Targeted agri-environment schemes significantly improve the population size of common farmland bumblebee species

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Abstract

Changes in agricultural practice across Europe and North America have been associated with range contractions and local extinction of bumblebees (*Bombus* spp.). A number of agri-environment schemes have been implemented to halt and reverse these declines, predominantly revolving around the provision of additional forage plants. Although it has been demonstrated that these schemes can attract substantial numbers of foraging bumblebees, it remains unclear to what extent they actually increase bumblebee populations. We used standardized transect walks and molecular techniques to compare the size of bumblebee populations between Higher Level Stewardship (HLS) farms implementing pollinator-friendly schemes and Entry Level Stewardship (ELS) control farms. Bumblebee abundance on the transect walks was significantly higher on HLS farms than ELS farms. Molecular analysis suggested maximum foraging ranges of 566 m for *Bombus hortorum*, 714 m for *B. lapidarius*, 363 m for *B. pascuorum* and 799 m for *B. terrestris*. Substantial differences in maximum foraging range were found within bumblebee species between farm types. Accounting for foraging range differences, *B. hortorum* (47 vs 13 nests/km^2) and *B. lapidarius* (45 vs 22 nests/km^2) were found to nest at significantly greater densities on HLS farms than ELS farms. There were no significant differences between farm type for *B. terrestris* (88 vs 38 nests/km^2) and *B. pascuorum* (32 vs 39 nests/km^2). Across all bumblebee species, HLS management had a significantly positive effect on bumblebee nest density. These results show that targeted agri-environment schemes that increase the availability of suitable forage can significantly increase the size of wild bumblebee populations.

Keywords: agri-environment schemes, *Bombus*, foraging range, nest density, pollinator conservation

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Introduction

Since the Second World War, many bumblebee species have suffered severe range contractions and local extinctions across Europe and North America (Kosior *et al.* 2007; Grixti *et al.* 2009; Williams & Osborne 2009). This decline has been particularly severe in arable areas, mirroring a wider decline in farmland biodiversity in this time period (Robinson & Sutherland 2002).

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The speed of this decline has been quite striking, with species considered widespread in Free & Butler (1959) described as rarities in Alford (1975).

The use of Fabaceae pollen by bumblebees and the decline in Fabaceae across the wider countryside has received a great deal of attention in recent years (Rasmont & Mersch 1988; Carvell *et al.* 2006a). Bumblebees that have declined most seriously tend to be medium- to long-tongued species that have more restricted diets and collect a greater proportion of their pollen from Fabaceae (Goulson *et al.* 2005). Analysis of pollen from museum specimens shows that in the 1950s, bumblebee

species that subsequently declined collected pollen from almost one-third fewer plant species than bumblebee species that have remained stable (Kleijn & Raemakers 2008). More generally, the loss of appropriate pollen host plants has been implicated as a key driver behind the loss of wild bee diversity (Biesmeijer *et al.* 2006; Scheper *et al.* 2014). In addition, it has been argued that certain species are inherently more susceptible to intensification as a result of their climatic and habitat specialization. Aspects of their life history such as emergence period have also been implicated, with late emerging species more vulnerable to a shortage of summer forage compared with better established spring emerging species (Williams *et al.* 2009). Consequently, to counteract these declines, the creation of Fabaceae-rich habitats has become an important part of the design and assessment of agri-environment schemes for bumblebees (Edwards 2003; Carvell *et al.* 2007).

Across the European Union, agri-environment schemes are now funded as part of the Common Agricultural Policy. In England, two tiers of environmental stewardship operated between 2005 and 2014. Entry Level Stewardship (ELS, Defra 2005a) was open to all farmers. Higher Level Stewardship (HLS, Defra 2005b) provided greater financial rewards for more substantial and rigorous agri-environmental schemes, often in targeted high-priority areas. There are three main pollinator-focused HLS options. HF4 pollen and nectar mixes are rotational plots or strips sown with a mixture of predominantly leguminous plants such as *Trifolium pratense* L. and *Trifolium hybridum* L., which tend to be re-sown within a 5-year period. HE10 floristically enhanced grass buffer strips are nonrotational grassland alongside fields and are composed of a mixture of grasses and plants such as *Centaurea nigra* L. and *Lotus corniculatus* L. HK6/7/8 focus on the maintenance, restoration and creation of species rich grassland. These schemes are aimed at increasing the availability of forage in farmed environments to support populations of bumblebees and other pollinators. A number of studies have assessed how well flower-rich agri-environment schemes establish and provide resources throughout the year, specifically for bumblebees (Carvell *et al.* 2006b, 2007; Pywell *et al.* 2006), but little work has been done to establish if these schemes are having a population level impact and are actually increasing the size of bumblebee populations, rather than simply redistributing existing foragers in the landscape (Holland *et al.* 2015).

As eusocial insects, bumblebees form colonies, and consequently, bumblebee effective population size is the number of colonies in an area. Nesting densities of wild bumblebees are poorly understood and direct surveys are infrequent and labour intensive (Fussell & Corbet 1992; Osborne *et al.* 2008a; O'Connor *et al.* 2012). Moreover, previous studies that have assessed how agri-environment schemes have affected pollinators predominantly use counts of foraging workers when ideally we need to know how the schemes affect the abundance of bumblebee colonies. Bumblebee colonies are founded by a single queen, and most species are monandrous (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000; Hughes *et al.* 2008). The resulting high relatedness between workers makes social bumblebees amenable to identification of sisters using molecular techniques, such as microsatellite sequencing. These techniques have been used to quantify foraging distance and nesting density (Darvill *et al.* 2004; Knight *et al.* 2005; Carvell *et al.* 2012), conservation genetics and population structuring (Ellis *et al.* 2006; Charman *et al.* 2010) and dispersal distances of reproductive castes (Kraus *et al.* 2009; Lepais *et al.* 2010). Moreover, the development of molecular capture–recapture models (e.g. Miller *et al.* 2005) has enabled estimation of the number of bumblebee colonies present in an area, allowing assessment of nest mortality rates (Goulson *et al.* 2010). These studies have provided a novel understanding of bumblebee ecology that could not have been achieved with conventional techniques.

The aim of this study was to evaluate the effectiveness of pollinator-targeted HLS schemes in boosting bumblebee populations by comparing farms with and without such HLS schemes using a combination of molecular techniques and direct surveys. The molecular studies of nest density and foraging range focus on the four most common bumblebee species on farmland in southern England: *Bombus hortorum*, *Bombus lapidarius*, *Bombus pascuorum* and *Bombus terrestris*. All species are members of the 'big six' bumblebee species that remain common in the UK, being found in almost every habitat (Goulson *et al.* 2006). They differ markedly in tongue length and hence the flowers that they visit. *B. lapidarius* and *B. terrestris* are ubiquitous short-tongued bumblebees and *B. pascuorum* has a medium-length tongue. *B. hortorum* has a much longer tongue than the other members of this group, of around 13 mm compared to 5–8 mm (Goulson *et al.* 2008a). Both *B. pascuorum* and *B. hortorum* have remained relatively common whilst most long- and medium-tongued bumblebees have declined markedly in western Europe in the last 60 years (Goulson *et al.* 2005; Kosior *et al.* 2007).

The relative success of these four contrasting bumblebees will therefore provide greater insight into the suitability of farm management for other bumblebee species and potentially other pollinators. In addition to the molecular studies of the nesting density of these four species, we investigate more broadly how the provision of additional forage for insect pollinators has

affected populations of all bumblebee species using standard field surveys. Our results are examined in relationship to management type and floral availability and have clear implications for the management of pollinator populations on agricultural land.

Materials and methods

Study area

Nine ELS and nine HLS farms were selected in north Hampshire and West Sussex, UK (Fig. 1). On average, HLS farms covered 256.22 ± 37.80 ha, significantly more than ELS farms which covered 156.67 ± 22.07 ha (*t*-test, $t_{16} = 2.565$, $P = 0.021$). Selected HLS farms had been implementing an average of 5.56 ± 0.13 ha of pollinator-focused flower-rich options representing $2.17 \pm 0.05\%$ of the farm area for a minimum of 3 years. Almost all farms in lowland England implement some form of agri-environment scheme, and consequently, basic ELS farms were selected as the control group. ELS farms were not implementing any pollinator-focused schemes. The centre points of the two closest farms were 5.4 km apart. At this distance, it is unlikely that bumblebees will be able to regularly travel between farms on foraging trips (see Knight *et al.* 2005; Osborne *et al.* 2008b). There was no pairing of farms. Farms were predominantly arable or mixed arable/

dairy with wheat, barley, oilseed rape and permanent/silage grassland as the major crops.

Line transect counts

A standardized 3 km line transect was designed for each farm. Transects on HLS farms were designed to pass through as many areas of flower-rich options as possible. Bumblebee activity was recorded along each transect following standard bee walk methodology (see Carvell *et al.* 2004). All bumblebees within 2 m of the recorder were recorded to species and caste level. The first flowering species visited and the purpose of the visit, for either pollen or nectar, was recorded. Bumblebees collect pollen by loading it onto specially adapted grooves on their hind legs. Many foraging bumblebees will visit a flower for nectar with pollen collected from different plant species, so only bees actively grooming pollen onto their hind legs were recorded as collecting pollen. For each transect, the number of flowering units of each plant species within 2 m of the recorder was estimated. This assessment followed Carvell *et al.* (2007) with one flower cluster (e.g. an umbel, a head, a capitulum) counted as a single unit.

Sixteen farms (8 HLS, 8 ELS) were surveyed in 2013. Transects were walked three times throughout the year, between 25th May and 5th June, 26th June and 15th July, and 3rd and 11th August. Seventeen farms (8
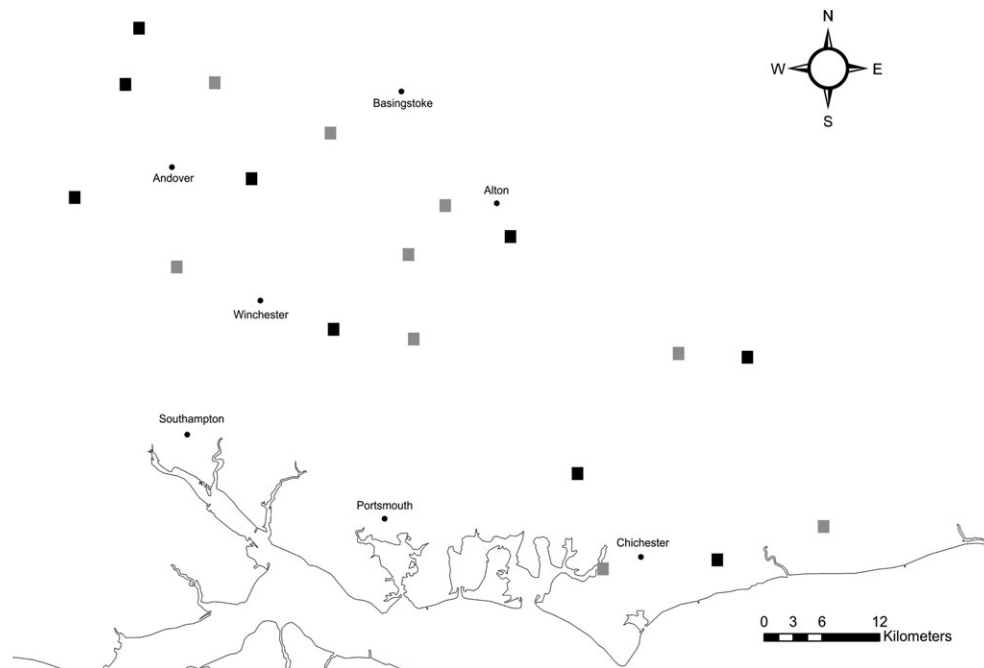

Fig. 1 Map of the study area showing Higher Level Stewardship farms (black squares) and Entry Level Stewardship Farms (grey squares).

HLS, 9 ELS) were surveyed in 2014. Transects were walked three times throughout the year, between 17th and 27th May, 21st June and 9th July and 3rd and 15th August. Each of these six sampling periods is henceforth referred to as sampling rounds. This sampling followed the UK butterfly Monitoring Scheme guidelines (Pollard & Yates 1993) with all surveys conducted between 0930 and 1700 hours when the temperature was above 13 °C with at least 60% clear sky, or above 17 °C with any sky conditions. Observation time was randomized between farms. No surveys were conducted when it was raining.

Genetic sample collection

Genetic samples were collected at the end of June and beginning of July as colony size should be approaching its peak at this time before the production of reproductive castes. Farms were sampled under the same climatic conditions as for the transects. *B. hortorum* and *B. terrestris* samples were collected from 8 HLS and 8 ELS farms between 26th June and 15th July 2013. HLS and ELS farms were visited on alternating days. *B. lapidarius* and *B. pascuorum* samples were collected from 8 HLS and 9 ELS farms between 21st June and 9th July 2014. From the centre of each farm, an area of a circle with radius 800 m was searched for bumblebee workers on 1 day for 6 h. The circle was divided into quarters, and each part was searched for 90 min to maximize the detection of unique colonies present on each farm. The total area covered the majority of each farm and all flower-rich options present. Nonlethal 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. A GPS reading accurate to the nearest few metres was taken at the location of each sample. If the sampled bee had been visiting flowers, the first flowering species the bee had been seen to visit and the purpose of the visit, for either pollen or nectar, was recorded for *B. lapidarius* and *B. pascuorum*, but not for *B. hortorum* and *B. terrestris*.

Molecular methods

DNA was extracted from the tarsal sample using the HotSHOT protocol (Truett *et al.* 2000) and amplified at nine polymorphic microsatellite loci (Estoup *et al.* 1995, 1996). All species used the same nine markers (*B100*, *B118*, *B132*, *B10*, *B11*, *B96*, *B119*, *B124*, *B126*), with the exception of *B100* replaced with *B121* for *B. pascuorum* and *B119* replaced with *B131* in *B. pascuorum* and *B. lapidarius*. Polymerase chain reactions (PCRs) were carried out on samples using the QIAGEN Multiplex PCR kit. Multiplex PCRs were run for combinations of the loci *B100*(VIC)/*B121*(VIC)-*B118*(NED)-*B132*(FAM), *B10*(VIC)-*B11*(NED)-*B96*(FAM) and *B119*(FAM)/*B131*(FAM)-*B124*(NED)-*B126*(PET) (fluorescent markers indicated in parenthesis). *B119* amplified weakly in *B. hortorum* and was removed from further analysis. PCRs 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 visualized 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).

Identification of unique colonies

COLONY v2.0.5.0 (Jones & Wang 2009; Wang 2013) was used to assign workers to unique colonies within each farm. This program uses maximum-likelihood methods to assign sibship or parent–offspring relationships and has been found to be the most reliable method available for assigning sibship in bumblebees (Lepais *et al.* 2010) and has been used extensively for this purpose (Charman *et al.* 2010; Carvell *et al.* 2012). The analysis was run globally with all farms included. Genotyping error was accounted for in the analysis by setting the error rate at 2% (allele dropout 0.5% and other errors 1.5%), rates typical for bumblebee genotyping (Goulson *et al.* 2010; Lepais *et al.* 2010). This procedure may regroup a small fraction of unrelated individuals into a common colony (Type I errors, Lepais *et al.* 2010). Type I errors for this number of loci with an average of 10 alleles per loci are expected to occur with a frequency of <0.005 (Wang 2013), which would lead to less than one worker from each species being falsely grouped. Global analysis resulted in a number of sister pairs identified between farms at distances of over 6 km (see Results), and these are highly likely to be spurious. The proportion of false sister pairs was 0.0207 for *B. hortorum*, 0.0181 for *B. lapidarius*, 0.0334 for *B. pascuorum* and 0.0202 for *B. terrestris*. These false-positive rates were used for the calculation of species-specific foraging range estimates. Given that any falsely accepted sisterhoods within farms would be randomly distributed, this was not considered to have a significant effect on the estimation of the number of unique colonies. Where markers departed from Hardy–Weinberg equilibrium (HWE) or were in linkage disequilibrium, they were individually removed and a separate analysis

conducted to determine their impact on sister pair identification.

Genetic parameters

Due to high relatedness between sister pairs, only one worker per colony was randomly selected for the following analyses. GENEPOP v4.2 (Rousset 2008) was used to calculate *F*-statistics, to test for genotypic linkage disequilibrium between pairs of loci, and the HWE of each locus on each farm. Bonferroni corrections for multiple tests were used to minimize type I errors (Rice 1989).

Estimation of number of unique colonies

DNA-based capture–recapture models allow for multiple sampling of an individual, and the frequency distribution of the number of times each individual is sampled can be used to estimate the population size. Instead of trying to estimate the number of individuals, we are interested in estimating the number of nests represented by our sample of workers. The program CAP-WIRE (Miller *et al.* 2005) can be used to obtain point estimates of the number of bumblebee colonies present at a farm from a subsample of workers (Goulson *et al.* 2010; Stanley *et al.* 2013). CAPWIRE uses two different estimation methods, an Even Capture Model (ECM) that assumes equal chance of sampling workers from the same colony and a Two Innate Rate Model (TIRM) that assumes unequal sampling chance. Heterogeneity of capture probability is a characteristic of bumblebee nests, so the TIRM model was chosen as it probably gives more accurate estimates of the number of nests present at a site (Goulson *et al.* 2010). Capwire models were run in 0.1 increments with capturability ratios of minimum 1; maximum 20; 95% confidence intervals for the estimate on population size based on 1000 bootstrap replicates; and a largest population size of 2000 for dimensioning.

Estimation of foraging range and nesting density

A foraging range estimate was calculated for each species on each farm type from an analysis of the distribution of identified sisters. GPS-marked sisters were plotted in Google Earth (Google Inc.), and the distance between them was measured. Sister pairs were ranked by distance of separation and plotted as a cumulative proportion of total sister pairs. A logarithmic line of best fit as measured by the highest adjusted R^2 was then plotted using the formula $y = a(\ln(x+b))+c$ and was used for foraging range calculations (Knight *et al.* 2005). The true separation distance was calculated for the point at which at which the proportion of sister pairs

falls to the false-positive rate as calculated for each species (see above). The resulting value was then halved to give an estimated maximum foraging range for each species, with the most conservative assumption being that two detected sisters were both foraging equidistant from their nest but in opposite directions. It is highly likely that bumblebees will forage over greater distances than this estimate, but the chances of detecting sister pairs at great distances becomes remote and hence, this estimate is best referred to as a minimum estimate of the maximum foraging distance (see Knight *et al.* 2005).

Whilst the area searched on each farm was a circle with radius 800 m, bumblebees are mobile organisms and the true area sampled is a function of the foraging range of each bumblebee species. A bumblebee sampled on the edge of the 800 m sampling area may have flown there from many hundreds of metres away. Consequently, estimates of nesting density were obtained by dividing the molecular estimate of the number of colonies present by the area of a circle of radius 800 m plus the estimated foraging range of the respective species on each farm type to more accurately reflect the size of the sampled area.

Data analysis

The effect of environmental stewardship on total bumblebee abundance and the abundance of the six most common bumblebee species was tested using generalized linear mixed-effect models (GLMMs) with management type as a fixed factor. Each sampling round, nested within sampling year, was included in the models as a random factor to account for the temporal data structure. Models were fitted with a negative binomial error structure. Differences in the number of genetic samples collected and the estimated nest density between farm types for each sampled bumblebee species were compared using Student *t*-tests. The effect of management type on estimated bumblebee nest density was tested in a GLMM as a fixed factor with bumblebee species included as a random factor. Models were fitted with a negative binomial error structure. The proportion of pollen visits to plants sown as part of HLS management and members of Fabaceae was calculated for *B. hortorum*, *B. lapidarius*, *B. pascuorum* and *B. terrestris* for all farms over the 2-year sampling period. For each bumblebee species, proportions were only calculated for farms with a minimum of five recorded pollen visits. Differences were tested in a GLMM with bumblebee species included as a random factor. Models were fitted with a binomial error structure.

All GLMMs were tested for over dispersion to ensure appropriate error structure selection. No models were

significantly over dispersed. Final models were compared by ANOVA with a null model containing the same random factors to test for significance. All data analyses were conducted in R version 3.1.1 (R Development Core Team) using the LME4 package for the GLMMs (Bates *et al.* 2014).

Results

Transect counts

A total of 9436 bumblebees of 15 species were recorded. The 15 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*, *B. ruderarius* and *B. ruderatus*. Five cuckoo bumblebee species (*Psithyrus*) were also recorded comprising *B. vestalis*, *B. barbutellus*, *B. rupestris*, *B. sylvestris* and *B. campestris*.

Significantly greater numbers of bumblebees were recorded on HLS farms over the 2-year sampling period (χ^2=8.5, $P = 0.004$). Of the six most abundant species, *B. lapidarius* (χ^2=11.5, $P < 0.001$), *B. pascuorum* (χ^2=8.4, $P = 0.004$) and *B. terrestris* (χ^2=8.3, $P = 0.004$) were significantly more abundant on HLS farms. *B. hortorum* (χ^2=0.3, $P = 0.589$), *B. lucorum* (χ^2=2.1, $P = 0.145$) and *B. pratorum* (χ^2=0.2, $P = 0.654$) showed no difference in abundance between farm types.

Genetic samples

A total of 386 *B. hortorum* (24.00 ± 3.69 samples per farm), 771 *B. lapidarius* (45.36 ± 7.02), 598 *B. pascuorum* (35.18 ± 4.44) and 593 *B. terrestris* workers (37.06 ± 4.45) were collected. There was no significant difference in the number of samples collected between farm types for *B. hortorum* (HLS 24.00 ± 4.83 against ELS 24.00 ± 5.93, $t_{10} = 0.118$, $P = 0.908$), *B. pascuorum* (32.75 ± 4.77 against 37.33 ± 7.44, $t_{15} = 0.183$, $P = 0.857$) and *B. terrestris* (36.00 ± 6.34 against 38.13 ± 6.65, $t_{10} = 0.057$, $P = 0.956$). Significantly more *B. lapidarius* samples were collected on HLS farms than ELS farms (72.75 ± 3.80 against 21.00 ± 4.27, $t_{8.071} = 5.953$, $P < 0.001$). Allelic diversity was high with an average of 18.88 ± 2.55 alleles per locus in *B. hortorum*, 9.56 ± 0.85 alleles per locus in *B. lapidarius*, 12.44 ± 2.29 alleles per locus in *B. pascuorum* and 14.67 ± 2.02 alleles per locus in *B. terrestris*.

At one farm, only one individual *B. hortorum* sample was taken, which was excluded from the following assessments. For *B. hortorum* (364 unique colonies), departures from Hardy–Weinberg were significant after Bonferroni correction in a total of 16 of 120 tests. Marker B118 showed departure at 10 of 15 farms. Signifi-

cant linkage disequilibrium was found for B100 & B126, B118 & B132, B126 & B132 and B96 & B126. For *B. lapidarius* (664 unique colonies), departure from Hardy–Weinberg was significant after Bonferroni correction in a total of 3 of 144 tests. No significant linkage disequilibrium was found. For *B. pascuorum* (488 unique colonies), departure from Hardy–Weinberg was significant after Bonferroni correction in a total of 6 of 153 tests. Significant linkage disequilibrium was found for B96 & B126 and B96 & B132. For *B. terrestris* (580 unique colonies), departure from Hardy–Weinberg was significant after Bonferroni correction in a total of 7 of 144 tests. Significant evidence of linkage disequilibrium was found for B124 & B126.

The inbreeding coefficient was low with F_{is}=0.0969 ± 0.0387 for *B. hortorum*, F_{is}=0.0091 ± 0.0113 for *B. lapidarius*, F_{is}=0.0468 ± 0.0171 for *B. pascuorum* and F_{is}=0.0948 ± 0.0139 for *B. terrestris*. Overall genetic differentiation between farms was very low with F_{st}=0.001 ± 0.001 for *B. hortorum*, F_{st}=0.001 ± 0.0001 for *B. lapidarius*, F_{st}=0.001 ± 0.001 for *B. pascuorum* and F_{st}=0.003 ± 0.001 for *B. terrestris*.

Identification of unique colonies

Eighty-five *B. hortorum* sister pairs ($n = 386$, 8 loci), 185 *B. lapidarius* sister pairs ($n = 771$, 9 loci), 193 *B. pascuorum* sister pairs ($n = 598$, 9 loci) and 58 *B. terrestris* sister pairs ($n = 593$, 9 loci) were identified. No 'noncircular' nests where sister A and sister B were both related to sister C, but not to each other, were identified. Global analysis identified a number of sister pairs between farms at distances over 6 km (8 pairs for *B. hortorum*, 14 pairs for *B. lapidarius*, 20 pairs for *B. pascuorum* and 12 pairs for *B. terrestris*). At these distances, such sister pairs are highly likely to be spurious. Further analysis with the removal of markers that departed from HWE and were in linkage disequilibrium identified some additional sister pairs between farms at large distance (over 10 km), and these are also highly likely to be spurious. No existing sister pairs within farms were discarded. The use of these linked markers does not appear to be affecting the identification of sisters within farms. Of the 16 farms studied in 2013, 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). Of the 17 farms studied in 2014, no sister pairs were found for *B. lapidarius* on one farm. Consequently, no estimate of undetected nests could be made for these farms, leaving a total of 12 farms (6 HLS, 6 ELS) for *B. hortorum* and *B. terrestris* and 16 farms (8 HLS, 8 ELS) for *B. lapidarius* for which we can estimate nest density.

Point estimates from Capwire of the number of colonies present on each farm ranged from 64 to 412 for

B. hortorum, 9 to 579 for *B. lapidarius*, 30 to 329 for *B. pascuorum* and 92 to 1,780 for *B. terrestris*. An average of 193 ± 63 nests of *B. hortorum*, 114 ± 37 nests of *B. lapidarius*, 152 ± 35 nests of *B. pascuorum* and 482 ± 185 nests of *B. terrestris* was detected on ELS farms. An average of 173 ± 36 nests of *B. hortorum*, 336 ± 55 nests of *B. lapidarius*, 140 ± 30 nests of *B. pascuorum* and 630 ± 236 nests of *B. terrestris* was detected on ELS farms.

Estimation of foraging range and nesting density

Foraging range estimates showed considerable differences between bumblebee species. *B. hortorum* was estimated to forage up to 566 m (Fig. 2a), *B. lapidarius* up to 714 m (Fig. 2b), *B. pascuorum* up to 363 m (Fig. 2c) and *B. terrestris* up to 799 m (Fig. 2d). Large differences in foraging range estimates were found between farm types, although the direction of change differed between species. On ELS farms, *B. hortorum* was estimated to forage up to 1415 m (Fig. 3a), *B. lapidarius* up to 484 m (Fig. 3b), *B. pascuorum* up to 313 m (Fig. 3c) and *B. terrestris* up to 1196 m (Fig. 3d). On HLS farms, *B. hortorum* was estimated to forage up to 282 m, *B. lapidarius* up to 746 m, *B. pascuorum* up to 377 m and *B. terrestris* up to 709 m.

Using these foraging range estimates, significant differences in bumblebee nest density were found between farm types (Fig. 4). Both *B. hortorum* and *B. lapidarius* were found at significantly greater nesting density on HLS farms than ELS farms ($t_{10} = 4.014$, $P = 0.002$ and $t_{13.983} = 2.232$, $P = 0.043$, respectively). There were no differences in the nesting density of *B. terrestris* or *B. pascuorum* between farm types ($t_{10} = 1.907$, $P = 0.0885$ and $t_{15} = 0.323$, $P = 0.751$, respectively). Overall, HLS farms had a significantly higher estimated bumblebee nesting density across all four species ($\chi^2 = 7.7$, $P = 0.006$).

Bumblebee foraging preferences

A total of approximately 9.69 million flowering units of 237 insect-pollinated flowering plant species were recorded on the transects. A total of 9288 foraging trips to 110 species were recorded by bumblebees with 102 species visited for nectar ($n = 7823$) and 47 species visited for pollen ($n = 1465$). *Centaurea nigra* was the most popular choice for nectar, accounting for 51% of visits. *Lotus corniculatus* was the most popular choice for pollen, accounting for 19% of visits. Nectar collection was dominated by visits to Asteraceae, whilst pollen collection was equally dominated by visits to Fabaceae (Table 1). Including pollen visitation data from the
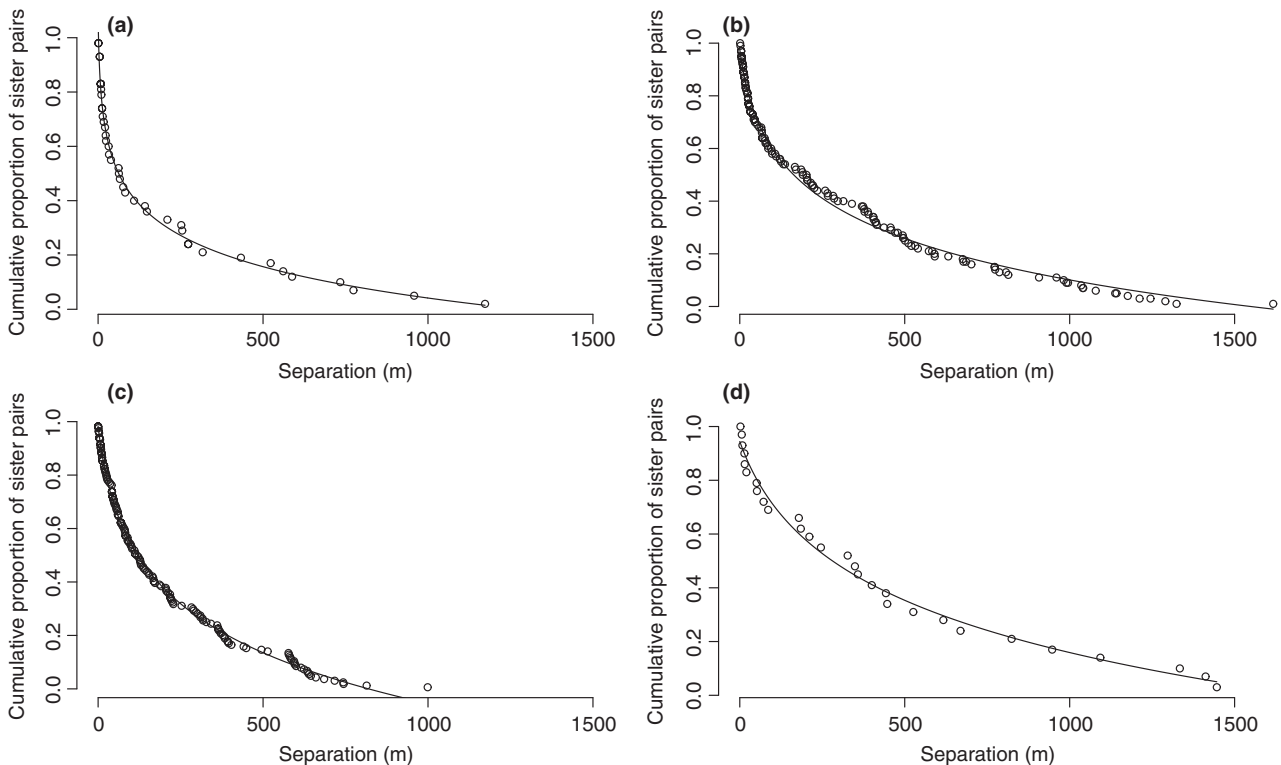

Fig. 2 Separation distance of sister pairs for a) *Bombus hortorum*, b) *Bombus lapidarius*, c) *Bombus pascuorum* and d) *Bombus terrestris* across all farm types.
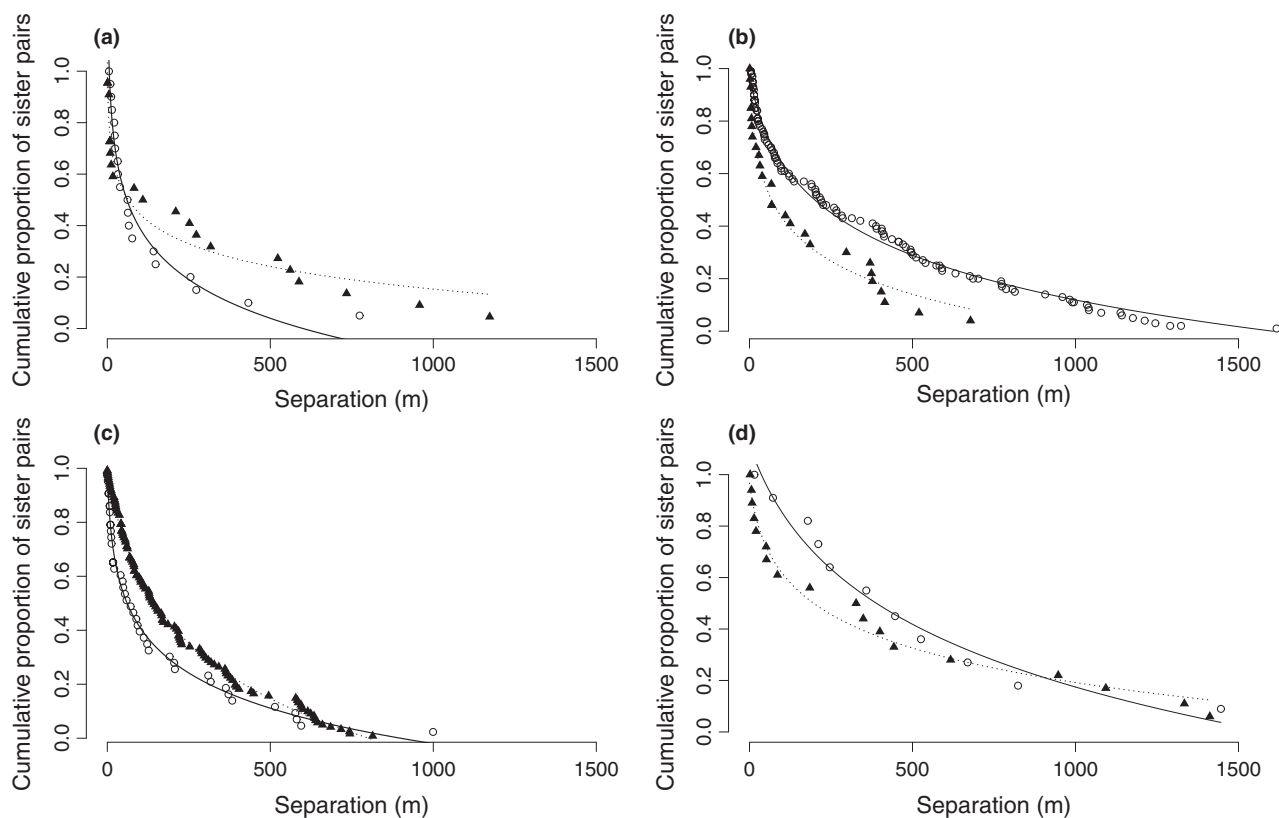
Fig. 3 Separation distance of sister pairs for a) *Bombus hortorum*, b) *Bombus lapidarius*, c) *Bombus pascuorum* and d) *Bombus terrestris* for Higher Level Stewardship farms (white circles with line of best fit) and Entry Level Stewardship farms (black triangles with dotted line of best fit).
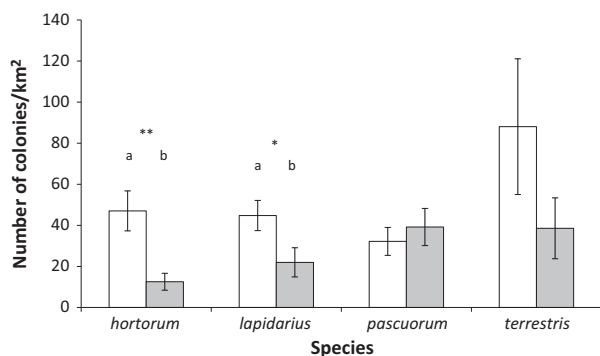

Fig. 4 Differences in the nesting density of four bumblebee species between Higher Level Stewardship farms (white bars) and Entry Level Stewardship farms (grey bars). Errors bars are ±1 standard error of the mean. Different letters above columns indicate farm types which differed significantly in a sampling round. *$P < 0.05$; **$P < 0.01$.

Table 1 Percentage of visits for pollen and nectar recorded by bumblebees on the transects. The top ten most popular flowering plant families for each group over the sampling period are shown

Family	Pollen (%)	Nectar (%)
Apiaceae	3.21	0.82
Asteraceae	2.12	66.33
Boraginaceae	0.68	3.13
Brassicaceae	0.89	—
Dipsacaceae	—	3.32
Fabaceae	60.27	12.58
Lamiaceae	15.09	7.27
Rosaceae	4.44	2.05
Orobanchaceae	11.13	1.05
Scrophulariaceae	—	1.60
Ranunculaceae	0.41	0.63
Resedaceae	1.09	—
Total	99.32	98.77

genetic sample collection during 2014 ($n = 898$), substantial differences in pollen preferences were found between the four most abundant bumblebee species across farm types (Table 2). All four species collected a significantly higher proportion of their pollen from

plants sown as part of HLS management on HLS farms compared with ELS farms (χ^2=11.8, $P < 0.001$). The proportion of pollen collected from Fabaceae was also significantly higher on HLS farms, increasing by an

Table 2 Percentage of pollen collected from different flowering plant species on different farm types by the four most abundant bumblebee species. Plant species highlighted in bold are commonly sown as part of pollinator-friendly HLS management and are also found growing wild on all farm types

Entry Level Stewardship farms

Flowering plant species (n = 8)	Bombus hortorum (visits n = 106)	Flowering plant species (n = 13)	Bombus lapidarius (visits n = 150)	Flowering plant species (n = 15)	Bombus pascuorum (visits n = 393)	Flowering plant species (n = 15)	Bombus terrestris (visits n = 78)
Lamium album	39.62	Trifolium repens	48.00	Stachys sylvatica	38.42	Rubus fruticosus agg.	23.08
Trifolium pratense	**30.19**	**Lotus corniculatus**	**11.33**	Odontites verna	20.10	Odontites verna	16.67
Stachys sylvatica	25.47	Rubus fruticosus agg.	10.67	Trifolium repens	17.56	Heracleum sphondylium	10.26
Other (<2%)	4.72	Reseda lutea	6.00	**Trifolium pratense**	**10.69**	Trifolium repens	10.26
		Trifolium hybridum	**5.33**	Lamium album	3.05	Rosa arvensis	7.69
		Brassica napus	4.67	**Lotus corniculatus**	**3.05**	Brassica napus	6.41
		Centaurea nigra	**4.00**	Ballota nigra	2.04	Lamium album	5.13
		Odontites verna	4.00	Rubus fruticosus agg.	2.04	Bryonia dioica	3.85
		Trifolium pratense	**3.33**	Other (<2%)	3.05	Plantago media	3.85
		Other (<2%)	2.67			Barbarea vulgaris	2.56
						Chaerophyllum temulum	2.56
						Pulicaria dysenterica	2.56
						Rhinanthus minor	2.56
						Other (<2%)	2.56
Total sown	30.19	Total sown	24.67	Total sown	15.01	Total sown	0.00
Total Fabaceae	30.19	Total Fabaceae	68.00	Total Fabaceae	33.59	Total Fabaceae	10.26

Higher Level Stewardship farms

Flowering plant species (n = 19)	Bombus hortorum (visits n = 93)	Flowering plant species (n = 23)	Bombus lapidarius (visits n = 936)	Flowering plant species (n = 23)	Bombus pascuorum (visits n = 438)	Flowering plant species (n = 14)	Bombus terrestris (visits n = 96)
Trifolium pratense	**49.46**	**Lotus corniculatus**	**39.64**	**Trifolium pratense**	**36.30**	Heracleum sphondylium	26.04
Stachys sylvatica	25.81	**Trifolium hybridum**	**33.33**	Stachys sylvatica	22.83	Trifolium repens	18.75
Clinopodium vulgare	7.53	**Trifolium repens**	**11.43**	**Lotus corniculatus**	**9.59**	**Trifolium hybridum**	**11.46**
Vicia sepium	6.45	**Melilotus officinalis**	**5.13**	Odontites verna	5.94	Rubus fruticosus agg.	10.42
Rosa arvensis	4.30	**Centaurea nigra**	**2.35**	**Trifolium hybridum**	**3.88**	**Lotus corniculatus**	**7.29**
Lamium album	2.15	Odontites verna	2.35	Trifolium repens	3.88	**Phacelia tanacetifolia**	**7.29**
Vicia sativa	**2.15**	**Trifolium pratense**	**2.24**	Lamium album	3.42	Odontites verna	6.25
Other (<2%)	2.15	Other (<2%)	3.53	**Vicia cracca**	2.51	Reseda lutea	4.17
				Medicago sativa	**2.28**	Genista tinctoria	3.13
				Other (<2%)	9.36	Other (<2%)	5.21
Total sown	51.61	Total sown	94.76	Total sown	58.68	Total sown	27.08
Total Fabaceae	58.06	Total Fabaceae	93.38	Total Fabaceae	64.61	Total Fabaceae	38.54

All farm types

	Bombus hortorum	Bombus lapidarius	Bombus pascuorum	Bombus terrestris
Total sown	41.21	75.32	36.58	16.09
Total Fabaceae	43.72	89.87	49.94	26.44

average of 26.9 ± 2.31 percentage points (χ^2=12.2, $P < 0.001$). Across all farm types, *B. lapidarius* visited Fabaceae most extensively, comprising 90% of total pollen visits, whilst still the most popular family, Fabaceae comprised between 26 and 49% of total pollen visits for the other three bumblebee species. *B. lapidarius* also visited plants sown as part of pollinator-friendly HLS management most extensively, with these plants comprising 75% of total pollen visits compared to between 16 and 41% for the other three species.

Discussion

A consistent problem in assessing the response of bumblebees to agri-environment schemes has been that it is unclear whether a high observed abundance of bumblebees was merely an attraction of workers to sown forage patches or a genuine population level increase. Here, we show for the first time that the management of uncropped land under Higher Level Stewardship can significantly increase the size of bumblebee populations, and specifically those of *B. hortorum* and *B. lapidarius*.

Whilst the observed numbers of bumblebee species recorded over the study were high, comprising over half of the extant species in Britain, no sightings were made of *B. sylvarum*, *B. humilis* or *B. muscorum*, and *B. ruderarius* and *B. ruderatus* were only observed in very low numbers. These five medium- to long-tongued bumblebees are the most threatened lowland England species and are most in need of assistance. All except *B. ruderatus* are members of the subgenus Thoracobombus. *Bombus pascuorum* is the final member of the Thoracobombus in Britain and showed no difference in nesting density between farm types, despite using sown plants more extensively than *B. hortorum*. Plants sown as part of HLS provided good bumblebee forage with all four common species favouring them for pollen collection when they were present, and the provision of large quantities of Fabaceae has clearly benefited Fabaceae specialists like *B. lapidarius*. However, this management does not appear to have benefited rarer species, which were formally found more extensively in this part of England.

It has been argued that dietary specialization alone is not sufficient to explain patterns of bumblebee decline. Species that have declined mostly emerge late from hibernation, and it has been suggested that such species may be more susceptible to a loss of food plants during mid- to late colony development (Williams *et al.* 2009). Moreover, it has long been suspected that bumblebees with smaller colonies forage over shorter distances (Free & Butler 1959). Large colonies require more resources and, all else being equal, should have to travel further to collect sufficient food. *Bombus pascuorum*, along with other members of the Thoracobombus, tends to have small nests with around 20–100 workers, whilst *B. lapidarius* and *B. terrestris* nests are larger, with 100–400 workers (Sladen 1912; Alford 1975). *B. hortorum* nests tend to be more similar to *B. pascuorum*, with seldom more than 100 workers produced (Sladen 1912). Studies suggest that Thoracobombus have short foraging ranges, with *B. muscorum* only observed foraging up to 125 m (Walther-Hellwig & Frankl 2000). Species with shorter foraging ranges may be more susceptible to habitat fragmentation because resources are spread out over larger distances in such landscapes. Both *B. muscorum* and *B. sylvarum* show significant population structuring between their remaining habitat patches (Ellis *et al.* 2006; Darvill *et al.* 2006), and such small, isolated populations are unlikely to be viable in the long term, with metapopulation breakdown likely to be behind the extinction of *B. subterraneus* in Britain (Goulson *et al.* 2008b). Providing extra foraging

Table 3 Estimated foraging distances of worker bumblebee species included in this study

Species	Maximum foraging distance (m)	Method	Reference
Bombus hortorum	566	Genetic markers	Present study
B. lapidarius	450	Genetic markers	Knight *et al.* (2005)
	714	Genetic markers	Present study
	1032	Genetic markers	Carvell *et al.* (2012)
	1500	Direct (marked workers)	Walther-Hellwig & Frankl (2000)
B. pascuorum	363	Genetic markers	Present study
	449	Genetic markers	Knight *et al.* (2005)
	990	Genetic markers	Carvell *et al.* (2012)
B. terrestris	631	Direct (radar tracking)	Osborne *et al.* (1999)
	758	Genetic markers	Knight *et al.* (2005)
	799	Genetic markers	Present study
	800	Direct (marked workers)	Wolf & Moritz (2008)
	1500	Direct (marked workers)	Osborne *et al.* (2008b)
	1750	Direct (marked workers)	Walther-Hellwig & Frankl (2000)

resources may be sufficient to benefit those bumblebees with greater foraging ranges, but such options have had low uptake across England (Clothier & Pike 2013), and such forage patches may be too few and too scattered in the landscape to benefit those species unable to cover larger distances. Concentrated efforts on agricultural land surrounding existing populations in semi-natural habitats should therefore be prioritized for these species. Additionally, improving the quality of existing buffer strips and hedgerows, many of which have become dominated by nitrophilous species, would provide a more homogenous supply of suitable forage compared to isolated pollinator-friendly agri-environment schemes.

Numerous studies have measured bumblebee foraging range using direct observations such as marking workers (Walther-Hellwig & Frankl 2000; Wolf & Moritz 2008) and genetic markers (Knight *et al.* 2005; Carvell *et al.* 2012; Table 3). Our suggested foraging ranges for *B. pascuorum* (363 m) and *B. terrestris* (799 m) compare reasonably well with previous studies. The situation is less similar for our estimate for *B. lapidarius* (714 m), though the authors of the previous study note that their estimate of 450 m was likely an underestimate (Knight *et al.* 2005). To the best of our knowledge, these are the first data presented on the foraging range of *B. hortorum* (566 m). Despite considerable variation, probably due to inherent difference in the various techniques used, the same broad trends are found, with bumblebee species with larger colony sizes foraging over greater distances.

It is noteworthy that there were considerable differences in foraging range within species in different landscapes. As highly mobile foragers, bumblebees are capable of making profitable foraging trips over long distances and have the ability to respond to an increase in floral availability at the landscape scale. When seeking to maximize the amount of pollen and nectar brought back to the nest, the most important factor in the final profitability of the trip is the time spent foraging, as whilst travelling longer distances incurs a slightly higher energetic cost, the bigger cost is the lost time that could have been spent foraging for resources (Beutler 1951). Consequently, we would expect bumblebees to favour shorter foraging trips wherever possible (Heinrich 1979). Using molecular markers, Carvell *et al.* (2012) found that the foraging range of both *B. lapidarius* and *B. pascuorum* increased as the availability of semi-natural habitat decreased, increasing the average distance between bumblebee nests and the nearest forage patch and forcing workers to make longer foraging trips. However, in our study, whilst *B. hortorum* and *B. terrestris* foraged over shorter distances on resource-rich HLS farms, *B. lapidarius* actually foraged further on HLS farms than on ELS farms. If highly rewarding forage patches are added to a landscape, the quality of the resources they provide may offset the time cost of travelling to these patches, enabling profitable foraging trips to be made over larger distances. However, it is unclear why different species should respond in opposite ways to the provision of extra foraging resources. Whilst it is difficult to draw wider conclusions with so few studies having assessed this question directly, it may be the case that bumblebees will not always prefer a closer patch of forage to a further patch if the quality of the latter is sufficiently high.

Conclusions

It is not clear how we might define what a desirable bumblebee population density should be, either from a conservation or an ecosystem services provisioning perspective. Are conservation schemes for pollinators aimed at conserving rare species, providing high seed set in commercial crops or ensuring adequate pollination for wild flowers? In identifying key knowledge needs in the conservation of wild pollinators, Dicks *et al.* (2013) highlighted the importance of understanding the relationship between pollinator populations and the ecosystem services they provide. Our work suggests than current HLS conservation measures significantly increase populations of at least two of the bumblebee species studied, and it seems likely that this will boost pollination services for crops and wildflowers. On the other hand, our study farms do not appear to be supporting viable populations of any rare bumblebee species, many of which were formally found in this region. These rarer species require more specific and geographically focused management that takes into account their shorter foraging range and other ecological requirements if they are to persist in agricultural landscapes.

Acknowledgements

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Data accessibility

- Bumblebee abundance on transect walks
- Microsatellite genotypes
- Pollen and nectar use by bumblebees
- Bumblebee genetic sample GPS locations

All data are available from the Dryad database http://dx.doi.org/10.5061/dryad.fr8g7