

THE BUMBLEBEE *BOMBUS HORTORUM* IS THE MAIN POLLINATING VISITOR TO *DIGITALIS PURPUREA* (COMMON FOXGLOVE) IN A U.K. POPULATION

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Abstract—Specialization in plant-pollinator systems represents an important issue for both the ecological understanding and conservation of these systems. We investigated the extent to which the bumblebee *Bombus hortorum* (Linnaeus) is the main potential pollinator of Common Foxglove, *Digitalis purpurea* L. Twenty *D. purpurea* patches were selected in North Yorkshire, U.K., ten each in woodland and garden or park habitat. All insects visiting *D. purpurea* within the patches were recorded over seventy 30-min bouts. The relative frequency of insect visitors to other flowering plant species within 15 m of each patch was also determined. *B. hortorum* and *B. pascuorum* were the two most frequent visitors to *D. purpurea*, accounting for 82 - 92% and 3 -17%, respectively, of all insect visits ($n = 1682$), depending on habitat. *B. hortorum* showed a significant preference for visiting *D. purpurea* relative to its frequency of visits to other available plant species. The relationship of *D. purpurea* with *B. hortorum*, which pollinates several plant species with long corollas, therefore represents a potential case of asymmetric specialization, albeit one that may vary spatially. Because *D. purpurea* reproduction appears dependent on insect pollination, *B. hortorum* and *B. pascuorum* may help underpin the viability of *D. purpurea* populations.

Keywords: Bumblebee, Foxglove, mutualism, pollination, specialization

INTRODUCTION

The degree to which plant-pollinator systems are specialized (reliant on one or a few plant or pollinator partners) is a major issue in the study of insect pollination (Waser and Ollerton 2006). Specialization informs our understanding of the ecology and evolution of these systems (Ollerton et al. 2006) and affects the provision of pollination services and the risk of extinction in both pollinators and plants in the face of population declines in their partners (Memmott et al. 2004; Waser and Ollerton 2006; Kleijn and Raemakers 2008). Investigating potential cases of specialization in plant-pollinator systems is particularly important when, unlike the situation in species such as insect-mimicking orchids (Proctor et al. 1996), floral morphology does not immediately suggest the presence of specialization.

Bumblebees (*Bombus* spp) are major insect pollinators of both wild flowers and flowering crops (Goulson 2010). In the U.K. *Bombus* fauna, *B. hortorum* (Linnaeus) is unusual in having the longest tongue (proboscis) among the remaining, widespread species (Prys-Jones and Corbet 1991; Goulson et al. 2011). Correspondingly, it is well known to favour flowering plant species with long corollas (Benton 2006). Long-tongued bumblebees represent a particularly important group in conservation terms, because wild flowers and flowering crops with long corollas may not receive

effective pollination in their absence (Goulson 2010). It is therefore worrying that, among bumblebees as a whole, long-tongued species, including *B. hortorum*, appear disproportionately prone to population decline and range contraction in modern agricultural landscapes (Goulson 2010; Bommarco et al. 2012). These considerations render the investigation of plant-pollinator specializations involving long-tongued bumblebees of particular interest.

One flowering plant with long corollas is Common Foxglove, *Digitalis purpurea* L., a biennial with elongate (42 - 43 mm), bell-shaped flowers that have basal nectaries and hang from tall, vertical inflorescences, widespread in its native Eurasia including the U.K. (Percival and Morgan 1965; Best and Bierzychudek 1982; Hill et al. 2004). *D. purpurea* appears to lack vegetative reproduction (Hill et al. 2004) and bagging experiments show that it requires insect pollinators to set seed (Nazir et al. 2008), suggesting that it is obligately dependent on insect pollination for reproduction. Although *D. purpurea* is not itself scarce (Hill et al. 2004), it therefore represents a potentially useful model with which to investigate plant-pollinator specialization. Previous studies have noted an association between *B. hortorum* and *D. purpurea* in the U.K. (Brian 1951, 1957; Edwards and Jenner 2005; Benton 2006) and elsewhere in Europe (Grindeland et al. 2005). However, none of these studies quantified the relative visitation rate of *B. hortorum* to *D. purpurea*. For example, Grindeland et al. (2005) found *B. hortorum* to account for > 52% of all *Bombus* visits to natural stands of *D. purpurea* in Norway, but, because this study had a different focus, whether this result simply reflected relative local abundances of *Bombus* species was not investigated. We therefore sought to investigate potential

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specialization in the relationship between *B. hortorum* and *D. purpurea* by quantifying the visitation rate of *B. hortorum* to *D. purpurea* relative to the visitation rate of other *Bombus* to *D. purpurea* and of *B. hortorum* and other *Bombus* to other available plant species.

MATERIALS AND METHODS

Twenty flowering patches of *Digitalis purpurea* in North Yorkshire, U.K., were selected, ten in woodland and ten in domestic gardens or parks ('non-woodland'). Only patches with five or more flowering plants per patch were included, a plant being defined as one or more inflorescences arising from a single basal rosette of leaves. Patches were spatially mixed with respect to habitat type and all patches lay within a radius of 11.5 km of the position, 54°01'25" N, 01°09'12" W. To maximize spatial independence, each was at least 2 km from its nearest neighbour. Within each patch, five *D. purpurea* plants were randomly chosen and individually labelled with a small tag near ground level. The white-flowered morph of *D. purpurea* (Blamey and Grey-Wilson 1989) was present at an overall mean frequency of 15%, with the number of patches having white morphs being the same (3/10) in both woodland and non-woodland habitat. Neither patch size nor within-patch plant density (i.e. for both morphs considered together) differed significantly between the two habitats (size: means = 6.1 and 6.2 m², respectively; two-sample *t*-test with equal variances, $t_{18} = 0.074$, $P = 0.942$; density: medians = 2.0 and 2.7 plants m⁻², respectively; Mann-Whitney *U*-test, $U = 47.0$, $m = m = 10$, $P = 0.82$).

Plants within each patch were observed simultaneously for 2 - 4 separate bouts of 30 min each between 11:00 and 20:00 from 25 June to 13 July 2010 (mean total observation = 105 min per patch). These dates include the peak annual flowering time of *D. purpurea* in central U.K. (Percival and Morgan 1965). Bout dates were rotated across patches and habitats, but, because of the limited flowering period of *D. purpurea*, some bouts (14/70) for a given patch took place on the same day (no more than two per day). Overall, therefore, the *D. purpurea* sample consisted of 100 plants (20 patches × 5 focal plants per patch) observed over 35 h (20 patches × 3.5 30-min observation bouts per patch). Bouts were conducted by recording, from 1.5 - 2.0 m away, each time an insect fully entered any corolla on the focal plants. Only insects approximately > 10 mm long and > 5 mm at their widest point were recorded, as smaller insects were considered unlikely to receive deposits of pollen by visiting *D. purpurea* flowers. Bumblebees were identified to species level (but not to sex or caste) where possible, using Edwards and Jenner (2005). Other insects were identified at least to family level.

To determine the relative frequency of all insect pollinators within the vicinity of the patches, eight equally spaced, linear 15 m transects (control transects) were laid out in a radial pattern centred on each patch. Each transect was walked for 2 min and every insect (again, approximately > 10 mm long and > 5 mm wide) seen visiting a flower within 1 m either side of the transect was recorded. The entire 16 minute-set of control transects was walked twice, on separate days for each patch, between 25 June and 13 July 2010

(83% of control transects) or between 14 and 20 July 2010 (17% of control transects). The plant species on control transects most frequently visited by insects were, in woodland habitat, *Arctium* spp, *Cirsium* spp, *Impatiens glandulifera*, *Rhododendron* spp and *Rubus* spp, and, in non-woodland habitat, *Cirsium* spp, *Crataegus* spp, *Lavandula* spp, *Trifolium repens* and a variety of other cultivated plant species.

For analysis of the proportion of visits made by focal *Bombus* species to *D. purpurea* relative to total visits made by *Bombus* species to *D. purpurea* (proportionate data; Fig. 1), bouts were pooled within patches to create datasets of 10 patches for each of woodland and non-woodland habitat. For analysis of the numbers of visits made by *Bombus* species to *D. purpurea* relative to total visits made by *Bombus* species to other plant species within the patches (Fig. 2), numbers of visits by bee species to *D. purpurea* were divided by the mean number of flowers that single *Bombus* workers visit per plant of *D. purpurea*, which was calculated as 23.2 (Best and Bierzychudek 1982; Nazir et al. 2008). This correction was applied to minimize effects of pseudoreplication arising from individual insects visiting multiple flowers of single *D. purpurea* plants within bouts. It was not applied to proportionate data, which were unaffected by multiple visits by single insects (i.e., if two *Bombus* species made 80% and 20%, respectively, of all *Bombus* visits to *D. purpurea*, these proportions would be unchanged whether measured at the plant or the flower level, provided the two species did not differ systematically in the number of flowers per plant visited by single bees). Datasets were analysed non-parametrically whenever they proved to be significantly non-normal (Kolmogorov-Smirnov tests).

RESULTS

In both habitats, *Bombus hortorum* was by a large margin the most frequent visitor to *Digitalis purpurea* (Fig. 1). Overall, *B. hortorum* accounted for 82% and 92% of total visits to *D. purpurea* ($n = 1682$) in woodland habitat (Fig. 1a) and non-woodland habitat (Fig. 1b), respectively, whereas the next most frequent visitor in both habitats, *B. pascuorum*, accounted for 17% and 3% of total visits in each respective habitat. Together, therefore, *B. hortorum* and *B. pascuorum* accounted for 95 - 99% of total visits to *D. purpurea* across both habitats. Within each of *B. hortorum* and *B. pascuorum*, the proportions of visits to *D. purpurea* in woodland and non-woodland habitat were not significantly different (Mann-Whitney *U*-tests: *B. hortorum*, $U = 54.5$, $m = m = 10$, $P = 0.73$; *B. pascuorum*, $U = 59.0$, $m = m = 10$, $P = 0.48$; Figs. 1a, 1b). Hence remaining data analyses were conducted using data from both habitats pooled.

In contrast to other insects, including *B. pascuorum*, *B. hortorum* was recorded visiting *D. purpurea* at a significantly much higher frequency than expected from its abundance as a visitor to other plant species on the control transects (chi-squared test, $\chi^2 = 248.9$, d.f. = 5, $P < 0.001$; Fig. 2). Therefore, *B. hortorum* was not the most abundant bumblebee at all plants in the vicinity of the patches but visited *D. purpurea* preferentially.

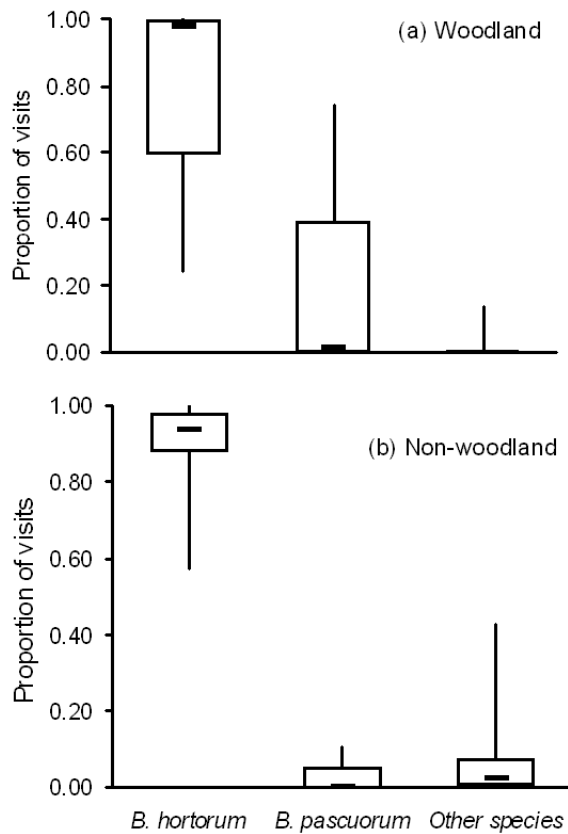


FIGURE 1. Proportion of visits to *Digitalis purpurea* by *Bombus hortorum*, *B. pascuorum* and other insect species in (a) woodland habitat and (b) non-woodland habitat. Median denoted by horizontal bar, quartiles by box and range by vertical lines. Per-patch mean (range) total numbers of insect visits were 66.8 (24 - 134) for woodland and 101.4 (15 - 241) for non-woodland habitat ($n = 10$ *D. purpurea* patches per habitat). Other species consisted of *Apis mellifera*, *Bombus lapidarius*, *B. terrestris* / *B. lucorum* (pooled), Megachilidae, Syrphidae, *Vespa* spp. and other Vespidae.

DISCUSSION

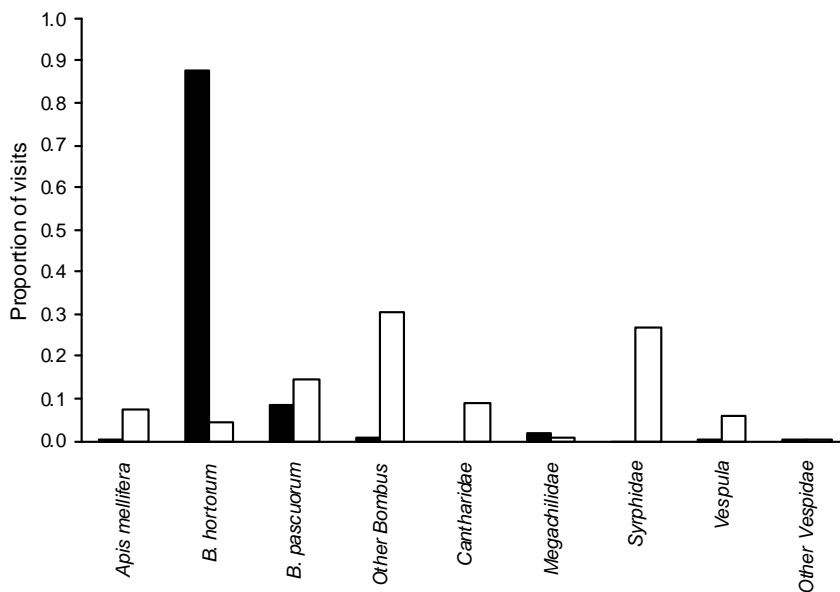


FIGURE 2. Comparison of proportion of visits by insect taxa to flowers of *Digitalis purpurea* (black columns) and to flowers of other plant species on control transects within 15 m of the focal patches of *D. purpurea* (white columns). Data from woodland and non-woodland habitat have been pooled. Total numbers of insect visits were 1682 to *D. purpurea* and 1133 to flowers on control transects. Figure is illustrative, with statistical analysis (chi-squared test) being conducted on corrected numbers of visits as described in 'Materials and methods' and reported in 'Results'. Other *Bombus* consisted of *B. hypnorum*, *B. lapidarius*, *B. pratorum*, *B. terrestris* / *B. lucorum* (pooled) and *B. (Psithyrus)* spp.

Our findings show that *B. hortorum* was the principal insect visitor to *D. purpurea* in the study population, with a significant preference for this plant, followed by *B. pascuorum*, which lacked such a preference. *B. pascuorum* has the second longest tongue among common U.K. bumblebees (Prys-Jones and Corbet 1991). The predominance of *B. hortorum* and *B. pascuorum* among *Bombus* visitors to *D. purpurea*, which was also noted by Grindeland et al. (2005), is therefore likely to have arisen because *D. purpurea*, through having long corollas, is preferentially attractive to long-tongued bumblebee species. Because it was the principal insect visitor, we infer that *B. hortorum* is likely to be the main pollinator of *D. purpurea* in the study population. This inference is subject to two qualifications. First, visitation does not equate to pollination. However, observations that *B. hortorum* workers completely enter *D. purpurea* flowers and emerge coated in pollen (Benton 2006; unpublished observations), and that *D. purpurea* pollen occurs in their pollen-loads (Brian 1951; Kleijn and Raemakers 2008), together with the experimental evidence showing that bagged *D. purpurea* flowers set no seed (Nazir et al. 2008), leave little doubt that *B. hortorum* and other *Bombus* visitors pollinate *D. purpurea*. Second, *D. purpurea* flowers may also be visited by nocturnal insects such as moths. This possibility, which we did not investigate, seems unlikely given that *D. purpurea* flowers lack features typical of flowers pollinated by nocturnal moths (Proctor et al. 1996). But since floral features are not necessarily reliable guides to pollinator taxon (Ollerton et al. 2009), the possibility remains that nocturnal insects also contribute to *D. purpurea* pollination.

With regard to diurnal pollinators, the relationship between *B. hortorum* and *D. purpurea* therefore provides a potential example of a high degree of specialization in a plant-pollinator system. Because *B. hortorum* visits a wide range of other plant species with long corollas (Benton 2006; Kleijn and Raemakers 2008), any association of *D. purpurea* with it is clearly asymmetric (Vázquez and Aizen

2004). Studies elsewhere have found that *D. purpurea* is also visited by other *Bombus* species (Brian 1957; Percival and Morgan 1965; Fussell and Corbet 1993; Grindeland et al. 2005; Nazir et al. 2008), including native *Bombus* species recently brought into contact with introduced *D. purpurea* populations (Best and Bierzychudek 1982). These studies, combined with our results, suggest that the extent to which *D. purpurea* has a specialized association with *B. hortorum* or other long-tongued bumblebees must vary geographically. Nonetheless, even spatially-limited asymmetric specialization of *D. purpurea* on one or a few *Bombus* species could be an important phenomenon given that *D. purpurea* appears obligately dependent on insect pollination for reproduction (Hill et al. 2004; Nazir et al. 2008) and that, among bumblebees as a whole, *B. hortorum* and other long-tongued species appear disproportionately prone to decline (see 'Introduction'). Hence our findings also show that *B. hortorum* and *B. pascuorum* or their functional equivalents may play a major role in the viability of *D. purpurea* populations. More broadly, if other species of flowering plant with long corollas likewise exhibit asymmetric, specialized relationships with one or a few long-tongued *Bombus* species, they too would be vulnerable to effects of declines in these bees.

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