

1 **Revealing the hidden niches of cryptic bumblebees in Great Britain: implications for**  
2 **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.

## Keywords

*Bombus*, PCR-RFLP, cryptic species, ecology, distribution, conservation

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## Introduction

Bumblebees (*Bombus*: Hymenoptera, Apidae) are ecologically and economically important as pollinators (Goulson 2010; Velthuis & 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.* 2007; Goulson *et al.* 2008a, Goulson 2010; Williams 1982; Williams & 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 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.* 2004b), 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 & 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; Fitzpatrick *et al.* 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

166 referring to data for multiple species and is therefore of limited value. Consequently, the only reliable  
 167 information we have on the ecology of these three species comes from Murray *et al.* (2008) and  
 168 Stanley *et al.* (2013) who used molecular methods to study the *lucorum* complex in Ireland and  
 169 Waters *et al.* (2010) who studied them in the Western Isles of Scotland. Niche-partitioning might be  
 170 expected between these species (Goulson *et al.* 2008b) and indeed some ecological differences have  
 171 been suggested. Specifically, Waters *et al.* (2010) found that *B. magnus* appeared to be strongly  
 172 associated with the heathland forage plant *Calluna vulgaris*. These studies suggest that the three taxa  
 173 are widespread throughout Ireland and the Western Isles of Scotland but have differing patterns of  
 174 geographic distributions. These studies have suggested some differences in the ecology, abundance  
 175 and distribution of the three taxa, which, given the ongoing concerns over bumblebee declines,  
 176 indicates the need for further work to reveal the biology of these species and reassess their  
 177 conservation status.

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

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### 191 *Sampling*

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

209 DNA extraction from the samples collected in 2010 was performed using a Chelex® 100 protocol  
 210 (Walsch *et al.* 1991) and from the 2011 samples using a HotShot protocol (Truett *et al.* 2000). For  
 211 species identification we followed a PCR-RFLP method based on amplification of the cytochrome  
 212 oxidase I (COI) gene developed by Murray *et al.* (2008). The pattern of digested fragments for each  
 213 individual was compared with the characteristic patterns associated with each of the cryptic species  
 214 and *B. terrestris* (see Fig. 3 in Murray *et al.* 2008), in order to determine their species identity.  
 215 To confirm RFLP identification; 108 individuals (46 *B. terrestris*, 55 *B. lucorum*, 2 *B. magnus*, 2  
 216 *B. cryptarum*, 2 *B. soroensis*, 1 *B. sylvestris*), collected from all but one of the 2010 sample sites,  
 217 were amplified using the PCR-RFLP primers. Resulting PCR amplicons were purified (ExoSAP;  
 218 Werle *et al.* 1994) and sent for sequencing (DNA Sequencing and Services, Dundee, U.K.).  
 219 Consensus sequences were aligned (Geneious v 6.1.7) then checked against the RFLP banding  
 220 pattern. For those samples that did not exhibit a clear RFLP banding pattern after two amplifications  
 (174 of 2 415), we used microsatellite data for species assignment (obtained from a separate study  
 comparing population structure of the three species, Scriven *et al.* in prep.). In brief, individuals were

221 genotyped at 13 microsatellite loci (Tables S 1 & 2). Structure v 2.3.4 (Pritchard *et al.* 2000) was used  
 222 to cluster the samples according to species. The USEPOPINFO model was applied to define “learning  
 223 samples” that are pre-defined as coming from particular clusters (the known species from RFLP  
 224 analysis) to assist ancestry estimation for the remaining individuals of unknown origin. The  
 225 Admixture and Independent Allele Frequency models were also used and the software was run with  
 226 four clusters (K, for the three *lucorum* complex species and *B. terrestris* using 50 000 burn-in periods  
 227 followed by 100 000 MCMC repetitions).

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### 230 *Analyses*

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

240 Other analyses were carried out using R version 3.0.2 (R Core Team 2013). Generalised  
 241 linear models with a binary error distribution were used to investigate the biogeographical and  
 242 climatic correlates (UK Meteorological Office 2014) of *lucorum* complex species presence at sites.  
 243 The response variable was the presence or absence of a species at a site. Explanatory variables tested  
 244 were habitat type (heathland or non-heathland), mean maximum daily temperature from March to  
 245 August (the approximate flight period of these species), elevation (m) and all two-way interactions.  
 246 Associations with average rainfall and the number of days of ground frost from March to August were  
 247 also investigated; however, they were negatively correlated with mean maximum temperature ( $r = -$   
 248  $0.55$  and  $-0.57$  respectively). These correlations meant we could not adequately distinguish their  
 249 effects, hence rainfall and frost were dropped from analyses because mean temperature has greater  
 250 explanatory power (at least 2 AIC points). These variables were chosen because previous studies have  
 251 shown them to influence bumblebee species distributions (Goulson 2010; Lye *et al.* 2010; Williams *et*  
 252 *al.* 2007). The preference of each species for the ericaceous plants *Calluna vulgaris* or *Erica spp.* was  
 253 examined using linear mixed effects models with individual bee as the unit of replication, and whether  
 254 the bee was recorded on a *Calluna vulgaris* or *Erica spp.* flower or not as the binary response. Linear  
 255 mixed-effect models were fit with lmer in the lme4 package (ver. 1.0-5; Bates *et al.* 2013) in R. The  
 256 fixed effects investigated the influence of the species that an individual bee belonged to and the  
 257 habitat type in which it was found, with location as a random effect. The most parsimonious  
 258 combination of fixed effects was determined using maximum likelihood (ML) rather than restricted  
 259 maximum likelihood (REML). Optimal models were selected to minimise AICc after using the  
 260 function dredge in the MuMIn package (ver. 1.9.5; Burnham & Anderson 2002) to run a complete set  
 261 of models with all combinations of fixed effects and their two-way interactions. Pairwise differences  
 262 between factor means were investigated using Tukey’s post hoc tests.

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## 266 **Results**

### 267 *Species identification*

268 Of the 2 415 bees sampled, 20.3% of the samples collected were identified as *B. terrestris*. These  
 269 were inadvertently collected during sampling as *B. terrestris* workers can be confused with *B.*  
 270 *lucorum* workers (Wolf *et al.* 2009) and represented an average of  $19.9 \pm 3.7\%$  SE (max. 72.5% and  
 271 min. 0%) of samples taken from each location. All *B. terrestris* samples were excluded from further  
 272 analyses. We did not include *B. terrestris* in this study because many *B. terrestris* individuals are  
 273 easily distinguished using morphological traits, so only a proportion of all *B. terrestris* individuals  
 274 (those that strongly resemble the *lucorum* complex species) were collected in our sampling. Of the

275 remaining 1 924 bees that belonged to the *lucorum* complex, 65.5% were identified as *B. lucorum*,  
 276 23.7% were *B. cryptarum*, and 10.8% were *B. magnus* (Table S 3).

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#### 278 *Geographic distributions and habitat use*

279 The three species exhibit marked differences in their distributions across the UK. *Bombus lucorum*  
 280 was found at every location sampled, from the Orkney Islands in the north, to Dartmoor in the south  
 281 west and East Sussex in the south east (Figure 1). *Bombus cryptarum* was found in almost all  
 282 locations sampled to the north of  $\sim 53^{\circ}\text{N}$ , hence including North Wales, northern England and  
 283 Scotland; it was the most abundant species present in Orkney and on the east coast of Aberdeenshire.  
 284 *Bombus cryptarum* was also found in small numbers in East Anglia, and was abundant on Dartmoor  
 285 in the southwest. *Bombus magnus* was the most restricted of the three species, found at 11 of 27  
 286 locations. Its distribution is similar to that of *B. cryptarum*, being largely found north of  $\sim 53^{\circ}\text{N}$ . It was  
 287 the most abundant species at four locations, three in the highlands and west of Scotland, and also on  
 288 Dartmoor in the southwest.

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

297 For *B. magnus* and *B. cryptarum*, we tested the biological and climatic correlates of species  
 298 presence or absence at each site (*B. lucorum* was present at all sites, so was excluded from this  
 299 analysis). For *B. cryptarum*, increasing average maximum daily temperatures significantly decreased  
 300 the likelihood of presence at a site; the negative effect of elevation was not quite significant (see Table  
 301 1 & Fig. 3a). For *B. magnus*, the likelihood of occurrence similarly declined significantly with  
 302 increasing average maximum daily temperature, (see Table 1 & Fig. 3). The likelihood of occurrence  
 303 for *B. magnus* was also significantly lower on non-heathland habitat: for a standardised summer  
 304 maximum temperature of  $15^{\circ}\text{C}$  the probability of *B. magnus* occurring at a non-heathland site is  
 305 approximately 0.1, whereas at a heathland site, it is approximately 0.8 (see Table 1 & Fig. 3b). Other  
 306 fixed effects (Table 1) and all two way interactions were not significant. The significant effect of  
 307 average maximum temperature remained when this analysis was performed on heathland (parameter  
 308 estimate =  $-1.24 \pm 0.63$ ,  $\chi^2_1 = 6.48$ ,  $P = 0.011$ ) and non-heathland sites separately (parameter estimate  
 309 =  $-2.68 \pm 0.63$ ,  $\chi^2_1 = 11.02$ ,  $P < 0.001$ ).

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#### 311 *Forage use*

312 *Bombus lucorum* queens and workers had the largest diet breadth (Table 2 & S 4), visiting a wide  
 313 range of species from 20 different plant families. *Bombus cryptarum* workers and queens were found  
 314 on a more restricted variety of species than *B. lucorum* workers. The majority (90.5%) of *B. magnus*  
 315 workers and queens were found foraging on *Calluna vulgaris* or *Erica cinerea* and *Erica tetralix*  
 316 (Table 2 & S 4) and consequently had the lowest diet breadth of the three species. The number of bees  
 317 feeding on *Erica spp.* and *Calluna vulgaris* (heather) compared to all other plant species differed  
 318 significantly across the 3 bumblebee species ( $\chi^2_2 = 253$ ,  $P < 0.001$ ). *Bombus magnus* individuals  
 319 foraged most often on heather (90.5%), followed by *B. cryptarum* (43.9%); *B. lucorum* individuals  
 320 foraged on these flowers least often (27.3%).

321 We tested whether this apparent preference was simply a consequence of *B. magnus*  
 322 occurring predominantly in heathland habitats where heather plants are most common, by assessing  
 323 how the probability of foraging on *Erica spp.* or *Calluna vulgaris* varied between bee species across  
 324 both habitat types. The likelihood of bees foraging on these flowers was significantly influenced by  
 325 which bumblebee species they belonged to ( $\chi^2_2 = 42.1$ ,  $P < 0.001$ ) and habitat type ( $\chi^2_1 = 210$ ,  $P <$   
 326  $0.001$ ). Furthermore, a significant interaction between species and habitat ( $\chi^2_2 = 10.6$ ,  $P < 0.01$ )  
 327 demonstrated that the differences between species in the extent of their preference for heather varied  
 328 between the habitats. Whilst *B. magnus* individuals were significantly more likely to forage on  
 329 heather when on heathland than either *B. cryptarum* (parameter estimate =  $-4.5 \pm 1.18$ ,  $P < 0.001$ ) or

330 *B. lucorum* (parameter estimate =  $-4.36 \pm 1.18$ ,  $P < 0.001$ , Fig. 5), on non-heathland habitats, all three  
 331 were equally likely to be found foraging on *Erica spp.* or *Calluna vulgaris* (Fig. 4). There was no  
 332 significant difference in the likelihood of *B. cryptarum* and *B. lucorum* foraging on these heather  
 333 flowers when on heathland (parameter estimate =  $-0.17 \pm 0.3$ ,  $P > 0.1$ , Fig. 4).

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### 337 Discussion

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

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

354 In the Western Isles of Scotland, *B. lucorum* was the least common of the *lucorum* complex  
 355 species (Waters *et al.* 2010). In contrast, in this study of mainland Great Britain, and also in Ireland  
 356 (Murray *et al.* 2008, Stanley *et al.* 2013), *B. lucorum* was the most common species (double the  
 357 proportion found in Waters *et al.* 2010). In the current study, *B. lucorum* was found at all sampled  
 358 sites, making it the most widespread of the species, although a greater proportion of individuals were  
 359 found in non-heathland than heathland habitat. Unlike in the Western Isles, where *B. cryptarum*  
 360 workers were shown to have the broadest diet (Waters *et al.* 2010), in our study *B. lucorum* workers  
 361 (and queens) exhibited the largest diet breadth, exploiting a greater number of plant species than  
 362 either *B. cryptarum* or *B. magnus*. Such a large diet breadth may be a reflection of the broad range of  
 363 habitats and locations that this species inhabits. Overall, *B. lucorum* appears to be the most  
 364 generalised of the three species, occupying the broadest climatic range, feeding on a wide range of  
 365 flowers, and is the only species of the three to be found in the intensively farmed and urbanized south  
 366 east of England.

367 *Bombus cryptarum* was the second most common species in this current study. However,  
 368 previous studies show that in Ireland it was the least common of the three (Murray *et al.* 2008),  
 369 whereas in the Western Isles, it was the most common (almost half of the individuals, Waters *et al.*  
 370 2010). It was also found to be the most polylectic in the Western Isles, visiting a wide range of food  
 371 plants belonging to many families, including non-native garden plants (Waters *et al.* 2010). In the rest  
 372 of Scotland, England and Wales, it also appears to be highly polylectic, but less so than *B. lucorum*,  
 373 possibly because its narrower geographic distribution inevitably means it encounters fewer plant  
 374 species.

375 In the Western Isles of Scotland (Waters *et al.* 2010) and Ireland (Murray *et al.* 2008), *B.*  
 376 *magnus* was the second most common of the three species, whereas in this study of mainland Great  
 377 Britain, *B. magnus* was the least abundant of the three species (approximately three times lower than  
 378 in the other two studies). It has previously been described as associated with upland, northerly, and  
 379 westerly areas, and thus the generally cooler, wetter regions in the UK (Benton 2006, Alford 1975).  
 380 Waters *et al.* (2010) and Murray *et al.* (2008) found that their data for *B. magnus* in Ireland and the  
 381 Western Isles of Scotland did not support this. Instead, Murray *et al.* (2008) found that this species  
 382 was present in both upland and lowland sites but was absent from urban areas and Stanley *et al.*  
 383 (2013) found that it was absent from mass flowering crops in Ireland. Our results for Great Britain  
 384 correspond to the findings of Waters *et al.* (2010) that *B. magnus* is strongly associated with

385 heathland, but is not restricted to upland areas. Waters et al. (2010) also found that *B. magnus* was  
 386 particularly associated with the forage plant *Calluna vulgaris*; our results indicate an association with  
 387 the three Ericaceae, *Calluna vulgaris*, *Erica cinerea* and *Erica tetralix*. This apparent preference for  
 388 these Ericaceous flowers leads to *B. magnus* exhibiting the lowest diet breadth.

389 Tight dietary specializations or biotope associations are unusual in European bumblebees.  
 390 In a study on the biotope associations of UK bumblebee species, Goulson et al. (2006) found that they  
 391 were all recorded in more than one, most being found in a broad range of different biotopes. Even  
 392 very rare species such as *B. sylvarum*, which is the second rarest extant species in the UK, do not  
 393 seem to have tight biotope associations. *B. jonellus*, *B. muscorum* and *B. soroensis* are also  
 394 associated with heathland to varying extents, especially in the north of the UK, but all three also have  
 395 significant populations in non-heathland habitats (Darvill 2006; Darvill et al. 2010; Goulson et al.  
 396 2006) and specialisation in habitat and food associations may often be related to the position of a site  
 397 within a species' global range (Williams et al., 2007). In this study, only 9.5% of *B. magnus*  
 398 individuals were found in habitat other than heathland, or on flowers other than *Erica spp.* or *Calluna*  
 399 *vulgaris*; all of these individuals were found very near to large areas of heathland, suggesting that they  
 400 were probably individuals spilling out from heathland habitat. This apparent tight association  
 401 exhibited by *B. magnus* could impose a serious disadvantage for a social organism that needs to  
 402 maintain colonies with high energy demands beyond the flowering season of any one (or two) plant  
 403 species (Williams 2005) and seems to be quite unusual amongst bumblebees.

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

430 We acknowledge that our diet breadth estimates are likely to be conservative, since fieldwork  
 431 targeted flower patches and times of day where bees were abundant enough to collect an adequate  
 432 sample size to accurately characterise feeding behaviour. This may have led us to miss a small  
 433 number of bees foraging on some rare flower species. However, it is unlikely to have strongly  
 434 affected the results; our estimates will be representative of foraging behaviour in the substantial  
 435 majority of individuals. There was no possibility that this introduced bias into our diet breadth  
 436 comparisons between the different *lucorum* complex species, as species identity was only determined  
 437 *post-hoc* by molecular methods. It should be noted that our analysis techniques cannot entirely  
 438 disentangle effects of habitat preference on observed diet breadth; localized species, or species with

439 specialized habitat preferences, will encounter fewer flower species and thus inevitably tend to have a  
 440 more restricted diet (see Williams 2005).

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

457 The lack of diagnostic characteristic traits for these species in Scotland and Ireland (Carolan  
 458 et al. 2012), as well as geographical variation in colour pattern across taxa, means that the potential  
 459 for misidentification of these species is very high. As a consequence, descriptions of the ecology and  
 460 distribution of these three species, obtained prior to the utilisation of molecular methods for species  
 461 identification, are likely to be problematic (see Rasmont 1984, Rasmont et al. 1986 and Pamilo et al.  
 462 1997 for European distributions). Therefore, the only reliable information available about the  
 463 worldwide distributions of these species comes from a study by Williams et al. (2012) of the subgenus  
 464 *Bombus s. str.* They find *B. lucorum* to be present from Iceland in the west, across Europe to the  
 465 mountains of Central Asia and in Mongolia. *Bombus cryptarum* appears to have the broadest  
 466 distribution of all *Bombus s. str.* species. It was found from Great Britain, across Europe and central  
 467 Asia to western North America. *Bombus magnus* is present in Great Britain, Spain, Denmark, Sweden  
 468 and near Moscow, Russia. Further work would evidently be beneficial.

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

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

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