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Revealing the hidden niches of cryptic bumblebees in Great Britain: Implications for conservation

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ABSTRACT

Bumblebees are ecologically and economically important, and some species have suffered dramatic population declines. The absence of morphological diagnostic characters for the identification of some species creates difficulties for basic ecological studies, and for conservation management. The widespread and commercially exploited bumblebee subgenus *Bombus sensu stricto* contains a cryptic species complex, known as the *lucorum* complex, which in Europe comprises *B. lucorum*, *B. cryptarum* and *B. magnus*. Little is known about these species and much of what has been reported is likely to have suffered from incorrect identification. Although the *lucorum* complex as a whole is common in Great Britain, we aimed to determine whether the populations of the individual species are vulnerable and require conservation action. Using genetic methods to distinguish them, we determined the geographic distribution and abundance of the *lucorum* complex species in Great Britain, and assessed the extent of niche differentiation between these species. We detected major differences in the geographic range, forage use and sensitivity to summer temperatures of the three species. *Bombus lucorum* was found to have the broadest distribution and diet, being present throughout mainland Great Britain, whereas *B. cryptarum* and *B. magnus* were absent from large areas of central and southern England. *Bombus cryptarum* and *B. magnus* were more likely to be found at sites with lower summer temperatures. *Bombus magnus*, the least abundant species, was found to exhibit an unusually tight biotope association with heathland habitat. This has conservation implications for *B. magnus* given the current threats to this habitat type.

1. Introduction

Bumblebees (*Bombus*: Hymenoptera, Apidae) are ecologically and economically important as pollinators (Goulson, 2010; Velthuis and van Doorn, 2006). Some species have recently suffered severe declines and range contractions across much of Western Europe and North America (Cameron et al., 2011; Fitzpatrick et al., 2007a,b; Goulson et al., 2008a; Goulson, 2010; Williams, 1982; Williams and Osborne, 2009). In the UK, seven out of the 27 species are listed as priority species in the UK post-2010 Biodiversity Framework (previously Biodiversity Action Plan), a higher proportion than known for any other invertebrate group (Goulson, 2010). *Bombus* species are also notorious for possessing convergent colour patterns and displaying high intraspecific variation, resulting in cryptic species (Williams, 2007). The inability to correctly identify such species creates difficulties for basic ecological and population genetic studies as well as for their conservation management.

Cryptic species can be defined as two or more distinct species that are similar or identical in morphology (Williams et al., 2012). Speciation is not always accompanied by morphological change, and as a result, the true number of biological species is likely to be greater than the current total of nominal species, most

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of which are delineated on a purely morphological basis (Bickford et al., 2007). The development of molecular genetic tools has enabled the detection of numerous cryptic species. Large genetic distances within traditionally recognised species, usually in combination with morphological, geographical, ecological or behavioural differences, have led to the discovery of cryptic species in a diverse range of organisms, from tropical butterflies (Hebert et al., 2004), to arctic flora (Grundt, 2006), fish (Feulner et al., 2006; Puckridge, 2013) and lemurs (Ravaoarimanana et al., 2004).

Theories on the ecological specialisation of species can be seriously challenged by the existence of cryptic species complexes. Studies of a range of insects have revealed that presumed dietary generalists are in fact complexes of dietary specialists (Hebert et al., 2004; Smith et al., 2007). The occurrence of cryptic species also has important repercussions for conservation; in an area of Southeast Asia with the highest relative rate of deforestation in any tropical region, studies of forest dwelling frogs have revealed at least 14 species within two nominal species. These were both thought to be geographically widespread, but instead represent multiple species with smaller geographic ranges, and therefore greater vulnerability to extinction (Stuart et al., 2006). Such findings illustrate the importance of accurate assessments of diversity and distributions to enable appropriate management and thereby reduce the risk of extinctions of evolutionary lineages. Cryptic species complexes in already endangered nominal species consequently pose more problems for conservation, as species that are already considered endangered may consist of multiple species with smaller distributions. Such cryptic species will be even rarer than the nominal species and may require different conservation strategies (Bickford et al., 2007).

The subgenus *Bombus sensu stricto* is a widespread and commercially exploited taxon of bumblebee, which contains five species in Europe, *B.* (*Bombus*) *cryptarum*, (Fabricius), *B.* (*B.*) *lucorum* (Linnaeus), *B.* (*B.*) *magnus* (Vogt), *B.* (*B.*) *sporadicus* (Nylander), *B.* (*Bombus*) *terrestris* (Linnaeus). The taxonomic status of the last two species is widely accepted but *B. lucorum*, *B. magnus* and *B. cryptarum* are morphologically indistinguishable in much of their range, triggering considerable debate about their status. *B. magnus* and *B. cryptarum* have been regarded as subspecies of *B. lucorum* and are often referred to collectively as the '*lucorum* complex' or simply synonymized to *B. lucorum* (Benton, 2006; Edwards and Jenner, 2005). Recent studies using CO1 barcode analysis show discrete differences between the three species (Carolan et al., 2012; Murray et al., 2008; Williams et al., 2012), in accordance with studies of labial gland secretions (Bertsch et al., 2005). Diagnostic morphological characters have also been previously reported for queens, but some of these have now been demonstrated to overlap considerably, and vary along a continuum, thus making them unreliable and leading to a high potential for misidentification (Carolan et al., 2012).

In Ireland, *B. lucorum* is classified as of Least Concern according to the IUCN Red List criteria. *Bombus cryptarum* and *B. magnus* cannot be assigned to a threat category because they are currently Data Deficient (Fitzpatrick et al., 2006, 2007b). The situation is no clearer in Great Britain, where the distribution of the three taxa is only known for the Western Isles of Scotland (Waters et al., 2010). The difficulty in identifying these species means that little is known about their ecological attributes; much of what can be found in standard texts will actually be referring to data for multiple species and is therefore of limited value. Consequently, the only reliable information we have on the ecology of these three species comes from Murray et al. (2008) and Stanley et al. (2013) who used molecular methods to study the *lucorum* complex in Ireland and Waters et al. (2010) who studied them in the Western Isles of Scotland. Niche-partitioning might be expected between these species (Goulson et al., 2008b) and indeed some ecological differences have been suggested. Specifically, Waters et al. (2010) found that *B. magnus* appeared to be strongly associated with the heathland forage plant *Calluna vulgaris*. These studies suggest that the three taxa are widespread throughout Ireland and the Western Isles of Scotland but have differing patterns of geographic distributions. These studies have suggested some differences in the ecology, abundance and distribution of the three taxa, which, given the ongoing concerns over bumblebee declines, indicates the need for further work to reveal the biology of these species and reassess their conservation status.

The aim of this study was to assess the distribution and abundance of the *lucorum* complex species in Scotland, England and Wales and establish whether the populations of the individual species are vulnerable and require conservation action. Genetic methods were used to distinguish the three species. We then tested for niche differentiation between them by assessing how climatic factors and habitat associations correlate with the distributions of the three species. Further, we assessed foraging behaviour and quantified the differences in diet breadth and forage use between the three species. In particular, we tested the specific hypothesis that *B. magnus* is a heathland specialist, using a paired sampling strategy where heathland and non-heathland sites were sampled at each location.

2. Materials and methods

2.1. Sampling

Queens, workers and males were sampled across Great Britain from June-September during the summers of 2010 and 2011. In July 2010, 13 locations were sampled along a North-South line through the approximate centre of Scotland and England; during June–August 2011, 14 further locations were sampled focussing on the periphery of the UK. The 2011 fieldwork tested the hypothesis that *B. magnus* is a heathland specialist (Murray et al., 2008; Waters et al., 2010) using a paired sampling design: 11 of the 14 locations comprised a pair of sites representing heathland and non-heathland habitats within 15 km of one another. All locations sampled in 2010 consisted of non-heathland habitat, although some were close to heathland. We aimed to catch at least 100 bees at each location, but occasionally this was not possible (mean = 89.4 ± 12.9 SE). For bees caught foraging on a flower (as were most), forage plant identity was recorded. Whole bees were stored in absolute ethanol. Thorax width of all individuals sampled in 2011 was measured using callipers to examine size differences between species.

2.2. Species identification

DNA extraction from the samples collected in 2010 was performed using a Chelex® 100 protocol (Walsch et al., 1991) and from the 2011 samples using a HotShot protocol (Truett et al., 2000). For species identification we followed a PCR-RFLP method based on amplification of the cytochrome oxidase I (COI) gene developed by Murray et al. (2008). The pattern of digested fragments for each individual was compared with the characteristic patterns associated with each of the cryptic species and *B. terrestris* (see Fig. 3 in Murray et al., 2008), in order to determine their species identity.

To confirm RFLP identification; 108 individuals (46 *B. terrestris*, 55 *B. lucorum*, 2 *B. magnus*, 2 *B. cryptarum*, 2 *B. soroeensis*, 1 *B. sylvestris*), collected from all but one of the 2010 sample sites, were amplified using the PCR-RFLP primers. Resulting PCR amplicons were purified (ExoSAP; Werle et al., 1994) and sent for sequencing (DNA Sequencing and Services, Dundee, UK). Consensus sequences were aligned (Geneious v 6.1.7) then checked against the RFLP

banding pattern. For those samples that did not exhibit a clear RFLP banding pattern after two amplifications (174 of 2415), we used microsatellite data for species assignment (obtained from a separate study comparing population structure of the three species, Scriven et al. unpublished data). In brief, individuals were genotyped at 13 microsatellite loci (Tables S1 and 2). Structure v 2.3.4 (Pritchard et al., 2000) was used to cluster the samples according to species. The USEPOPINFO model was applied to define "learning samples" that are pre-defined as coming from particular clusters (the known species from RFLP analysis) to assist ancestry estimation for the remaining individuals of unknown origin. The Admixture and Independent Allele Frequency models were also used and the software was run with four clusters (K, for the three *lucorum* complex species and *B. terrestris* using 50 000 burn-in periods followed by 100 000 MCMC repetitions).

2.3. Analyses

Differences in habitat use and forage use between the three bumblebee species were examined using χ^2 tests of association on data pooled across all sites in contingency tables. For habitat use, data from all castes were included; for forage use, only data from queens and workers were used. Males often rest upon flowers when not foraging or searching for queens (Alford, 1975), so they were not included in the analysis of forage use. Diet breadth was calculated and compared between bumblebee species using rarefaction: 100 samples were randomly drawn from those recorded for each species, without replacement, and the number of forage plants represented in this subsample recorded; 100 replicates were performed per species to estimate the mean number of plant species each bee species would be expected to visit in the specified number of flower visits.

Other analyses were carried out using R version 3.0.2 (R Core Team, 2014). Generalised linear models with a binary error distribution were used to investigate the biogeographical and climatic correlates (UK Meteorological Office, 2014) of *lucorum* complex species presence at sites. The response variable was the presence or absence of a species at a site. Explanatory variables tested were habitat type (heathland or non-heathland), mean maximum daily temperature from March to August (the approximate flight period of these species), elevation (m) and all two-way interactions. Associations with average rainfall and the number of days of ground frost from March to August were also investigated; however, they were negatively correlated with mean maximum temperature ($r = -0.55$ and -0.57 respectively). These correlations meant we could not adequately distinguish their effects, hence rainfall and frost were dropped from analyses because mean temperature has greater explanatory power (at least 2 AIC points). These variables were chosen because previous studies have shown them to influence bumblebee species distributions (Goulson, 2010; Lye et al., 2010; Williams et al., 2007). The preference of each species for the ericaceous plants *Calluna vulgaris* or *Erica* spp. was examined using linear mixed effects models with individual bee as the unit of replication, and whether the bee was recorded on a *Calluna vulgaris* or *Erica* spp. flower or not as the binary response. Linear mixed-effect models were fit with lmer in the lme4 package (ver. 1.0-5; Bates et al., 2013) in R. The fixed effects investigated the influence of the species that an individual bee belonged to and the habitat type in which it was found, with location as a random effect. The most parsimonious combination of fixed effects was determined using maximum likelihood (ML) rather than restricted maximum likelihood (REML). Optimal models were selected to minimise AICc after using the function dredge in the MuMIn package (ver. 1.9.5; Burnham and Anderson, 2002) to run a complete set of models with all combinations of fixed effects and their two-way interactions. Pairwise differences between factor means were investigated using Tukey's post hoc tests.

3. Results

3.1. Species identification

Of the 2415 bees sampled, 20.3% of the samples collected were identified as *B. terrestris*. These were inadvertently collected during sampling as *B. terrestris* workers can be confused with *B. lucorum* workers (Wolf et al., 2010) and represented an average of 19.9 ± 3.7% SE (max. 72.5% and min. 0%) of samples taken from each location. All *B. terrestris* samples were excluded from further analyses. We did not include *B. terrestris* in this study because many *B. terrestris* individuals are easily distinguished using morphological traits, so only a proportion of all *B. terrestris* individuals (those that strongly resemble the *lucorum* complex species) were collected in our sampling. Of the remaining 1924 bees that belonged to the *lucorum* complex, 65.5% were identified as *B. lucorum*, 23.7% were *B. cryptarum*, and 10.8% were *B. magnus* (Table S3).

3.2. Geographic distributions and habitat use

The three species exhibit marked differences in their distributions across the UK. *Bombus lucorum* was found at every location sampled, from the Orkney Islands in the north, to Dartmoor in the south west and East Sussex in the south east (Fig. 1). *Bombus cryptarum* was found in almost all locations sampled to the north of ~53°N, hence including North Wales, northern England and Scotland; it was the most abundant species present in Orkney and on the east coast of Aberdeenshire. *Bombus cryptarum* was also found in small numbers in East Anglia, and was abundant on Dartmoor in the southwest. *Bombus magnus* was the most restricted of the three species, found at 11 of 27 locations. Its distribution is similar to that of *B. cryptarum*, being largely found north of ~53°N. It was the most abundant species at four locations, three in the highlands and west of Scotland, and also on Dartmoor in the southwest.

There was a marked difference in the strength of association of the three species with heathland habitats (Fig. 2, $\chi^2_2 = 435.94$, $P < 0.001$). *Bombus magnus* exhibited striking habitat specialisation, occurring almost exclusively on heathland (Fig. 2). When samples were collected from paired heathland and non-heathland habitats, *B. magnus* was almost always found in only the heathland habitat: only at two of 11 locations was *B. magnus* detected in the non-heathland habitat and then either only one or two individuals were found. Both *B. lucorum* and *B. cryptarum* were found more commonly in non-heathland than heathland habitats, but a greater proportion of *B. cryptarum* (46.4%) than *B. lucorum* (20.1%) were detected on heathland (Fig. 2).

For *B. magnus* and *B. cryptarum*, we tested the biological and climatic correlates of species presence or absence at each site (*B. lucorum* was present at all sites, so was excluded from this analysis). For *B. cryptarum*, increasing average maximum daily temperatures significantly decreased the likelihood of presence at a site; the negative effect of elevation was not quite significant (see Table 1 and Fig. 3a). For *B. magnus*, the likelihood of occurrence similarly declined significantly with increasing average maximum daily temperature, (see Table 1 and Fig. 3). The likelihood of occurrence for *B. magnus* was also significantly lower on non-heathland habitat: for a standardised summer maximum temperature of 15 °C the probability of *B. magnus* occurring at a non-heathland site is approximately 0.1, whereas at a heathland site, it is approximately 0.8 (see Table 1 and Fig. 3b). Other fixed effects (Table 1) and all two way interactions were not significant. The significant

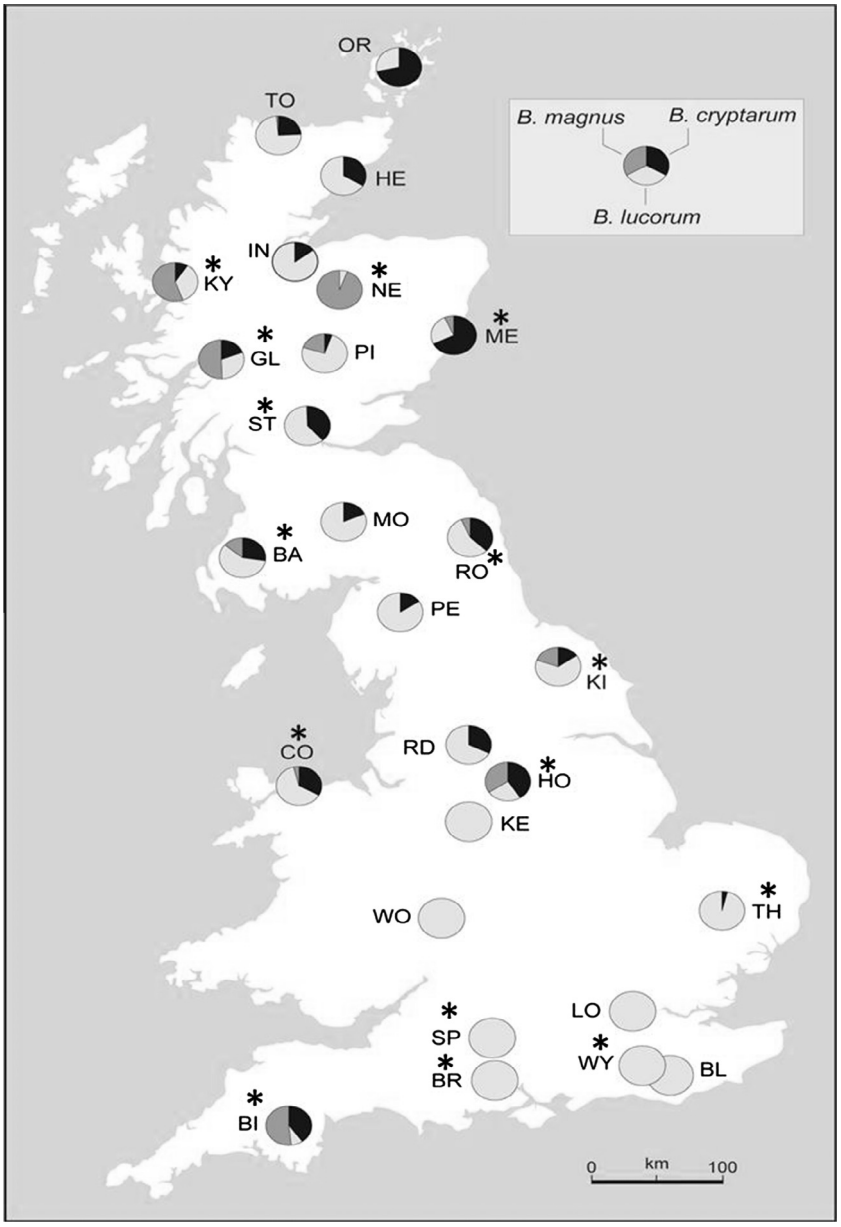

Fig. 1. The distribution of *Bombus lucorum* complex species across Great Britain. Sites marked with a * were sampled in 2011. The number of specimens identified per site, and habitat types sampled, are shown in Table S1.

effect of average maximum temperature remained when this analysis was performed on heathland (parameter estimate = −1.24 ± 0.63, $\chi^2_1 = 6.48$, $P = 0.011$) and non-heathland sites separately (parameter estimate = −2.68 ± 0.63, $\chi^2_1 = 11.02$, $P < 0.001$).

3.3. Forage use

Bombus lucorum queens and workers had the largest diet breadth (Tables 2 and S4), visiting a wide range of species from 20 different plant families. *Bombus cryptarum* workers and queens were found on a more restricted variety of species than *B. lucorum* workers. The majority (90.5%) of *B. magnus* workers and queens were found foraging on *Calluna vulgaris* or *Erica cinerea* and *Erica tetralix* (Tables 2 and S4) and consequently had the lowest diet breadth of the three species. The number of bees feeding on *Erica* spp. and *Calluna vulgaris* (heather) compared to all other plant species differed significantly across the 3 bumblebee species ($\chi^2_2 = 253$, $P < 0.001$). *Bombus magnus* individuals foraged most often on heather (90.5%), followed by *B. cryptarum* (43.9%); *B. lucorum* individuals foraged on these flowers least often (27.3%).

We tested whether this apparent preference was simply a consequence of *B. magnus* occurring predominantly in heathland habitats where heather plants are most common, by assessing how the probability of foraging on *Erica* spp. or *Calluna vulgaris* varied between bee species across both habitat types. The likelihood of bees foraging on these flowers was significantly influenced by which bumblebee species they belonged to ($\chi^2_2 = 42.1$, $P < 0.001$) and habitat type ($\chi^2_1 = 210$, $P < 0.001$). Furthermore, a significant interaction between species and habitat ($\chi^2_2 = 10.6$, $P < 0.01$) demonstrated that the differences between species in the extent of their preference for heather varied between the habitats. Whilst *B. magnus* individuals were significantly more likely to forage on heather when on heathland than either *B. cryptarum* (parameter estimate = −4.5 ± 1.18, $P < 0.001$) or *B. lucorum* (parameter estimate = −4.36 ± 1.18,

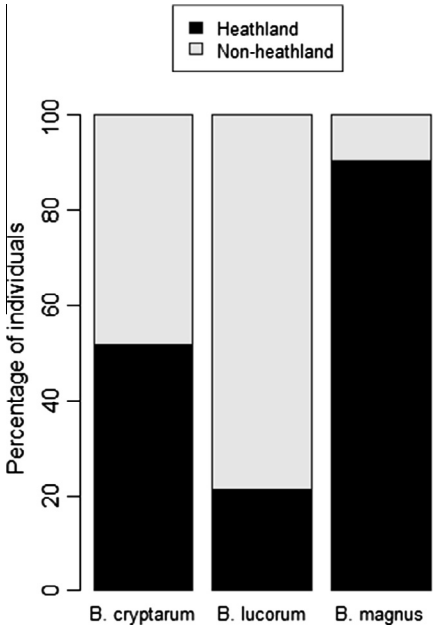

Fig. 2. Habitat use by all castes of *B. lucorum*, *B. magnus* and *B. cryptarum*, indicated by the percentage of bees caught in each habitat type, pooled for all sample sites.

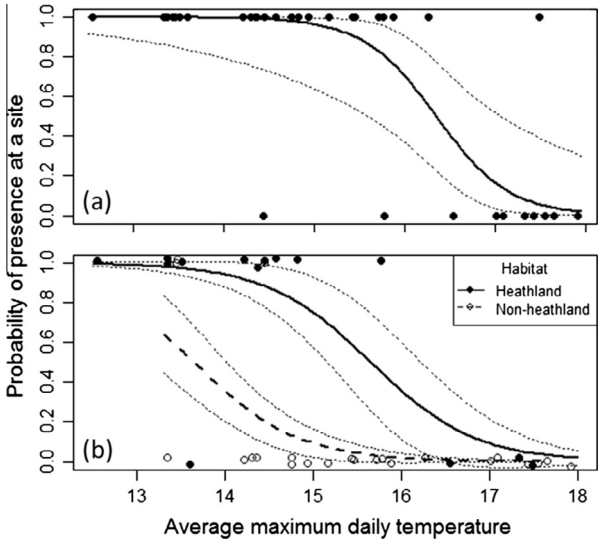

Fig. 3. (a) Probability of *B. cryptarum* presence at sites as a function of mean maximum daily temperature (°C) from March to August, and (b) of *B. magnus* presence as a function of mean maximum daily temperature (°C) on heathland (filled circles) and non-heathland habitat (non-filled circles). Bold lines represent the relationship between the presence of the species and the mean maximum daily temperature estimated from a generalised linear model. Small dashed lines represent 95% confidence intervals (CI) around this estimated relationship.

$P < 0.001$), on non-heathland habitats, all three were equally likely to be found foraging on *Erica* spp. or *Calluna vulgaris* (Fig. 4). There was no significant difference in the likelihood of *B. cryptarum* and *B. lucorum* foraging on these heather flowers when on heathland (parameter estimate = −0.17 ± 0.3, $P > 0.1$, Fig. 4).

4. Discussion

This study has substantially enhanced our understanding of the distribution of the three cryptic members of the *lucorum* species complex in Britain. Previous authors studying more restricted geographic areas in Ireland (Murray et al., 2008) and Western Scotland (Waters et al., 2010) concluded that *B. lucorum*, *B. cryptarum* and *B. magnus* are common, widely distributed and sympatric. By undertaking a more wide-ranging study, we demonstrate that across the UK *B. magnus* and *B. cryptarum* are associated with cooler climates than *B. lucorum*, being found most commonly in northern and western Britain and that they are absent from a large portion of the south and east. Our data also demonstrate that *B. magnus* exhibits a tight association with heathland habitats.

The absence of morphological diagnostic characters leads to a lack of even basic knowledge about the ecology and distribution of cryptic species. Without ecological knowledge of cryptic species, we have no way of discerning whether populations are stable or establishing effective conservation management strategies when necessary. This is particularly true for pollinator groups such as bumblebees, which are important both ecologically and economically, and comprise species that are suffering dramatic declines resulting from habitat loss and fragmentation (Goulson, 2010) and agricultural intensification (Goulson et al., 2006; Williams, 1986). This study therefore contributes vital information for this purpose.

In the Western Isles of Scotland, *B. lucorum* was the least common of the *lucorum* complex species (Waters et al., 2010). In contrast, in this study of mainland Great Britain, and also in Ireland (Murray et al., 2008; Stanley et al., 2013), *B. lucorum* was the most common species (double the proportion found in Waters et al., 2010). In the current study, *B. lucorum* was found at all sampled sites, making it the most widespread of the species, although a greater proportion of individuals were found in non-heathland than heathland habitat. Unlike in the Western Isles, where *B. cryptarum* workers were shown to have the broadest diet (Waters et al., 2010), in our study *B. lucorum* workers (and queens) exhibited the largest diet breadth, exploiting a greater number of

Table 2
Forage use and measures of diet breadth for *B. lucorum* complex queens and workers pooled across sample sites. Diet breadth is measured via rarefaction to estimate the number of plant species each bee species would be expected to visit in a total of 100 flower visits.

	B. lucorum	*B. cryptarum*	*B. magnus*	All bee species
Total sample size	689	321	188	1198
No. of plant taxa visited	43	25	6	47
Diet breadth (±SD)	22.57 ± 2.24	15.20 ± 1.88	4.76 ± 0.85	

Table 1
The probability of *B. cryptarum* and *B. magnus* individuals being found at a site, in relation to multiple independent variables. Summary of the results of a generalised linear model that investigated the effects of habitat type (heathland or non-heathland), mean maximum daily temperature from March to August and elevation. Significant results are shown in italics.

Parameter	*B. cryptarum*				*B. magnus*			
	Estimate	SE	χ^2	Prob > χ^2	Estimate	SE	χ^2	Prob > χ^2
Elevation (m)	−0.015	0.009	3.551	0.060	−0.002	0.009	0.065	0.799
Average max. daily temperature (°C)	−2.475	0.925	25.204	*5.157×10^{-7}*	−1.694	0.614	16.324	*5.339×10^{-5}*
Habitat: Non-heathland	−1.256	1.458	0.755	0.385	−3.398	1.325	10.169	*0.001*

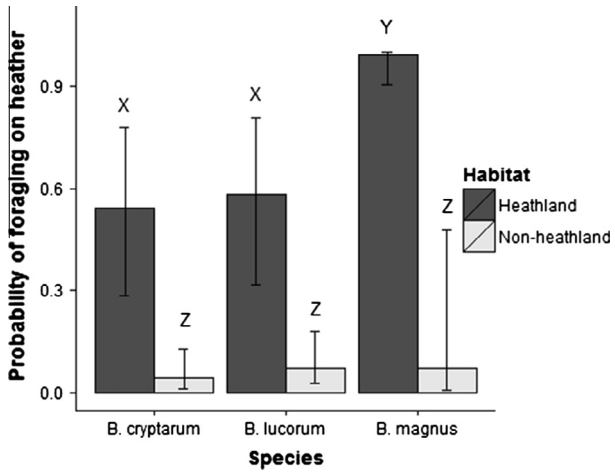

Fig. 4. The probability of individuals (queens and workers) of each taxa foraging on *Calluna vulgaris*, *Erica tetralix* or *Erica cinerea* compared to all other plant species, according to habitat type. Porbabilities were estimated from a linear mixed effect model. Error bars show 95% confidence intervals. Probabilities with different letters are significantly different ($P < 0.001$).

plant species than either *B. cryptarum* or *B. magnus*. Such a large diet breadth may be a reflection of the broad range of habitats and locations that this species inhabits. Overall, *B. lucorum* appears to be the most generalised of the three species, occupying the broadest climatic range, feeding on a wide range of flowers, and is the only species of the three to be found in the intensively farmed and urbanised south east of England.

Bombus cryptarum was the second most common species in this current study. However, previous studies show that in Ireland it was the least common of the three (Murray et al., 2008), whereas in the Western Isles, it was the most common (almost half of the individuals, Waters et al., 2010). It was also found to be the most polylectic in the Western Isles, visiting a wide range of food plants belonging to many families, including non-native garden plants (Waters et al., 2010). In the rest of Scotland, England and Wales, it also appears to be highly polylectic, but less so than *B. lucorum*, possibly because its narrower geographic distribution inevitably means it encounters fewer plant species.

In the Western Isles of Scotland (Waters et al., 2010) and Ireland (Murray et al., 2008), *B. magnus* was the second most common of the three species, whereas in this study of mainland Great Britain, *B. magnus* was the least abundant of the three species (approximately three times lower than in the other two studies). It has previously been described as associated with upland, northerly, and westerly areas, and thus the generally cooler, wetter regions in the UK (Benton, 2006; Alford, 1975). Waters et al. (2010) and Murray et al. (2008) found that their data for *B. magnus* in Ireland and the Western Isles of Scotland did not support this. Instead, Murray et al. (2008) found that this species was present in both upland and lowland sites but was absent from urban areas and Stanley et al. (2013) found that it was absent from mass flowering crops in Ireland. Our results for Great Britain correspond to the findings of Waters et al. (2010) that *B. magnus* is strongly associated with heathland, but is not restricted to upland areas. Waters et al. (2010) also found that *B. magnus* was particularly associated with the forage plant *Calluna vulgaris*; our results indicate an association with the three Ericaceae, *Calluna vulgaris*, *Erica cinerea* and *Erica tetralix*. This apparent preference for these Ericaceous flowers leads to *B. magnus* exhibiting the lowest diet breadth.

Tight dietary specializations or biotope associations are unusual in European bumblebees. In a study on the biotope associations of UK bumblebee species, Goulson et al. (2006) found that they were all recorded in more than one, most being found in a broad range of different biotopes. Even very rare species such as *B. sylvarum*, which is the second rarest extant species in the UK, do not seem to have tight biotope associations. *B. jonellus*, *B. muscorum* and *B. soroeensis* are also associated with heathland to varying extents, especially in the north of the UK, but all three also have significant populations in non-heathland habitats (Darvill, 2006; Darvill et al., 2010; Goulson et al., 2006) and specialisation in habitat and food associations may often be related to the position of a site within a species' global range (Williams et al., 2007). In this study, only 9.5% of *B. magnus* individuals were found in habitat other than heathland, or on flowers other than *Erica* spp. or *Calluna vulgaris*; all of these individuals were found very near to large areas of heathland, suggesting that they were probably individuals spilling out from heathland habitat. This apparent tight association exhibited by *B. magnus* could impose a serious disadvantage for a social organism that needs to maintain colonies with high energy demands beyond the flowering season of any one (or two) plant species (Williams, 2005) and seems to be quite unusual amongst bumblebees.

In Great Britain there are two types of heathland habitat, lowland and upland heath. The lowland heaths of southern England make up 14% of this habitat type in Europe (Groves et al., 2012), yet around 80% has been lost since 1800 due to agriculture, urbanisation and changes in land management (Price, 2003). Upland heath is a sub-montane habitat characterised by common or ling heather *Calluna vulgaris*, found mostly in the British Isles, and along parts of the western seaboard of the northwest European mainland. *Calluna vulgaris* occurs much more widely than this but the massive extent of rotationally burned heather is unique to the UK and Ireland (Thompson et al., 1995). In the UK, large proportions of upland heath have also been lost to afforestation and over-grazing by sheep (Thompson et al., 1995). Consequently, both lowland and upland heathland are listed as UK post-2010 Biodiversity Framework priority habitats, meaning that they have been identified as being the most threatened and requiring conservation action. Habitat degradation can have considerable implications for the species that are associated with it. In fact habitat loss is widely agreed to be the most important factor driving bee declines (Brown and Paxton, 2009). A direct result of habitat loss is habitat fragmentation, which impacts surviving populations through genetic isolation and subsequent inbreeding (Whitehorn et al., 2011; Zayed, 2009) or simply the inability of small remaining habitat fragments to support viable bee populations (e.g., Ellis et al., 2006b). In this case, *B. magnus* may already have suffered from past losses of heathland and further loss of this habitat is likely to lead to population declines. The apparent dietary specialisation of *B. magnus* could make this especially problematic. Only a small number of bumblebee species (six in the UK) appear to have been largely unaffected by changes to the environment in the last 60 years. These species seem to have more generalised foraging preferences than some of the rare species, which may mean they have a greater ability to adapt to changing forage resources (Goulson et al., 2005). In addition, species with narrow diet breadth have access to fewer resources, so, as biotopes become degraded and floral resources decline, these specialists are likely to be the first to disappear (Goulson et al., 2006). Presently, we have no way of knowing whether the populations of the species within the *lucorum* complex are currently stable or if they have experienced population changes in the past.

We acknowledge that our diet breadth estimates are likely to be conservative, since fieldwork targeted flower patches and times of day where bees were abundant enough to collect an adequate sample size to accurately characterise feeding behaviour. This may have led us to miss a small number of bees foraging on some rare flower species. However, it is unlikely to have strongly affected the

results; our estimates will be representative of foraging behaviour in the substantial majority of individuals. There was no possibility that this introduced bias into our diet breadth comparisons between the different *lucorum* complex species, as species identity was only determined *post-hoc* by molecular methods. It should be noted that our analysis techniques cannot entirely disentangle effects of habitat preference on observed diet breadth; localised species, or species with specialised habitat preferences, will encounter fewer flower species and thus inevitably tend to have a more restricted diet (see Williams, 2005).

Bombus cryptarum and *B. magnus* occurred more commonly where temperatures were lower and were found to be generally more common at northerly latitudes, a preference that was not detected for *B. lucorum*. They were consequently absent from much of the south and east of England. Heathland habitats were sampled in this area but *B. magnus* was not found to be present (though Williams et al., 2012 report a specimen from the heathland of Dungeness in the South East of England). It may be that these sites are too warm, or that *B. magnus* used to occur there in the past when the heathland area was larger and less fragmented. The south-east of England is also highly urbanised. Urban areas can support diverse pollinator assemblages but they can also have negative impacts on pollinator species (Bates et al., 2011). One obvious outlier in the distributions of both *B. cryptarum* and *B. magnus* is the Birch Tor site on Dartmoor in the south-west of England (Fig. 1), where *B. magnus* and *B. cryptarum* were more abundant than *B. lucorum*. This appears incongruous (Fig. 1) but due to the high altitude the temperature at this site is actually much lower than at other sites with similar latitude, meaning the presence of *B. magnus* and *B. cryptarum* at Birch Tor is consistent with their preferences. Further sampling in the southwest of England and in Wales would help reveal whether these are isolated populations of *B. magnus* and *B. cryptarum*, or whether they are actually present in suitable areas throughout the western side of Great Britain.

The lack of diagnostic characteristic traits for these species in Scotland and Ireland (Carolan et al., 2012), as well as geographical variation in colour pattern across taxa, means that the potential for misidentification of these species is very high. As a consequence, descriptions of the ecology and distribution of these three species, obtained prior to the utilisation of molecular methods for species identification, are likely to be problematic (see Rasmont, 1984; Rasmont et al., 1986 and Pamilo et al., 1997 for European distributions). Therefore, the only reliable information available about the worldwide distributions of these species comes from a study by Williams et al. (2012) of the subgenus *Bombus s. str*. They find *B. lucorum* to be present from Iceland in the west, across Europe to the mountains of Central Asia and in Mongolia. *Bombus cryptarum* appears to have the broadest distribution of all *Bombus s. str.* species. It was found from Great Britain, across Europe and central Asia to western North America. *Bombus magnus* is present in Great Britain, Spain, Denmark, Sweden and near Moscow, Russia. Further work would evidently be beneficial.

This study has revealed that while these species have a sympatric distribution across much of northern England, Northern Wales and Scotland, they exhibit clearly discernible differences in their ecological characteristics. This demonstrates the importance of correctly identifying cryptic species, not just amongst important pollinators such as bumblebees (e.g. Ellis et al., 2006a; Williams, 2007) but in insects in general, where they are also common (e.g. Hebert et al., 2004; Smith et al., 2007). Failure to account for cryptic diversity could result in missing the causal link between changes in species distribution and environmental variation, incorrect delineation of units for conservation and consequently, serious repercussions for their management.

Further studies of these three species would be required to determine whether the observed differences are the result of preference or the outcome of inter-specific competition. In addition, it would be interesting to determine what *B. magnus* feeds on during the periods when *Erica* spp. and *Calluna vulgaris* are not in flower on heathland habitats. A long term study would be able to establish whether the populations of these three species are stable or declining, particularly focussing on the response of *B. magnus* populations to past and present heathland loss/degradation. Our ongoing research is investigating the population genetics of this species complex to provide insight into differences in genetic diversity, and reveal whether the highly specialised *B. magnus* is suffering from population fragmentation as a result of its tight association with a declining and fragmented habitat type.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.11.027.

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