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Short Communication

Inter-Order Interactions Between Flower-Visiting Insects: Foraging Bees Avoid Flowers Previously Visited by Hoverflies

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INTRODUCTION

In all insects, the ability to perceive and respond to the environment is at least partly dependent on olfaction. Our current understanding of the ways in which insects use olfactory information has developed mainly through studies of pheromonal communication within species (e.g., within the social Hymenoptera—Van der Meer *et al.*, 1998; Ayasse *et al.*, 2001) and of consumer-resource interactions (e.g., between phytophagous insects and plants—Bernays and Chapman, 1994; Dicke, 2000). The potential for olfactory cues to mediate interactions between species competing for shared resources is less well researched, particularly in cases where competition occurs between distantly related taxa. There is some evidence that insects can detect con-generic or con-familial competitor species using olfaction. For example, the host-marking pheromones of parasitoids and phytophagous insects can be used by closely related species as indicators of the activity of competitors (Nufio and Papaj, 2001). However, there appears to be no published evidence that olfactory information can play a direct role in interactions between competitors in different insect orders.

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Olfactory information plays an important role in decision making by insects foraging for nectar. For example, there is good evidence that social and solitary bees (Hymenoptera: Apidae) use scent cues to determine if a flower has recently been visited by a conspecific (Nunez, 1967; Ferguson and Free, 1979; Cameron, 1981; Kato, 1988; Williams, 1998; Gilbert *et al.*, 2001; Goulson *et al.*, 2001). By avoiding recently visited flowers, which are likely to be nectar-depleted, bees can increase their foraging efficiency (Kato, 1988; Williams, 1998). Different bee species frequently exploit the same floral resources, and it is not surprising therefore that several species are known to use scent cues to detect and avoid flowers that have recently been visited by heterospecifics in the family Apidae (Goulson *et al.*, 1998; Stout *et al.*, 1998; Stout and Goulson, 2001). Although bees also compete for food with a range of more distantly related insect taxa, it is not known if bees can detect and avoid flowers recently visited by members of other insect families or orders.

In this paper we test the hypothesis that the likelihood that foraging bees will visit a flower is influenced by previous visits to that flower by members of a different insect order. Hoverflies (Diptera: Syrphidae) are generalist flower-visitors (Branquart and Hemptinne, 2000) that often forage on the same plants as bees, and as such are potentially important competitors for nectar and pollen. If the ability of bees to assess flower status using scent cues reflects a general strategy for avoiding flowers whose resources have been depleted by competitors, we predict that bees will reject flowers that have recently been visited by hoverflies.

METHODS

We investigated the behaviour of insects foraging on six large bramble bushes (*Rubus fruticosus* L. agg.), each with several hundred flowers, on White Hill, Surrey, UK between 0800 and 1800 hrs BST from 28th June to 2nd July 2003. Visiting insects were pooled into three categories: bumble bee workers (*Bombus terrestris*, *Bombus lucorum*, *Bombus pascuorum* and *Bombus lapidarius*), honey bee workers (*Apis mellifera*), and hoverflies (*Chrysotoxum cautum*, *Eristalis tenax* and *Helophilus pendulus*).

We observed the responses of bumble bees, honey bees and hoverflies ("test insects") when offered individual bramble flowers that had either recently been visited by another foraging insect ("test flowers"), or isolated in a muslin bag for the previous 24 h ("control flowers"). The effects of repellent olfactory cues deposited on flowers by foraging insects typically wear off well within 24 h (Stout and Goulson, 2001; Stout and Goulson, 2002), and we thus assume that our bagged flowers control for any effects of previous forager activity on test insect behaviour. In addition to any olfactory cues left on a flower after visitation, the depletion of nectar caused by a foraging insect could provide a direct visual cue to the rewards offered by a flower (Thorp *et al.*, 1975; Marden, 1984). Since control flowers will have had ample time to compensate for any depletion of nectar caused by visitation prior to bagging, we used a second type of bagged control to distinguish between the effects of visual and olfactory cues on forager behaviour. We offered test insects flowers that had been bagged for 24 h and then drained of nectar artificially using a microcapillary tube ("drained control flowers").

We adopted a design similar to Goulson *et al.* (1998) and Stout *et al.* (1998) when presenting test and control flowers to insects. Flowers were cut from bramble plants, leaving enough stem for them to be handled with forceps, and offered immediately to test insects. We removed the nectar from drained control flowers immediately before they were cut. Test flowers were always cut immediately after a visit by a foraging insect, the identity of the visitor being recorded. In all trials, a cut flower was held within a few centimetres of a flower upon which a test insect was already feeding, in a position chosen to coincide with the anticipated flight path of the insect. If, having left its original flower, the test insect landed on and probed the flower being offered, this was classed as "acceptance." If the offered flower was approached but not landed on, this was classed as "rejection." When the test insect left its original flower in a different direction to the offered flower, nothing was recorded. The proportion of acceptances for each class of experimental flower was compared to the two controls using a G-test with William's correction (Sokal and Rohlf, 1995). Probabilities were adjusted with a sequential Bonferroni correction (Rice, 1989) to account for multiple comparisons.

Flowers of different types were offered to test insects in a haphazard order. We could not fully randomise the sequence, since the range of test flowers available at any one time was constrained by the identity of foraging insects in the vicinity of the experimenter. In general, we cannot be sure that we did not use the same individual test insect more than once during the experiment, but the chances of pseudo-replication were minimal since populations of all the species we used at the field site were large (see also Williams, 1998; Stout and Goulson, 2002).

RESULTS

Bumble bees and honey bees landed on the majority (~80%) of control flowers offered to them (Fig. 1). They were significantly less likely to accept a flower that had recently been visited by another bee, regardless of

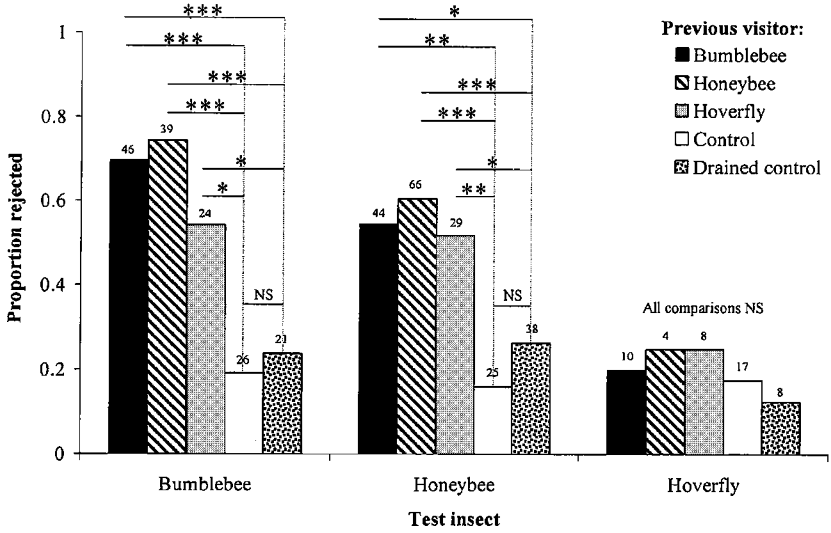

Fig. 1. The proportions of different types of flowers that, when offered to three types of test insect, were rejected. The five flower types are: those that had recently been visited by three types of insect, control flowers that were bagged for 24 h prior to their use ("control"), and control flowers that were bagged for 24 h and then drained of all nectar prior to their use ("drained control"). Significance levels arising from pre-planned pair-wise comparisons using G-tests are shown (***$p < 0.001$; ** $p < 0.01$; *$p < 0.05$; NS $p > 0.05$; probabilities are adjusted with a sequential Bonferroni correction). Numbers above bars represent sample size (n).

its identity ($G \geq 10.31; p \leq 0.002$ for all comparisons). They were also more likely to reject flowers that had previously been visited by hoverflies (honey bees: $G = 6.56, p = 0.011$; bumble bees: $G = 7.65, p = 0.006$). There was no evidence that bee behaviour was directly affected by the amount of nectar present in flowers—the rejection rate for drained control flowers was not significantly higher than the rate for controls that had not been drained ($G \geq 0.92; p \geq 0.26$ for both species). Owing to small sample size, a detailed analysis of hoverfly behaviour is problematic. Hoverflies rejected relatively few of the test or control flowers that they approached (≤ 25 %). Even when the two controls and all test flowers were pooled, no significant effect of prior visitation could be detected on the rejection rate (5 out of 22 test flowers rejected versus 4 out of 25 controls, $G = 0.054, p > 0.9$).

DISCUSSION

Although initial reports suggested that repellent scent cues used by flower-visiting insects were species specific (Williams, 1998), recent research has shown that bumble bees and honey bees can interpret each others' scents and avoid recently visited flowers accordingly (Stout and Goulson, 2001). Our results support this finding, with bumble bees showing a strong aversion to flowers recently visited by honey bees, and vice versa. Furthermore, our results strongly suggest that both bumble bees and honey bees can tell when flowers have been visited by hoverflies. Although we do not know whether the insects that we observed were foraging for nectar or pollen, there is certainly the potential for strong competition between bees and hoverflies for the resources offered by flowers.

Our results indicate that even when bees are competing with a taxonomically diverse range of flower-visiting insects, they will be capable of avoiding resource-depleted flowers. Such behaviour is likely to lead to increased overall foraging efficiency, assuming that there are significant costs, in terms of energy or time, associated with landing on unrewarding flowers (Kato, 1988; Schmitt and Bertsch, 1990; Williams, 1998).

Our data are consistent with the hypothesis that bees can avoid recently visited flowers on the basis of the remote perception of scent cues deposited by hoverflies. It has been suggested, however, that other types of cue can be used by bees to assess flower status without landing (e.g., Thorp *et al.*, 1975; Heinrich, 1979; Goulson *et al.*, 2001). Given that bees did not distinguish between control and drained control flowers, we can exclude the possibility that bees in our experiment directly assessed nectar volume by sight or smell, but other changes in flower appearance or scent (e.g., those related to the depletion of pollen) may have been indicative of a recent visit and cannot be ruled out as factors influencing bee behaviour. Nevertheless, individual chemicals isolated from honey bee mandibular glands (Vallet *et al.*, 1991), and from bumble bee tarsal extracts (Stout *et al.*, 1998; Goulson *et al.*, 2000), can elicit exactly the kind of flower rejection behaviour we observed in our study. We believe that the most parsimonious explanation for our results is that the detection of similar chemicals by bees leads to the rejection of flowers that have recently been visited by hoverflies. Chemical cues might be left behind on flowers accidentally by hoverflies, or might even be deposited deliberately, perhaps to help individuals recognise flowers that they have already visited.

Our findings strongly suggest that olfactory information can pass between species in different insect orders, and that this may have important consequences for the shaping of competitive interactions among members of the same guild. Research into the mechanisms that mediate interspecific competition has largely focussed on interactions between closely related species, and it is not clear to what extent our results can be generalised. Perhaps social bees, because they have evolved highly sophisticated mechanisms for communication between related individuals, are in an unusually good evolutionary position to exploit olfactory cues deposited by other species. The ability to detect chemical signals from distantly related taxa could also influence other types of interactions. For example, might bees be able to use chemical cues to avoid predatory insects or parasitoids? Such possibilities merit further attention.

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