e.g. Westphal et al. 2003; Herrmann et al. 2007). It has been argued that as the proportion of arable land increases the abundance of semi-natural forage is reduced, resulting in sown forage patches being relatively more exploited because they represent a greater proportion of the total forage available in an area (Heard et al. 2007), making it difficult to assess the true benefit of semi-natural habitats to bumblebee populations. This is borne out by Carvell et al. (2011) who found that densities of *B. lapidarius*, *B. pascuorum*, *B. hortorum* and *B. terrestris* agg. on sown forage patches were significantly higher in areas with a greater proportion of arable land. Moreover, bumblebees also exhibit a degree of site constancy once lucrative locations have been found (Osborne et al. 1999), leading to a greater accumulation of workers where alternative forage is scarce. This 'ecological contrast' between sown floral resources and the resources present in the wider environment has been shown to affect how strongly pollinators respond to agri-environment schemes (Scheper et al. 2013). Given the strong aggregative effect of highly contrasting floral resources and the lack of similarity between different population measurements, it is not clear that a strong response to agri-environment schemes necessarily translates into a positive population level impact for bumblebees.

Whilst these aggregative effects may influence the distribution of foraging bumblebees throughout the landscape, they should not affect the distribution of workers from the same colony. Unlike honeybees, bumblebees lack a directional recruitment mechanism between foragers (Dornhaus and Chittka 1999), so sister workers should be distributed randomly throughout the environment. Consequently, even if samples are collected from an aggregation of foraging bees, it should still be representative of the number of colonies present in an area. The molecular nest estimates for both *B. hortorum* and *B. terrestris* showed a positive association with the percentage of the landscape covered with oilseed rape and field beans. It has been argued that these

mass flowering crops are good for providing resources for bumblebees in intensively farmed environments, and their presence has been associated with higher observed densities of bumblebees on sown forage patches (Westphal et al. 2003) and on transect walks (Herrmann et al. 2007). Whilst this may also be as a result of bumblebee aggregation, our molecular estimates suggest that there may be a real beneficial effect of mass flowering crop on the number of colonies founded in an area.

However, the molecular population estimates obtained in this study show marked variation, particularly for *B. terrestris*. True nesting densities of wild bumblebees are poorly understood, so it is not possible to comment on whether these colony abundance estimates are reasonable, but the lack of detection of sister pairs from 4 sites suggests that sampling the same site over multiple days may be required to detect sufficient sister pairs in order to make more comprehensive population estimates. Additionally, the low similarity between the results obtained from the molecular estimates and those from the direct sampling techniques may be due to differences in average nest size between farms. Bumblebee colonies placed in resource-rich areas develop faster than those in resource-poor areas, though their reproductive output was unaffected (Goulson et al. 2002; Williams et al. 2012). Colony growth of wild bumblebee nests is poorly understood, but with variable emergence and colony establishment dates for individual queens, it seems unlikely that consistent differences in nest size would exist between farms, but this is an issue that requires further study. Despite these shortcomings, the molecular technique does not appear to be biased by floral abundance in the study area and is likely to be a more accurate measure of bumblebee population size than the direct survey techniques.

The presence of general grass habitats was negatively associated with population estimates for the long-tongued *B. hortorum* across all three sampling techniques. The majority of the general grass biotope comprised 2- to 6-m grass margins, usually located alongside field edges. This negative association may also be as a result of dilution, with *B. hortorum* preferring these habitats, leading to a reduced detection in other areas. However, this seems unlikely, as from personal observation (T.J.W.) during the transects and the collection of sampling techniques, few *B. hortorum* were seen foraging in general grass habitats because these areas did not provide suitable forage plants, in contrast to hedgerows and flower-rich grassland. General grass margins tend to be comprised of coarse grasses which may repress forage plants such as *Stachys sylvatica*. This is a typical hedgerow plant that is not included in conservation mixes and was the most popular forage plant for *B. hortorum* in this study, representing 62 % of worker foraging visits during the collection of genetic samples and 38 %

of visits by all castes over the whole survey period. Given that the majority of the conservation priority bumblebee species in Britain are also long tongued and favour similar plant species to *B. hortorum* (Goulson et al. 2005), a potentially negative impact of general grass margins requires further investigation.

Conclusions

Distinguishing between factors that have a genuine population level effect and factors that simply cause a redistribution of individuals around the landscape is vitally important if the drivers of bumblebee population size at the farm scale are to be identified, understood and integrated into contemporary management schemes. Our results suggest that the bumblebee population size estimates from both transects and pan traps are greatly affected by the availability of forage both in absolute terms and in relationship to the floral resources present in the wider environment, potentially leading to an overestimation of the population in intensively farmed areas and an underestimation of the population in less intensively farmed areas. Whilst the molecular technique does not appear to be affected by bumblebee aggregation, it is substantially more expensive and time consuming and may not produce useful results if insufficient samples to detect sister pairs are collected. However, in the absence of a true population measurement, it appears to be the best technique. Further detailed work is needed to determine a practical and cost-effective way to accurately measure bumblebee populations in agricultural regions.

Author contribution statement T.J.W., J.M.H. and D.G. conceived the initial idea and designed the experiments. T.J.W. conducted the fieldwork and molecular work, analysed the data and wrote the manuscript and J.M.H. and D.G. provided editorial advice.

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Conflict of interest The authors declare that they have no conflict of interest.

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