This is the peer reviewed version of the following article: Edmunds, M. & Reader, T. (2014). Evidence for Batesian mimicry in a polymorphic hoverfly. Evolution. 68-3: 827–839, which has been published in final form at http://onlinelibrary.wiley.com/doi/10.1111/evo.12308. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Evidence for Batesian mimicry in a polymorphic hoverfly

Malcolm Edmunds[1*] and Tom Reader[2]

1. School of Built and Natural Environment, University of Central Lancashire, Preston, Lancashire, PR1 2HE, U.K. Email: medmunds@phonecoop.coop. Tel. ++44 (0)1772 865796.

2. School of Biology, University of Nottingham, Nottingham, NG7 2RD, U.K. Email: tom.reader@nottingham.ac.uk. Tel. ++44 (0)115 9513213.

*Corresponding author

Running title: Evidence for Batesian mimicry

Key words: Natural selection, adaptation, *Volucella bombylans*, polymorphism, distribution.

Word count: 5685. Tables: 6. Figures: 8. Supplementary Tables: 5.

Data Archival Location: Dryad Digital Repository doi:10.5061/dryad.ms172

Abstract

Palatable Batesian mimics are avoided by predators because they resemble noxious or defended species. The striking resemblance of many hoverflies to noxious Hymenoptera is a "textbook" example of Batesian mimicry, but evidence that selection by predators has shaped the evolution of hoverfly patterns is weak. We looked for geographical and temporal trends in frequencies of morphs of the polymorphic hoverfly *Volucella bombylans* which would support the hypothesis that these morphs are Batesian mimics of different bumblebee species. The frequency of the black and yellow hoverfly morph was significantly positively related to the frequency of black and yellow bumblebees across 52 sites. Similarly, the frequency of the red-tailed hoverfly morph was positively related to the frequency of red-tailed bumblebees. However, the frequencies of hoverfly morphs were positively spatially autocorrelated, and after controlling for this, only one of the two common hoverfly morphs showed a significant positive relationship with its putative model. We conclude that the distribution of *V. bombylans* morphs probably reflects geographical variation in selection by predators resulting from differences in the frequencies of noxious bumblebee species.

Introduction

Hoverflies (Diptera, Syrphidae) are abundant insects in temperate regions and many of them bear a close resemblance to social hymenopterans. The hypothesis that hoverflies derive protection from this resemblance through Batesian mimicry is paradigmatic in evolutionary biology (Gilbert, 2005; Rotheray and Gilbert, 2011). Nevertheless, despite considerable research effort over the past 150 years (Edmunds, 2008), definitive evidence that natural selection by predators has led to the evolution of Batesian mimicry in hoverflies remains elusive.

Batesian mimicry is where a palatable animal (the mimic) gains protection from predators because they mistake it for a noxious or unpalatable animal (the model). It was first described by (and is now named after) Henry Bates (1862) based on his studies of South American butterflies, and there have been numerous reviews of the topic since then (e.g. Cott, 1940; Edmunds, 1974; Ruxton, Sherratt & Speed, 2004). While some hoverflies bear a very close resemblance to their hymenopteran models (‘good’ mimics) others have a much less precise similarity (‘poor’ or ‘imperfect mimics’) so that it has been questioned whether they really do gain protection from this resemblance (Edmunds, 2000). While the existence of imperfect mimics may point to other explanations for hoverfly patterns that do not involve predators generalising avoidance behaviours learned after attacking noxious models, several plausible hypotheses are consistent with the idea that even taxa which do not closely resemble their putative models are Batesian mimics (Gilbert, 2005; Penney *et al.* 2012).

Empirical evidence supporting the idea that hoverflies are Batesian mimics comes mostly from studies of predator behaviour under controlled conditions. Mostler (1935) showed that different species of hoverflies resembling honeybees (*Apis mellifera*), wasps (principally in the family Vespidae) or bumblebees (*Bombus* spp.) are palatable to insectivorous birds, and that prior experience of the noxious model caused the birds to reject at least some of the mimics (data summarised by Gilbert, 2005). These experiments were in captivity, but Dlusskii (1984) worked in the field, exposing pairs of tethered insects to local birds. He showed that many birds could distinguish the models from the mimics, avoiding the former and eating the latter, but that some birds were deceived by the mimicry and avoided at least some of the mimics.

Obtaining evidence for the effectiveness of mimicry in natural populations is much more difficult. Possible support for hoverflies as Batesian mimics comes from Howarth, Edmunds & Gilbert (2004), who found a positive relationship between hoverfly abundance and the abundance of their putative hymenopteran models for ten out of 18 species studied. However, the association between population sizes of mimics and their models offers only very indirect evidence of selection by predators for mimicry. We might reason that where noxious models are abundant, predators quickly learn to avoid them and other similar-looking taxa, leading to a reduced predation rate on mimics, but the study of population dynamics tells us that reduced predation does not necessarily lead to increased population size. Even in prey populations tightly regulated by density-dependent predation (which may or may not be the case in mimetic taxa), population size can fluctuate dramatically and counter-intuitively for both deterministic and stochastic reasons (e.g. Abrams, 2009); it is therefore inherently risky to infer cause and effect from the study of population sizes alone.

More direct evidence for Batesian mimicry in natural populations could come from the study of polymorphic species, where the effectiveness of mimicry in individual morphs might vary depending on the environment. If mimicry really is protective, we would expect selection to favour morphs in environments in which their mimicry is most effective, and hence predation is least common. Under such circumstances, selection might exclude all but the most effective morph in a given population, but gene flow among populations experiencing different selection, or negatively frequency-dependent selection by predators, could easily allow less effective morphs to persist at lower frequencies (Bond, 2007). Thus, we would predict a positive relationship across sites between the frequency of a morph and the effectiveness of its mimicry. Here, we examine this prediction in populations of the polymorphic hoverfly *Volucella bombylans* (L. 1758) across the U.K.

The morphs of *V. bombylans* are strikingly different, with each resembling one or more species of bumblebee (Stubbs & Falk, 1983; Howarth, Clee & Edmunds, 1999). The commonest morph in the U.K., *V. bombylans plumata*, resembles black and yellow bumblebees (*Bombus lucorum, B. terrestris* and *B. hortorum*). The other morph that is widespread in the U.K. is *V. bombylans bombylans*, which is black with a red tail and closely resembles *Bombus lapidarius*. A scarce third morph, *V. bombylans haemorrhoidalis*, has both red and yellow bands, and resembles *Bombus pratorum*. The identity and frequency of the species which make up the bumblebee community varies considerably across the sites at which *V. bombylans* is found. If the appearance of *V. bombylans* is the result of selection for mimicry of bumblebees, and if selection is still occurring, we hypothesised that the effectiveness of a morph's mimicry, and hence its relative frequency in the population, will be positively related to the frequency or abundance of the bumblebee taxa that it most closely resembles. We tested this hypothesis using data describing the frequencies of *V. bombylans* morphs and their putative bumblebee models at a large number of sites in the U.K. We also looked for a positive association between model and mimic frequencies across years at a single site where *V. bombylans* was particularly abundant.

Method

Fifty-two sites in Britain where *Volucella bombylans* has been recorded were visited by ME during the flight season (normally June-July) between 2000 and 2011.Twenty nine sites were visited in only one year, 13 were visited in 2 – 6 years, and 10 were visited in more than six years (full details of sites are given in Table S1). All morphs of *V. bombylans* seen resting on flowers or on nearby vegetation were counted, as were all bumblebees visiting the same species of flower. Most bumblebees were identified to species and allocated to one of four common groups according to their appearance (see Table 1).

Of the three principal morphs of *Volucella bombylans* in Britain *V. bombylans plumata* is the commonest: it is typically black with a U-shaped fringe of yellow hairs on the thorax, yellowish hairs at the front of the abdomen, and white hairs at the tip. The amount of yellow on the thorax varies partly because yellow hairs fade and are shed in older, worn insects, but also because of variation in how much of the thorax is covered with yellow hairs. In a small number of insects the central black area is minute or absent, while the hue varies from dull yellow to yellowish brown or occasionally reddish brown so that a few insects resemble *Bombus pascuorum* rather than *Bombus terrestris* (Fig. 1 A – C). However none of the insects we recorded had the brownish abdomen of the brown morph illustrated in Stubbs & Falk (1983). *V. bombylans bombylans* is black with a red tip to the abdomen, but in older worn insects the red fades to dull yellow (Fig. 1 E, F). Very occasionally (just one insect in the present study) individuals are found with white hairs at the tip of the abdomen (Fig. 1 G). It is possible that this represents a distinct rare morph rather than an extreme fading of the red, but this insect was included in *V. bombylans bombylans* in the present study. The third morph, *V. bombylans haemorrhoidalis*, is exactly like var. *plumata* but with a red tail, and in this form too the yellow hairs on the thorax become sparse in worn insects while the red tail fades to yellowish (Fig. 1 I – L). *V. bombylans plumata* resembles the *Bombus terrestris* group of bumblebees, *V. bombylans bombylans* resembles the *Bombus lapidarius* group of bumblebees, and *V. bombylans haemorrhoidalis* resembles the *Bombus pratorum* group of bumblebees.

The latitude and longitude of each site was recorded to allow consideration of spatial (geographical) autocorrelation among the frequencies of the *Volucella bombylans* morphs.

Statistical analysis

Geographic and temporal patterns in the frequencies of *Bombus* spp. and *V. bombylans* morphs were analysed using general linear models (GLMs) in R Version 2.14.0 (R Development Core Team, 2011). Binomial response variables were constructed describing the proportion of all *V. bombylans* individuals which belonged to each morph, and the proportion of all bumblebees which belonged to each taxon. To test our main hypothesis that the frequency of mimic morphs is determined by the frequency or abundance of appropriate model taxa, the relative frequencies and abundances of putative model *Bombus* taxa were fitted as independent variables. Relative frequency was calculated as the proportion of all bumblebees recorded at a site that were of the relevant taxon. Because sampling effort varied among sites, an unbiased measure of absolute abundance was not available; instead, bumblebee abundance was calculated as the number of bees of the relevant taxon observed per individual *V. bombylans* observed.

In preliminary descriptive analysis of bumblebee and *V. bombylans* morph distributions, we used GLMs to test for simple linear effects of latitude and longitude on the probability of occurrence. Exploratory analysis suggested that more complex polynomial effects of latitude or longitude were not present. The significance of terms was tested by deletion from a saturated model (including the interaction between latitude and longitude), with terms which appeared to have the least explanatory power deleted first. Non-random sampling in space meant that there was partial collinearity between latitude and longitude; the results should be interpreted with care in this context.

We tested our main hypothesis in a spatial context by looking at the relationship between model *Bombus* taxa and their putative mimic *V. bombylans* morphs across sites. This analysis was complicated by the possibility that morph frequencies in neighbouring sites were autocorrelated. Such spatial autocorrelation might result, for example, from gene flow among populations, and would mean that sites are not statistically independent, thus increasing the chances of making a type-1 error when testing our hypothesis. To deal with this problem, we examined both the "raw" relationships between the frequencies of the model and mimic taxa, and the relationships which remained once the effects of spatial autocorrelation had been removed. This was achieved by employing spatial eigenvector mapping, following Dormann *et al.* (2007); see also Bivand *et al.* (2013) and Griffith & Peres-Neto (2006). First, we fitted a GLM for each *V. bombylans* morph, with the frequency or abundance of the putative model species as a predictor. Eigenvectors representing the spatial patterns of our sampling sites were then generated using the spdep package in R (Bivand, 2011). Those eigenvector(s) which substantially reduced spatial autocorrelation in the residuals of the fitted GLMs were selected and added as predictors to the model. We used all the eigenvectors required to leave no significant autocorrelation in the model residuals (Moran's I: $\alpha = 0.05$; usually only one eigenvector was needed); in cases where there was no strong autocorrelation initially, we adopted a conservative approach by increasing α to a level at which at least one eigenvector was required, except in one case where there was no detectable autocorrelation even at $\alpha = 0.5$.

Both before and after adding the eigenvetors as predictors, the effect of the frequency or abundance of the putative model species on the frequency of the relevant hoverfly morph was tested by deletion of the relevant term from the model. F-tests or Chi-squared tests, and binomial or quasi-binomial error-structures, were used depending on whether there was evidence of strong over-dispersion (see Crawley, 2007). One-tailed p-values were used to test the relationships between the frequencies of model *Bombus* taxa and their putatively mimetic *V. bombylans* morphs because the prediction *a priori* was that these relationships would be positive. It is important to note that the data describing the frequencies of the different *Bombus* taxa, and of the different *V. bombylans* morphs, are not independent, because an individual which belongs to one taxon cannot by definition belong to the other taxa. Thus, the p-values presented for the different taxa are not statistically independent, and they should be interpreted with caution in this context. A conservative approach to the interpretation of the results would be to consider only the statistics presented for the most common *Bombus* taxon (*B. terrestris* group) and the commonest *V. bombylans* morph (*V. bombylans plumata*). In both the temporal and geographic analyses, we focussed on the putative model *Bombus* groups as predictors of each *V. bombylans* morph frequency, lumping other bumblebees together as non-models in each case. For comparison, however, we also ran analyses where the frequencies of common *Bombus* groups which were *not* the putative models for each *V. bombylans* morph were fitted as independent variables. The results of these analyses are presented in the supplementary information.

Because most sites were only surveyed in a subset of the 12 years for which the study ran, a complete simultaneous analysis of geographic and temporal patterns in the frequencies of the taxa of interest was not possible. We therefore pooled data across years for an analysis which considered geographic variation across all sites, before examining temporal patterns at the three most comprehensively sampled sites (clustered near Bispham, Lancashire) in detail. The Bispham sites were visited three times each year for 11 years, with at least 12 days between visits. Using this method, the chances of recording the same insect on successive visits were minimised: the occasional rarer morph, var. *haemorrhoidalis*, was never found at the same site on consecutive visits, while a mark-release-recapture study of a population of *V. bombylans* in Northamptonshire found that no insects were recaptured after more than 7 days, and there was a daily survival rate of 0.71 (Ball & Morris, 2004).

For the analysis of temporal patterns, GLMs were first fitted with year as a covariate, sampling date (early, mid-season or late) as a fixed factor, and the interaction between year and date. Early samples were taken between 4th and 22nd of June; mid-season samples were taken between 23rd June and 6th July, and late samples were taken between 7th July and 8th August. Exploratory analysis suggested that, while some linear trends were evident over the years, there was not a strong case for the inclusion of polynomial temporal effects in the models. Terms were deleted from the saturated model until no non-significant terms remained, and we then tested the significance of adding the frequency or abundance of the putative model species as a predictor. For comparison, we also tested the frequency or abundance of the putative model in the absence of temporal effects. We checked for remaining temporal structure in the data by testing whether model residuals for samples which were close together in time were either more or less alike than would be expected at random using Mantel tests.

Results

Flowers used for nectar

Table S1 shows the flowers on which *V. bombylans* was found at all of the sites. At almost all sites the flies were on or resting close to just one species of flower, so the bumblebees recorded were also on the same species of flower. Most of the *V. bombylans* were on bramble (*Rubus fruticosus* agg.), marsh thistle (*Cirsium palustre*) or occasionally ragged robin (*Lychnis flos-cuculi*), more rarely on other nearby flowers. Almost all insects were on red, purple or white flowers and only one insect was seen briefly on a yellow flower (*Ranunculus repens*) before flying to its usual flower.

Geographical patterns across sites

Before examining the geographical distribution of the different morphs of *V. bombylans* at sites across the U.K., we looked for patterns in the distribution of the different bumblebee groups. Overall, the *B. terrestris* group was the most frequently encountered (57.0 % of 18,117 bees), followed by *B. pratorum* (17.4 %), *B. pascuorum* (12.3 %) and *B. lapidarius* (9.0 %). *B. monticola* (a montane red-tailed bumblebee very similar to *B. lapidarius*) and unidentified all-black *Bombus* spp. were scarce (4.3 % combined), and were not considered further in the analysis. There were significant latitudinal and/or longitudinal gradients in the frequencies of *B. terrestris*, *B. pratorum* and *B. lapidarius* (see Table 2; Figures 2a, 2b and 3). *B. terrestris* was relatively more common in the north, while the reverse was true for *B. pratorum*. *B. lapidarius* was generally more common in the west, with the opposite being true for *B. pratorum*. The frequency of *B. pascuorum* did not vary significantly with either latitude or longitude.

The most common *V. bombylans* morph seen was *V. bombylans plumata* (83.1 % of 2,098 insects), followed by *V. bombylans bombylans* (15.1 %). The third morph, *V. bombylans haemorrhoidalis*, was very rare (1.8 %). The two common morphs showed reciprocal geographic patterns: *V. bombylans plumata* was relatively more common in eastern and northern sites, while the reverse was true for *V. bombylans bombylans* (see Table 3; Figures 4a, 4b and 5). Before and after accounting statistically for spatial autocorrelation, there was a significant positive relationship between the frequency of *V. bombylans plumata* and both the frequency and the abundance of its putative model, the *B. terrestris* group (see Figure 6a and Table 4). A similar pattern was seen for *V. bombylans bombylans*, the frequency (but not abundance) of which was positively related to the frequency of its model *B. lapidarius* (see Figure 6b), but this relationship was not significant after accounting for spatial autocorrelation and was further weakened (slightly) if data for the rare red-tailed *B. monticola* were combined with those for *B. lapidarius* (results not shown). The distribution of *V.*

bombylans haemorrhoidalis did not show any clear geographic pattern, or any relationship with the frequency or abundance of its putative model, *B. pratorum*, although both were generally less common later in the season.

When analyses were run with non-model *Bombus* groups as predictors, significant negative relationships with the frequency of *V. bombylans plumata* were revealed, both before and after (with one exception) accounting for spatial autocorrelation (see Tables S2 and S3); these negative relationships can be interpreted simply as the reciprocals of the observed *positive* relationships involving the putative model *B. terrestris*. The expected negative relationships between the frequency of the *B. terrestris* group and the frequencies of *V. bombylans bombylans* and *V. bombylans haemorrhoidalis* were also significant, although the latter was not significant after controlling for autocorrelation. Surprisingly, there were also *positive* relationships between the rarer two morphs and the other non-model taxa, some of which remained even after controlling for spatial autocorrelation.

Temporal patterns at Bispham over eleven years

There were no overall differences in *V. bombylans* morph frequencies among the three Bispham sites (Chi-squared = 2.085, p = 0.353, n = 1993), and more detailed preliminary investigations showed no evidence of an effect of site as a factor, so we pooled the data from the three sites for the main analysis.

Before looking for temporal patterns in the frequency of *V. bombylans* morphs at Bispham, we examined patterns in bumblebee frequencies (see Table 5). Overall, the frequencies of the different *Bombus* groups encountered mirrored those seen at all sites combined (see above); about half (51.8 % of 5,156 individuals) were from the *B. terrestris* group, while *B. pratorum* (16.4 %), *B. lapidarius* (18.1 %) and *B. pascuorum* (13.8 %) groups were roughly equal in abundance. There were no long-term trends in the frequencies of *B. terrestris* and *B.*

pratorum groups across the 11 years of sampling, but there were reciprocal significant seasonal differences in the frequencies of these two groups (see Figures 7a and 7b). *B. terrestris* was more abundant relative to the other groups later in the season, while the opposite was true for *B. pratorum*. *B. lapidarius* was significantly less abundant early in the season than later, and was slightly more common in recent years. Finally, there was a small but significant interaction between the effects of year and season on the frequency with which *B. pascuorum* was encountered: it was seen less frequently late in the season in recent years.

Of the three *V. bombylans* morphs, *plumata* was the most frequently seen at Bispham (77.1 % of 1,016 individuals), followed by *bombylans* (20.3 %) and the much rarer *haemorrhoidalis* (2.7 %). Before accounting for seasonal and yearly differences, there were no obvious relationships between the frequencies of any of the morphs and the frequencies or abundances of the appropriate model bumblebee species (fourth and sixth lines of Table 6). However, the frequencies of the two common *V. bombylans* morphs at Bispham varied significantly with season and across years (see Figures 8a and 8b). *V. bombylans plumata* was seen more frequently earlier in the season, and in recent years, while the opposite was true for *V. bombylans bombylans*. For both common morphs, samples in which frequencies were higher than expected given the effects of year and season tended to be those in which higher frequencies of the appropriate model bumblebee species were observed, but these effects were not significant (fifth and seventh lines of Table 6). Frequencies of *V. bombylans haemorrhoidalis* showed no significant patterns either seasonally or across years. The frequencies of the three *V. bombylans* morphs showed no significant associations with non-model bee taxa at Bispham (see Tables S4 and S5).

Once the effects of year and season were accounted for statistically, there was no evidence of additional temporal autocorrelation, which might be expected if negative frequency dependence was influencing changes in morph frequency over time (Mantel tests of the residuals from the minimum adequate generalised linear models: $p > 0.1$ for all morphs).

Discussion

Batesian mimicry

Our results show that the frequencies of *V. bombylans plumata* at sites from south Wales to northern England and southern Scotland are positively related to the frequencies and abundances of the *B. terrestris* group of bumblebees whilst being, if anything, negatively related to the frequencies of other bumblebee groups. This finding supports the hypothesis that the commonest *V. bombylans* morph gains protection through Batesian mimicry of black and yellow bumblebees. Our results also show that frequencies of the less common morph, *V. bombylans bombylans*, are positively related to the frequencies of its putative model, *B. lapidarius,* but negatively related to the frequencies of the *B. terrestris* group. This again is exactly what we predicted we would find if *V. bombylans* gains protection through Batesian mimicry of bumblebees. However, our findings were not entirely clear-cut. After controlling for spatial autocorrelation, the relationship between *V. bombylans bombylans* and its putative model was no longer statistically significant, and the frequencies of this morph also showed unexpected positive relationships with the frequencies of some non-model taxa. Although the results are not unequivocal, to our knowledge our study is the first to have identified a positive association between model and mimic frequencies at a large geographical scale. Our findings thus provide some support for the long-held but seldom tested hypothesis that hoverflies are Batesian mimics of the aversive Hymenoptera that they resemble.

Because this is an observational study, there are of course other possible explanations for the relationships we have observed. Model and mimic frequencies may correlate because they are both influenced by factors other than predation which vary geographically. For example, bumblebee and hoverfly colouration may influence thermoregulation (e.g. Holloway, 1993) or crypsis (although this seems unlikely given their conspicuous yellow, red and black colouration), which in turn may influence fitness, and ultimately relative abundance, in different ways in different locations. Given the intricate and subtle ways in which *V. bombylans* morphs and other syrphids resemble their supposed models, however, such hypotheses seem to us much less plausible than the idea that mimicry explains hoverfly morphology.

It is possible that mimicry in *V. bombylans* is not Batesian. The larvae live in bumblebee nests, feeding on its contents, sometimes including host larvae, although probably only when they are undefended and not of use to the colony (Rupp, 1989; F. S. Gilbert, unpublished). While it is not clear whether this behaviour reduces host fitness, and there is no evidence that *V. bombylans* morphs specialise in inhabiting the colonies of matching host species, it is possible that the resemblance of the adult hoverfly to the host helps *V. bombylans* evade detection and attack by the host colony when laying eggs. Further experiments are required to investigate this hypothesis of “aggressive” mimicry, but at present it seems less plausible than the idea that *V. bombylans* is a Batesian mimic (F. S. Gilbert, unpublished).

Residuals from preliminary GLMs of *V. bombylans* morph frequencies were spatially autocorrelated, and controlling for this autocorrelation weakened the statistical support for some of the predicted relationships (most notable the positive relationship between *V. bombylans bombylans* and *B. lapidarius*). There are many possible sources of spatial structure in the residuals, but an obvious explanation is that neighbouring hoverfly populations are not independent because they are connected by dispersal. The persistence of the predicted positive association between *V. bombylans plumata* and *B. terrestris* when autocorrelation was removed suggests, however, that this association is not a statistical artefact. Nevertheless, while much work has been done recently to devise methods to account for the effects of spatial autocorrelation on type-1 error rates in observational studies of spatial patterns in biology (Dormann *et al.*, 2007), the causal relationships underpinning observed correlations in space will remain unconfirmed unless their study is augmented by appropriate manipulative experiments.

Experimental manipulation of mimic frequencies and direct measurement of selection coefficients could confirm once and for all that hoverflies are Batesian mimics, but such experiments are extremely difficult to conduct. Close parallels, however, can be found in studies which have sought to demonstrate the adaptive value of cryptic colouration. In several polymorphic cryptic species, it has been shown that on appropriate backgrounds, better camouflaged morphs receive less predation than more conspicuous morphs. Most of these studies involved observations of attacks by captive predators on prey such as praying mantids, grasshoppers, fish, moths and caterpillars placed against appropriate backgrounds (e.g. Edmunds, 1974 for references to earlier experiments; Mariath, 1982; Edmunds & Grayson, 1991). Because they are conducted in artificial conditions, such experiments do not provide direct evidence of selection pressures acting on natural populations. The best known study of selective predation on different morphs in wild populations is that of Sheppard (1951) on the banded snail (*Cepaea nemoralis*). He found that, in April, song thrushes (*Turdus merula*) took many more yellow snails (yellow-green in life) because these were conspicuous on the brown woodland floor, but by late May when the ground was green with low-growing plants they took fewer yellow and more brown snails because by then the browns were more conspicuous than the yellows. So in this instance the direction of selective predation varies seasonally, but over the geographical range of the snail there are many other factors known to be important in determining the fitness of different morphs, including the effect of shell banding, apostatic selection and climate (e.g. Ożgo & Schilthuisen, 2012, who give references to many earlier papers).

The only study that we know of involving selective predation of a polymorphic mimetic insect involved the diadem butterfly (*Hypolimnas misippus*) in tropical and southern Africa, the females of which resemble different morphs of the African queen (*Danaus chrysippus*). The African queen is now considered to be a superspecies comprising four semispecies which evolved in different parts of the continent, but which meet and hybridise in east and central Africa (recently reviewed by Gordon, Edmunds, Edgar, Lawrence & Smith, 2010). The diadem has four morphs corresponding to these four semispecies, but, contrary to expectation if they are Batesian mimics, all morphs occur throughout sub-saharan Africa, irrespective of the local model, with the same two morphs predominating in all populations. Initial work on a population in Ghana showed that when the white hind-winged model was common, the diadems with some white on the hind wings were at a relatively high frequency in the population and had a high survival rate, but when the model became scarce the white hind-winged diadems became rarer and had a lower survival rate (Edmunds, 1969). This supports the contention that diadems in Ghana gain protection through Batesian mimicry for part of the year. More extensive studies on populations in Ghana and Tanzania showed that the situation is much more complex: in both populations, occasional changes in morph frequency favouring rarer and mimetic morphs were followed by linkage disequilibrium between forewing and hindwing patterns (Gordon *et al*., 2010). In both populations the evidence was consistent with selective predation of non-mimetic forms and selection for perfection of mimicry of the hindwings in Ghana and of forewings in Tanzania, but such selection only occurred occasionally.

Although the frequencies of *V. bombylans* morphs were also positively associated with those of their putative bumblebee models across years at our best sampled site(s), these temporal relationships were not significant. It is possible that this is the result of a similar situation to that seen in the diadem: if differential selective predation on one or other morph only occurs occasionally, a longer time-series of observations or a different approach such as mark-release-recapture studies might be required to detect it.

Alternatively, lags in the effect of relative abundance of models on predation rates may make the influence of selection difficult to detect in our time-series. Further analysis showed no evidence of a seasonally- or annually-lagged relationship between model and mimic frequencies (data not shown), but ultimately a longer time-series is needed to investigate fully temporal feedback between model and mimic relative abundances. It may also be the case that the temporal resolution of three sampling periods per year was not appropriate to detect the effects of interest: a study of three sites in northern England with hourly sampling found evidence of associations at a finer temporal scale between the frequencies of mimetic hoverflies, including *V. bombylans*, and their models (Howarth *et al.*, 2004). These associations are suggestive of behavioural mimicry by hoverflies, but are generally consistent with the geographic patterns in *V. bombylans* morph frequencies.

Seasonal and geographical patterns in relative abundance

We found both seasonal and geographical variations in the relative abundances of different species of bumblebee. It is well known that *Bombus pratorum* starts its colonies early in the season and rears males and fertile females in early summer so that the colonies decline in mid- to late summer, well before most other species of bumblebee (Prŷs-Jones & Corbet, 1987), and our results confirm this. However, our findings that the *B. terrestris* group is more frequent in the north relative to *B. pratorum* while *B. lapidarius* is more frequent in the west relative to *B. pratorum* do not appear to have been reported before. *V. bombylans* also shows geographical variation, with *V. bombylans plumata* more frequent in the east and north while *V. bombylans bombylans* is more frequent in the south and west. There were insufficient data on the third morph, *V. bombylans haemorrhoidalis,* to draw any conclusions, but we note that this morph is widespread in France; for example at two sites in Brittany in 2011 it replaced *V. bombylans bombylans* as the second most frequent morph, yet the frequencies of the bumblebee taxa were very similar to those in the U.K. (ME unpublished data).

Most bumblebee species forage from a wide variety of flowers according to availability, with the relative frequencies foraging on a particular flower varying in different species of bumblebee (Benton, 2006). At Bispham and some other sites it was noticeable that *B. lapidarius* and *B. pascuorum* were more commonly seen on low growing Fabaceae (e.g. *Trifolium* and *Lotus* spp.) than were species in the *B. terrestris* group, but we only counted those bees that were seen on the plant used by *V. bombylans* for feeding and resting (*Rubus fruticosus* at Bispham). Thus the relative numbers of the different species of bumblebee at each site may have been different from those recorded here, but we justify this on the grounds that if there is selective predation of *Volucella* morphs then it is likely to be in the vicinity of the plant where it is most commonly found. Whether bumblebee frequency or abundance is a more important determinant of predator behaviour towards putative mimics is unknown; if birds, for example, really do learn to avoid mimetic hoverflies through prior experience with aversive model taxa, both the relative and absolute rates of encounter with models could conceivably influence the effectiveness or speed of learning.

Conclusion

Our results provide indirect evidence that the remarkable resemblance of *V. bombylans* morphs to common bumblebee species provides them with protection from predation, and hence that bumblebee community composition determines equilibrium morph frequencies in any given *V. bombylans* population. This and other recent research underlines the fact that the study of conspicuously polymorphic animals, which has a rich history stretching back over

150 years, continues to contribute to our understanding of the selective forces which have shaped the evolution of phenotypes in natural populations.

Acknowledgements

We wish to thank everyone who has helped with facilitating visits to sites and other advice and encouragement, especially Staff of Natural England and the Field Studies Council (Preston Montford), Stuart Ball, Barry Brigden, Janet Edmunds, Francis Gilbert, Brigitte Howarth and Yvonne Golding. We are also grateful to two anonymous reviewers for comments on an earlier version of the manuscript.

Table 1. Bumblebee groups identified in surveys of sites for *V. bombylans* morphs and their putative models. The vast majority of bees recorded were *Bombus* spp., but a few cuckoo bees (*Psithyris* spp.) were encountered. In addition to those species in the four groups listed, *Bombus monticola*, a black bumblebee with a large red tail, was seen at low frequencies at upland sites, and a small number of unidentified all-black bumblebees were encountered (possibly *B. ruderatus*, but more likely a black mutant of a common bumblebee); these scarce taxa were excluded from analyses.

Group	Taxa included	Description
Bombus terrestris	*B. terrestris*, *B. lucorum*, *B. hortorum*, *B. soroeensis*, *B. (Psithyris) vestalis*, *B. (P.) barbutellus*, *B. (P.) bohemicus*	Black and yellow bumblebees
Bombus lapidarius	*B. lapidarius* & *B. (P.) rupestris*	Black bumblebees with red tails
Bombus pratorum	*B. pratorum* & *B. (P.) sylvestris*	Black and yellow bumblebees with rusty red tails
Bombus pascuorum	*B. pascuorum*, *B. hypnorum* & *B. (P.) campestris*	Reddish or yellowish brown bumblebees, though with some black, especially in worn specimens

Table 2. Results of generalised linear models with quasi-binomial errors testing the effect of latitude and longitude on the proportion of different species of bumblebees seen at sites in the U.K. Statistics are reported for the effect of deleting the term of interest from the model during backwards step-wise model selection. Statistically significant results are in bold.

Term	*Bombus terrestris*			*Bombus pratorum*			*Bombus lapidarius*			*Bombus pascuorum*		
	F	df	P	F	df	P	F	df	P	F	df	P
Latitude	12.458	1,51	< **0.001**	11.542	1,51	**0.001**	1.131	1,50	0.293	2.217	1,51	0.143
Longitude	0.111	1,50	0.740	1.9591	1,50	0.168	6.643	1,51	**0.013**	1.106	1,50	0.298
Latitude x longitude	0.176	1,49	0.677	0.177	1,49	0.676	0.069	1,49	0.794	1.726	1,49	0.195

Table 3. Results of generalised linear models with quasi-binomial errors testing the effect of latitude, longitude on the proportion of different morphs of *V. bombylans* seen at sites in the U.K. Statistics are reported for the effect of deleting the term of interest from the model during backwards step-wise model selection. Statistically significant results are in bold.

Term	*Volucella bombylans plumata*			*Volucella bombylans bombylans*			*Volucella bombylans haemorrhoidalis*		
	F	df	P	F	df	P	F	df	P
Latitude	3.228	1,50	0.078	4.653	1,50	**0.036**	0.101	1,51	0.752
Longitude	12.053	1,50	**0.001**	12.353	1,50	< **0.001**	3.676	1,50	0.061
Latitude x longitude	2.339	1,49	0.133	2.815	1,49	0.100	1.192	1,49	0.280

Table 4. Results of generalised linear models with quasi-binomial errors testing the effect of the relative frequency and abundance of the appropriate model bumblebee species on the proportion of different morphs of *V. bombylans* seen at sites in the U.K. The effect of the frequency/abundance of the appropriate model species was tested both with and without spatial filters (generated by spatial eigenvector mapping) fitted as covariates to remove spatial autocorrelation. All tests are one-tailed because the null hypothesis for each was directional (relationships were predicted to be positive). Statistically significant results are in bold.

Morph	Bumblebee frequency as predictor						Bumblebee abundance as predictor					
	Without eigenvector maps			With eigenvector maps			Without eigenvector maps			With eigenvector maps		
	F	df	P	F	df	P	F	df	P	F	df	P
Volucella bombylans plumata	27.728	1,51	**< 0.001**	20.655	1,50	**< 0.001**	17.308	1,51	**< 0.001**	No detectable autocorrelation		
Volucella bombylans bombylans	9.070	1,51	**0.001**	0.017	1,50	0.552	0.003	1,51	0.494	0.209	1,50	0.325
Volucella bombylans haemorrhoidalis	0.089	1,51	0.384	0.400	1,50	0.265	8.278	1,51	0.503	8.434	1,50	0.502

Table 5. Results of generalised linear models with quasi-binomial errors testing the effect of year and sampling date (early-, mid- and late-season) on the proportion of different groups of bumblebees seen at Bispham. Statistics are reported for the effect of deleting the term of interest from the model during backwards step-wise model selection. Statistically significant results are in bold.

Term	*Bombus terrestris*				*Bombus pratorum*				*Bombus lapidarius*				*Bombus pascuorum*			
	Residual deviance	Change in deviance	df	P	Residual deviance	Change in deviance	df	P	Residual deviance	Change in deviance	df	P	Residual deviance	Change in deviance	df	P
Year	156.73	0.822	1	0.710	399.51	35.643	1	0.134	193.74	43.037	1	**0.015**	78.697	5.209	1	**0.023**
Sampling date	157.55	47.839	2	**0.016**	435.15	400.47	2	**< 0.001**	193.74	178.58	2	**< 0.001**	78.697	4.686	2	0.096
Year x sampling date	151.73	5.002	2	0.670	380.86	18.642	2	0.564	189.92	3.8126	2	0.780	72.126	6.571	2	**0.038**

Table 6. Results of generalised linear models with binomial errors testing the effect of year, sampling date (early-, mid- and late-season) and the relative frequency and abundance of the appropriate model bumblebee species on the proportion of different morphs of *V. bombylans* seen at Bispham. Statistics are reported for the effect of deleting the term of interest from the model during backwards step-wise model selection. The effect of the appropriate model species was tested both before and after accounting for variation among years and sample dates (fifth and seventh lines). Statistically significant results are in bold.

Term	*Volucella bombylans plumata*				*Volucella bombylans bombylans*				*Volucella bombylans haemorrhoidalis*			
	Residual deviance	Change in deviance	df	P	Residual deviance	Change in deviance	df	P	Residual deviance	Change in deviance	df	P
Year	39.375	7.363	1	**0.007**	26.190	16.913	1	< **0.001**	27.663	3.469	1	0.063
Sampling date	39.375	6.833	2	**0.033**	26.190	12.611	2	**0.002**	27.055	0.609	2	0.738
Year x sampling date	37.483	1.893	2	0.388	25.985	0.204	2	0.903	26.246	0.809	2	0.667
Frequency of model species alone	54.552	0.456	1	0.250[a]	56.486	0.009	1	0.538[a]	31.009	0.123	1	0.725
Abundance of model species alone	54.988	0.020	1	0.444[a]	61.222	0.288	1	0.296[a]	31.092	0.040	1	0.421[a]
Frequency of model species with year and sampling date	37.326	2.050	1	0.076[a]	26.163	0.027	1	0.435[a]	NA (year and sampling date not significant)			
Abundance of model species with year and sampling date	38.098	1.277	1	0.129[a]	38.936	0.527	1	0.234[a]	NA (year and sampling date not significant)			

[a] one-tailed p-value

References

Abrams, P. A. 2009. When does greater mortality increase population size? The long history and diverse mechanisms underlying the hydra effect. *Ecology Letters* **12**: 462-474.

Ball, S. G. and R. K. A. Morris. 2004. A mark-release-recapture study of *Volucella bombylans* (Linnaeus), *V. inflata* (Fabricius) and *V. pellucens* (Linnaeus) (Diptera, Syrphidae). *Dipterists Digest* **10**: 73-83.

Bates, H. W. 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. *Trans. Linn. Soc. London* **23**: 495-566.

Benton, T. 2006. *Bumblebees*. Collins New Naturalist, London, U.K.

Bivand, R. 2007. spdep: Spatial dependence: weighting schemes, statistics and models. R package, Version 0.5 – 60.

Bivand, R. S., Pebesma, E. and V. Gómez-Rubio. 2013. *Applied Spatial Data Analysis with R*. Springer. New York, USA.

Bond, A. B. 2007. The evolution of color polymorphism: crypticity, searching images, and apostatic selection. *Ann. Rev. Ecology, Evolution and Systematics* **38**: 489-514.

Cott, H. B. 1940. *Adaptive Coloration in Animals*. Methuen, London, U.K.

Crawley, M. J. 2007. *The R Book.* Wiley-Blackwell. London, U.K.

Dlusskii, G. M. 1984. Are dipterous insects protected by their similarity to stinging hymenopterans? (in Russian). *Byulleten' Moskovskogo Obshchestva Ispytatelei Priorody, Otdel Biloogicheskii* **89**: 25-40.

Dormann, C. F., McPherson, J. M., Araújo, M. B., Bivand, R., Bolliger, J., Carl, G., Davies, R. G., Hirzel, A., Jetz, W., Kissling, W. D., Kühn, I., Ohlemüller, R., Peres-Neto, P. R., Reineking, B., Schröder, B., Schurr, F. M. and R. Wilson. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. *Ecography* **30**: 609-628.

Edmunds, M. 1969. Polymorphism in the mimetic butterfly *Hypolimnas misippus* L. in Ghana. *Heredity* **24**: 281-302.

Edmunds, M. 1974. *Defence in Animals: a Survey of Anti-predator Defences*. Longman, Harlow, U.K.

Edmunds, M. 2000. Why are there good and poor mimics? *Biol. J. Linn. Soc.* **70**: 459-466.

Edmunds, M. 2008. Hoverflies: the garden mimics. *Biologist* **55**: 202-207.

Edmunds, M. and J. Grayson. 1991. Camouflage and selective predation in caterpillars of the poplar and eyed hawkmoths (*Laothoe populi* and *Smerinthus ocellata*). *Biol. J. Linn. Soc.* **42**: 467-480.

Gilbert, F. 2005. The evolution of imperfect mimicry. Pp 231-288 *in* Fellowes, Holloway Rolff eds. Insect Evolutionary Ecology. .) CABI, Wallingford, U.K.

Gordon, I. J., Edmunds, M., Edgar, J. A., Lawrence, J. and D. A. S. Smith. 2010. Linkage disequilibrium and natural selection for mimicry in the Batesian mimic *Hypolimnas misippus* (L.) (Lepidoptera: Nymphalidae) in the Afrotropics. *Biol. J. Linn. Soc.* **100**: 180-194.

Griffith, D. A., and Peres-Neto, P. R. (2006). Spatial modeling in ecology: the flexibility of eigenfunction spatial analyses. *Ecology*, **87**: 2603-2613.

Holloway, G. J. 1993. Phenotypic variation in colour pattern and seasonal plasticity in *Eristalis* hoverflies (Diptera: Syrphidae). *Ecological Entomology* **18**: 209–217.

Howarth, B., Clee, C. and M. Edmunds. 1999. The mimicry between British Syrphidae (Diptera) and aculeate Hymenoptera. *British J. Ent. Nat. Hist.* **13**: 1-39.

Howarth, B., Edmunds, M. and F. Gilbert. 2004. Does the abundance of hoverfly (Syrphidae) mimics depend on the numbers of their hymenopteran models? *Evolution* **58**: 367-375.

Mariath, H. A. 1982. Experiments on the selection against different colour morphs of a twig caterpillar by insectivorous birds. *Zeitschrift fűr Tierpsychologie* **60**: 135-146.

Mostler, G. 1935. Beobachtungen zur Frage der Wespenmimikry. *Zeitschrift fűr Morphologie und Őkologie der Tiere* **29**: 381-454.

Ożgo, M. and M. Schilthuizen. 2012. Evolutionary change in *Cepaea nemoralis* shell colour over 43 years. *Global Change Biology* **18**: 74-81.

Penney, H. D., Hassall, C., Skevington, J. H., Abbott, K. R. and T. N. Sherratt. 2012. A comparative analysis of the evolution of imperfect mimicry. *Nature*, **483**: 461-464.

Prŷs-Jones, O. E. and S. A. Corbet. 1987. *Bumblebees*. Cambridge Univ. Press, Cambridge, U.K.

R Development Core Team. 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rotheray, G. and F. Gilbert. 2011. *The Natural History of Hoverflies*. Forrest Text, Tresaith, U.K.

Rupp, L. 1989. Die mitteleuropäischen Arten der Gattung *Volucella* (Diptera, Syrphidae) als Kommensalen und Parasitoide in den Nestern von Hummeln und sozialen Wespen: Untersuchungen zur Wirtsfindung, Larvalbiologie und Mimikry. Dissertation. Albert-Ludwigs-Universität, Freiburg-im-Breisgau.

Ruxton, G. D., Sherratt, T. N. and M. P. Speed. 2004. *Avoiding Attack. The Evolutionary Ecology of Crypsis, Warning Signals & Mimicry*. Oxford Univ. Press, Oxford, U.K.

Stubbs, A. E. and S. J. Falk. 1983. *British Hoverflies: an Illustrated Identification Guide*. British Entomological & Natural History Society, London, U.K.

Figure Legends

Figure 1. The three morphs of *Volucella bombylans*: A-C *plumata*; E-G *bombylans*; I-K *haemorrhoidalis*, and examples of putative model bumblebee taxa (D, H & L). A, E & I are fresh insects with bright colours, A & I with almost no black in centre of thorax; B is unusually reddish brown; B & J have typical U-shaped yellow mark on thorax; C, F & K are worn insects with faded colours; G is possibly a different morph rather than var. *bombylans* with white tail. D is *B. hortorum*, from the *B. terrestris* group, H is *B. lapidarius* and L is *B. pratorum*.

Figure 2. Effect of a) latitude and b) longitude on frequencies of different *Bombus* groups (data pooled across sites).

Figure 3. Distribution of different *Bombus* groups across sites in the U.K. Sample size is indicated by the size of the pies: small n < 20, medium 20 < n < 200, large 200 < n < 4200.

Figure 4. Effect of a) latitude and b) longitude on frequencies of different *V. bombylans* morphs (data pooled across sites).

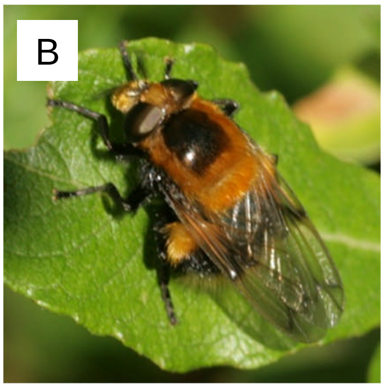
Figure 5. Distribution of different *V. bombylans* morphs across sites in the U.K. Sample size is indicated by the size of the pies: small n < 10, medium 10 < n < 100, large 100 < n < 700.

Figure 6. Relationship between the frequency of two mimetic *V. bombylans* morphs and their putative bumblebee models across sites in the U.K: a) *V. bombylans plumata* and its putative model *B. terrestris* and b) *V. bombylans bombylans* and its putative model *B. lapidarius*. The diameter of each data point is proportional to the sample size for *V. bombylans*.

Figure 7. Effect of a) year and b) sampling date on frequencies of different *Bombus* groups at Bispham.

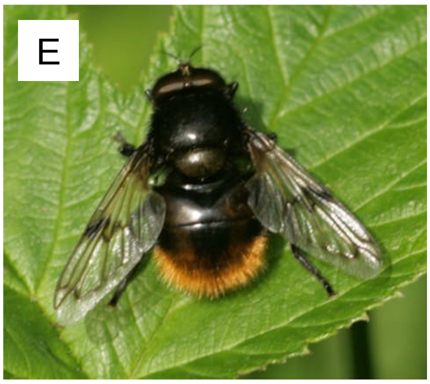
Figure 8. Effect of a) year and b) sampling date on frequencies of different *V. bombylans* morphs at Bispham.


A


B


C


D


E


F


G


H

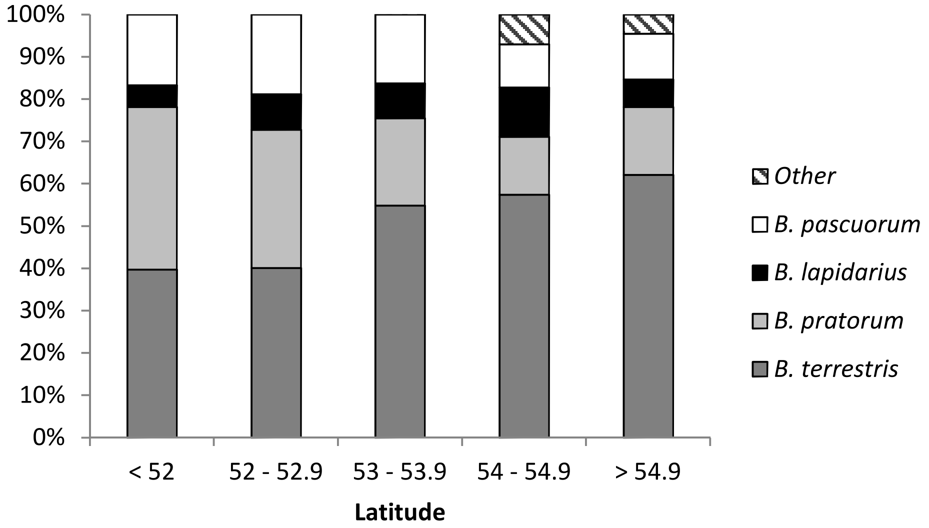

I


J

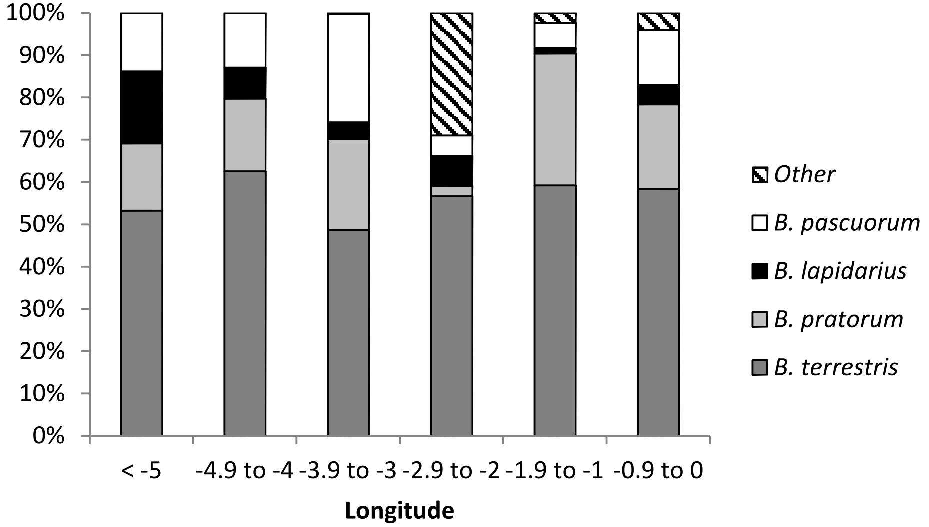

K

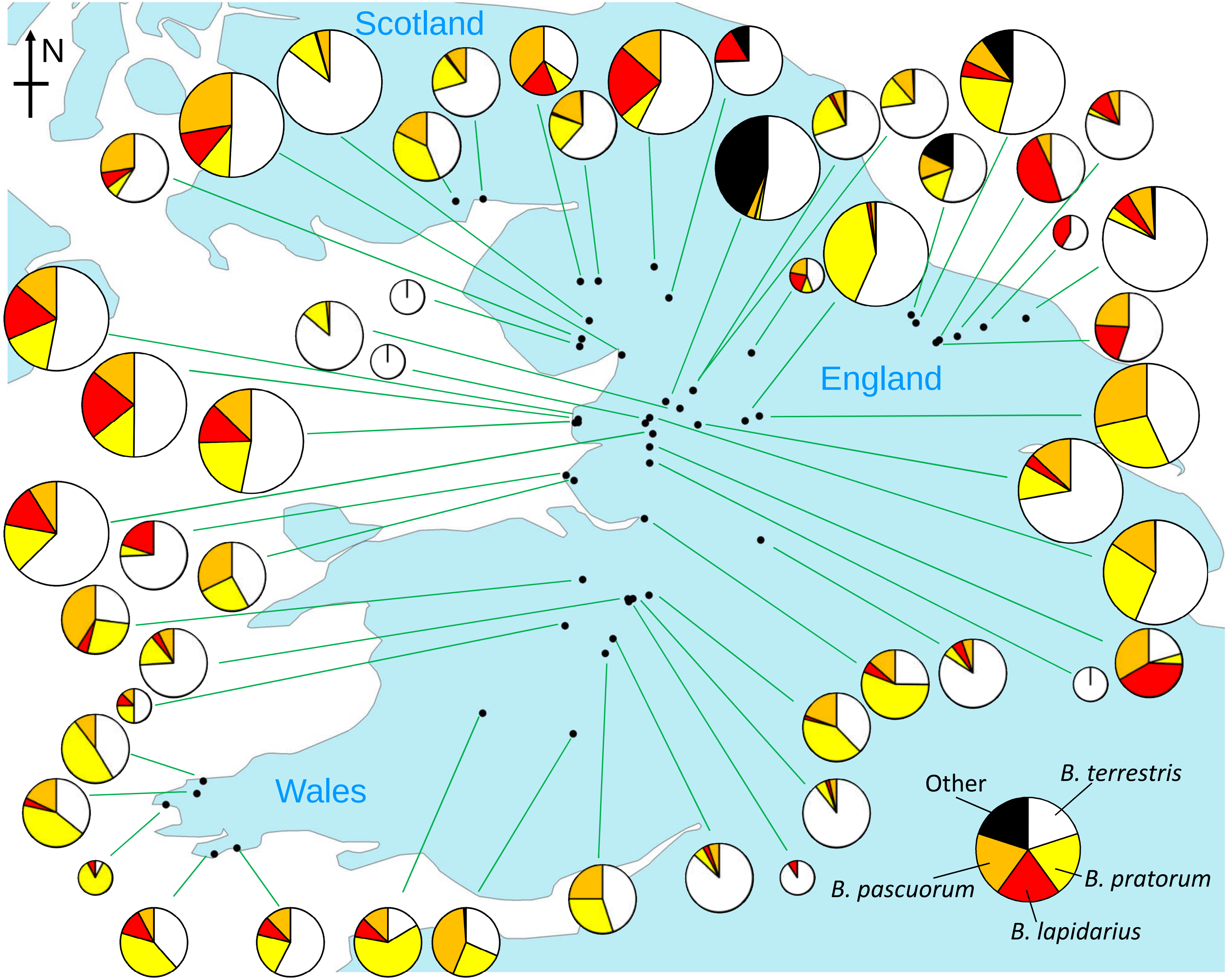

L

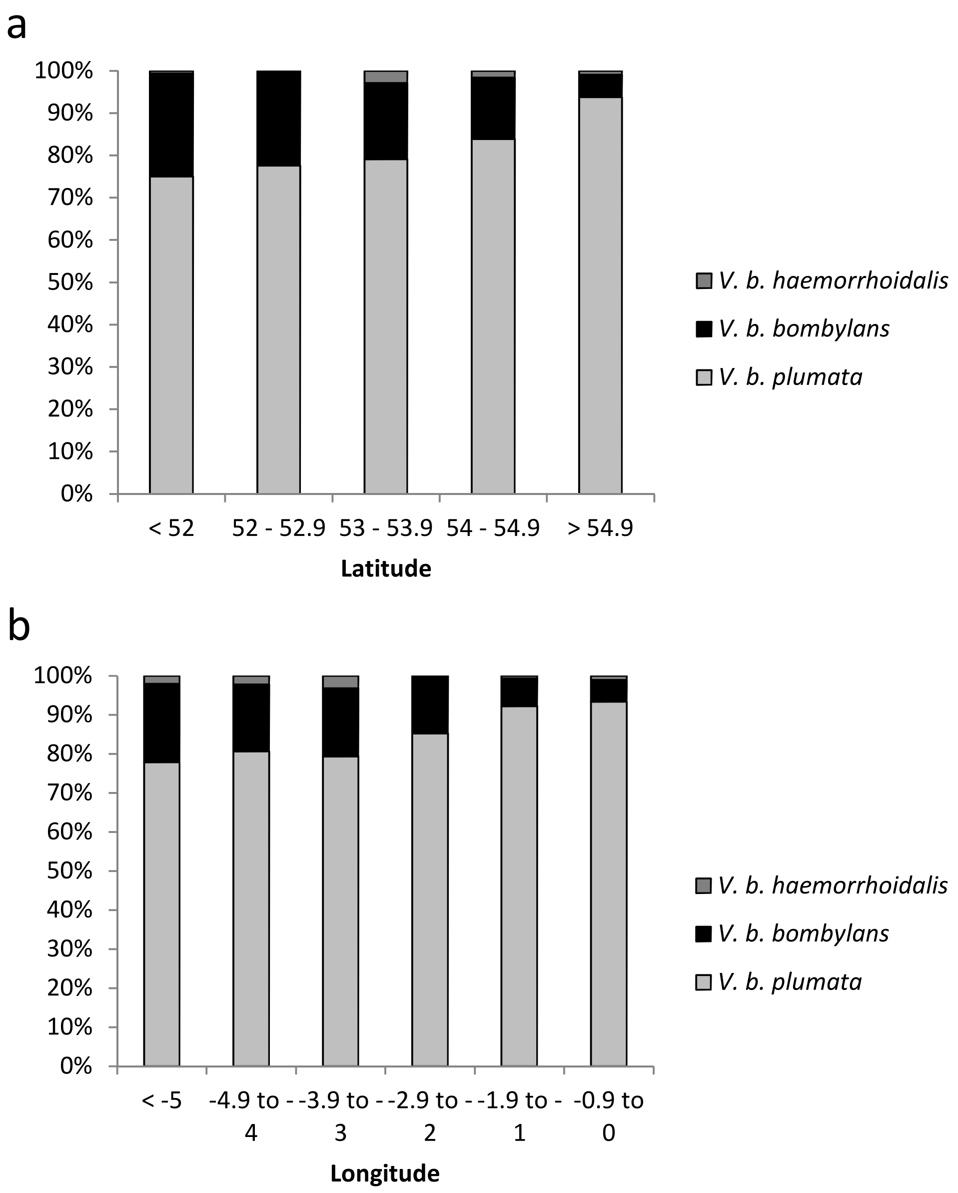
a

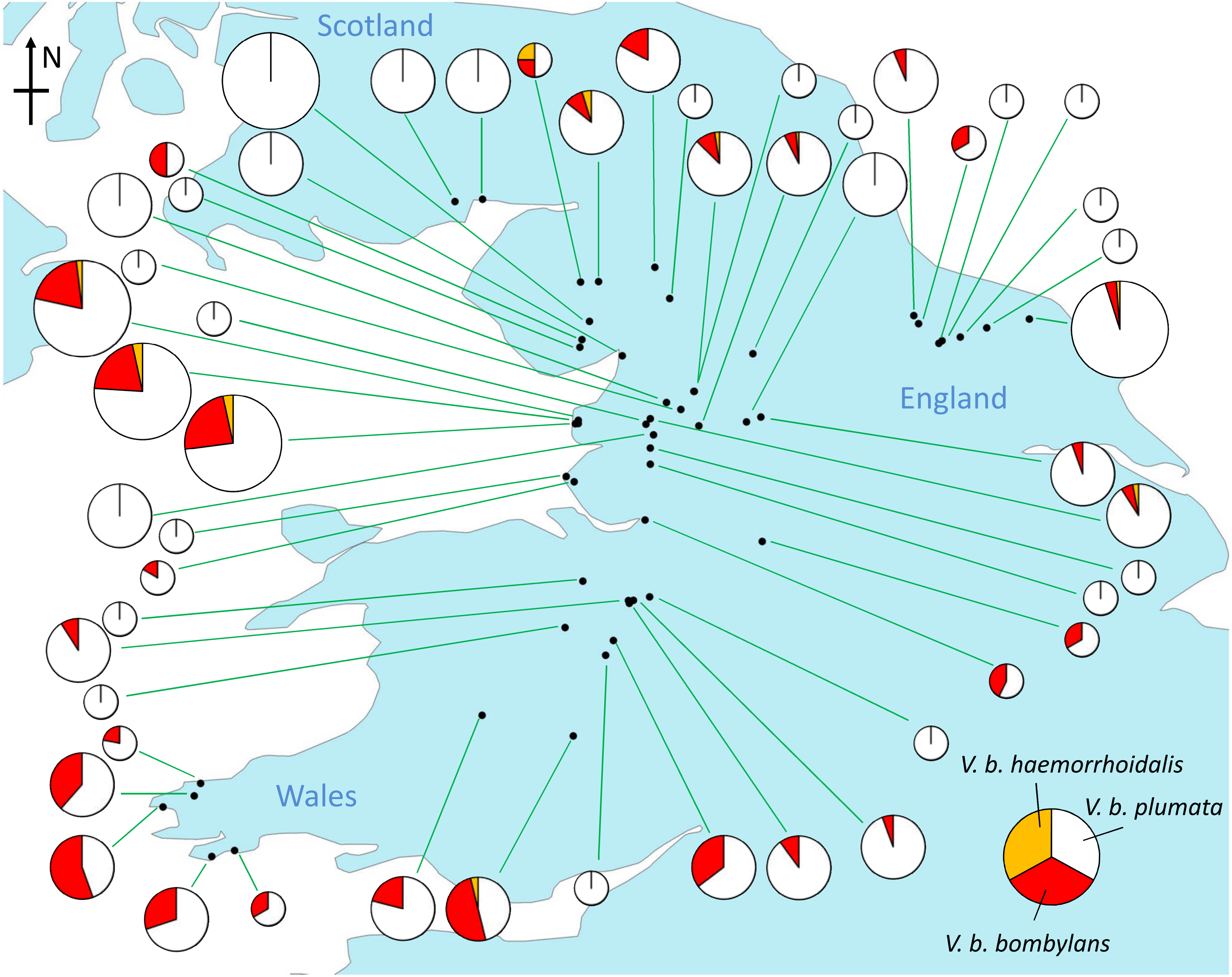

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
< 52
52 - 52.9
53 - 53.9
54 - 54.9
> 54.9
Latitude
Other
B. pascuorum
B. lapidarius
B. pratorum
B. terrestris

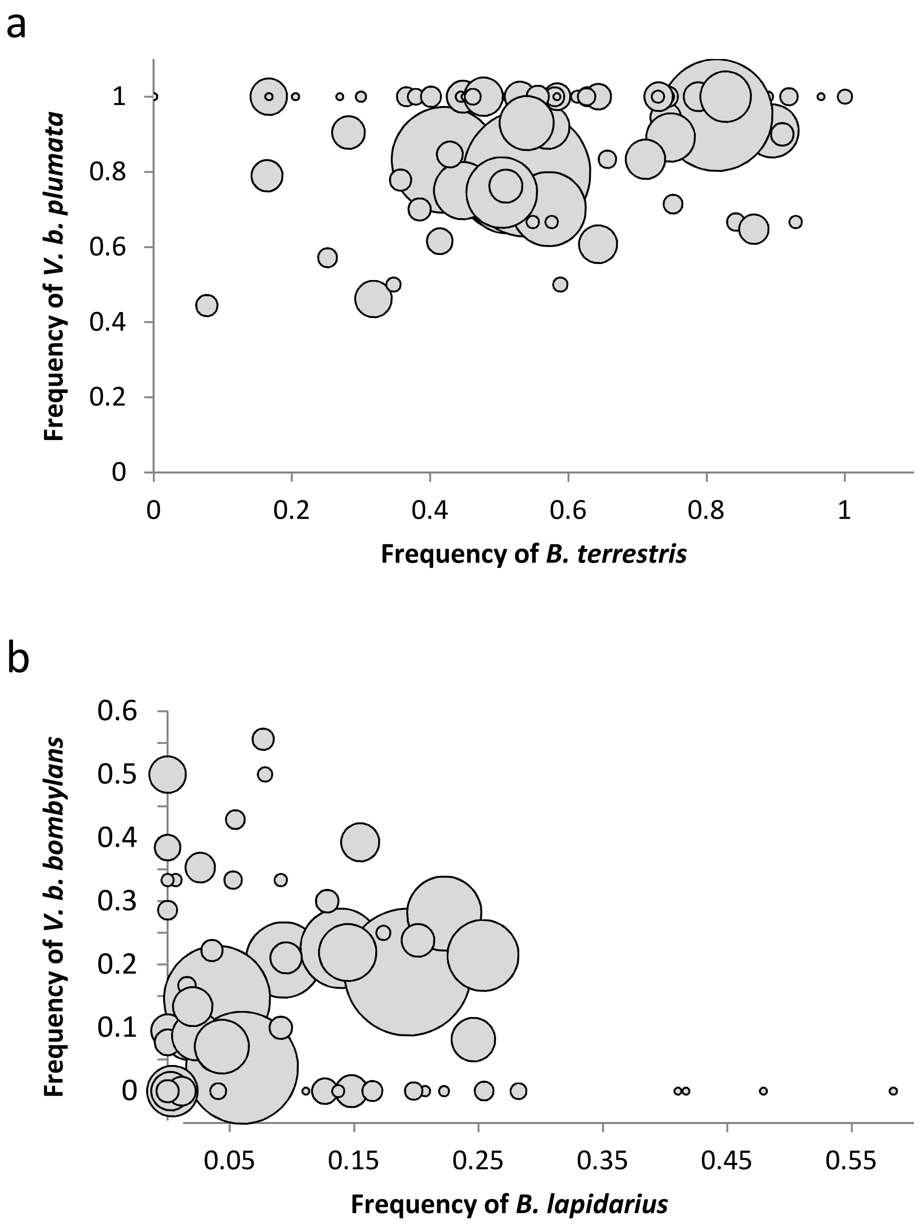
b


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
< -5
-4.9 to -4
-3.9 to -3
-2.9 to -2
-1.9 to -1
-0.9 to 0
Longitude
Other
B. pascuorum
B. lapidarius
B. pratorum
B. terrestris

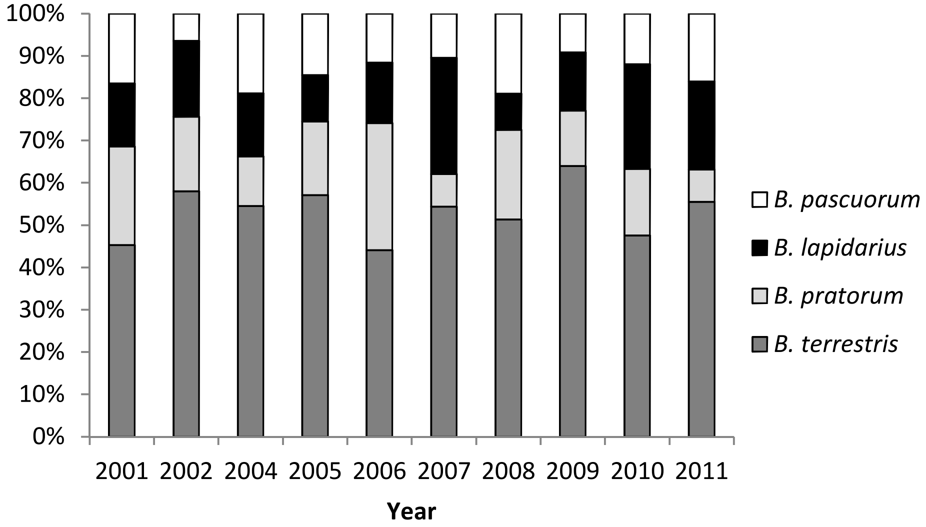

N
Scotland
England
Wales
Other
B. terrestris
B. pascuorum
B. pratorum
B. lapidarius


a
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
< 52
52 - 52.9
53 - 53.9
54 - 54.9
> 54.9
Latitude
▨ *V. b. haemorrhoidalis*
■ *V. b. bombylans*
□ *V. b. plumata*
b
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
< -5
-4.9 to - 4
-3.9 to - 3
-2.9 to - 2
-1.9 to - 1
-0.9 to 0
Longitude
▨ *V. b. haemorrhoidalis*
■ *V. b. bombylans*
□ *V. b. plumata*

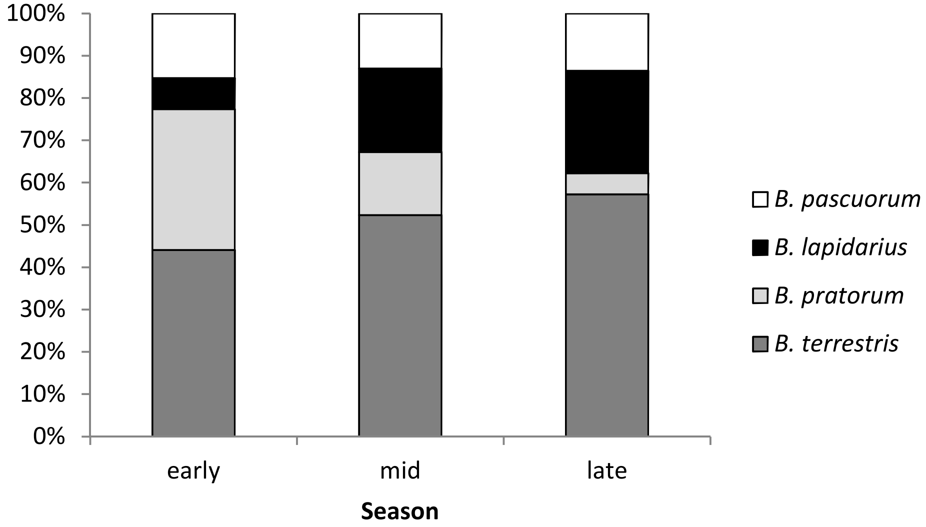

N
Scotland
England
Wales
V. b. haemorrhoidalis
V. b. plumata
V. b. bombylans


a
Frequency of *V. b. plumata*
0
0.2
0.4
0.6
0.8
1
0
0.2
0.4
0.6
0.8
1
Frequency of *B. terrestris*
b
Frequency of *V. b. bombylans*
0
0.1
0.2
0.3
0.4
0.5
0.6
0.05
0.15
0.25
0.35
0.45
0.55
Frequency of *B. lapidarius*

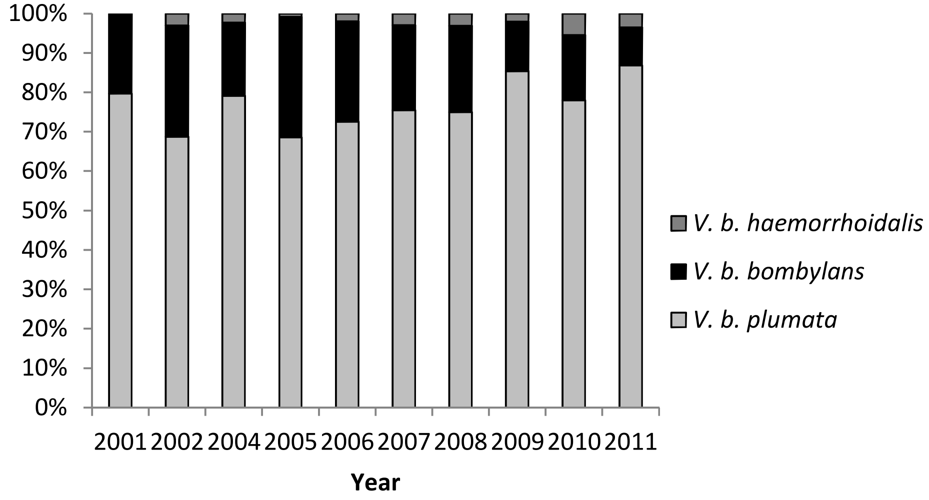
a


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2001
2002
2004
2005
2006
2007
2008
2009
2010
2011
Year
□ B. pascuorum
■ B. lapidarius
□ B. pratorum
■ B. terrestris

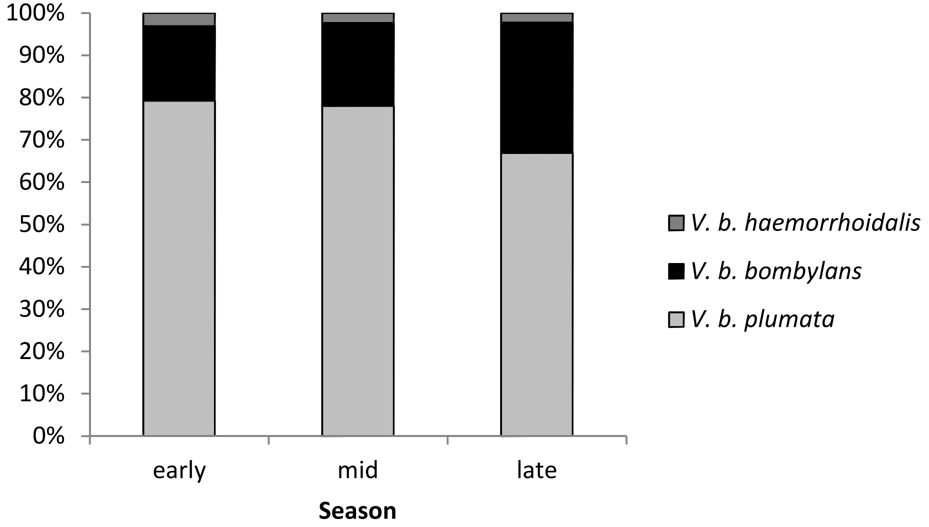
b


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
early
mid
late
Season
□ B. pascuorum
■ B. lapidarius
□ B. pratorum
■ B. terrestris

a


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2001
2002
2004
2005
2006
2007
2008
2009
2010
2011
Year
▨ *V. b. haemorrhoidalis*
■ *V. b. bombylans*
□ *V. b. plumata*

b


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
early
mid
late
Season
▨ *V. b. haemorrhoidalis*
■ *V. b. bombylans*
□ *V. b. plumata*