

# The use of heterospecific scent marks by the sweat bee *Halictus aerarius*

Tomoyuki Yokoi · Dave Goulson · Kenji Fujisaki

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**Abstract** To forage effectively amongst flowers, some bee species utilize olfactory cues left by previous visitors in addition to direct assessment of visual cues to identify rewarding flowers. This ability can be more advantageous if the bees can recognize and use scent marks left by heterospecifics, not just marks left by members of their own species. We conducted field experiments to investigate whether the sweat bee *Halictus aerarius* avoids visiting flowers of trailing water willow *Justicia procumbens* emptied by other bee species. We found that *H. aerarius* rejected the flowers visited by both heterospecifics and conspecifics. They also rejected visited flowers artificially replenished with nectar. Our results demonstrate that social bees outside the Apidae can detect marks left on flowers by heterospecifics but that (on this plant species) they are unable to discriminate against flowers by directly detecting nectar volume. *H. aerarius* exhibited different rejection rates according to the identity of the previous bee species. We suggest that the frequency of rejection responses may depend on the amount of chemical substances left by the previous bee. In general, the use of scent marks left by previous visitors is almost certainly advantageous, enabling foragers to avoid flowers with depleted nectar levels and thereby improving their foraging efficiency.

**Keywords** Sweat bee · Scent marks · Heterospecifics · Foraging · Floral resources

## Introduction

Foraging bees can improve their foraging efficiency when foraging amongst flowers that have previously been visited by other insects by using chemical cues such as scent marks to assess the availability of resources. It has been shown that honeybees and bumblebees detect attractant or repellent marks left by conspecifics (or by themselves) and utilize them to assess flower resources (e.g. Free and Williams 1979, 1983; Schmitt and Bertsch 1990; Giurfa et al. 1994; Goulson et al. 1998). Although it depends on the plant species and the experimental situation, the revisitation rate to the flowers foraged by previous visitors increased after between 20 and 60 min and 24 h in the field (Williams 1998; Stout and Goulson 2001, 2002). Moreover, several studies have found evidence for use of scent marks by solitary bees (Frankie and Vinson 1977; Gilbert et al. 2001). The studies on stingless bees used artificial food sources and found attracting scent marks, although their responses to scent marks on natural flowers have not been examined (e.g. Aguilar and Sommeijer 2001; Nieh et al. 2003; Jarau et al. 2004; Schmidt et al. 2005). Attractant scent marks have also been found in bumblebees but again only in laboratory experiments (Cameron 1981; Schmitt and Bertsch 1990; Schmitt et al. 1991; Williams 1998; Goulson et al. 2000). With regards to the role of scent marks, Saleh and Chittka (2006) showed that the scent marks left by bumblebees could be interpreted as attractive and repellent signals based on the reward level of the resource and the context in which they were presented.

T. Yokoi (✉) · K. Fujisaki  
Laboratory of Insect Ecology, Graduate School of Agriculture,  
Kyoto University,  
Kitashirakawa, Sakyo,  
606-8502 Kyoto, Japan  
e-mail: tomoyoko@kais.kyoto-u.ac.jp

D. Goulson  
School of Biological and Environmental Sciences,  
University of Stirling,  
Stirling FK9 4LA, UK

It is not known how widespread interspecific interactions via floral scent marks are (Goulson et al. 2000). Bumblebees avoid flowers visited by congeners (Goulson et al. 1998). Stout and Goulson (2001) also found that honeybees and bumblebees were able to detect marks left by one another. However, in a different floral system, Williams (1998) reported that bumblebees and honeybees showed no interspecific repellent responses.

The stingless bee, *Trigona spinipes*, utilizes the scent mark of *Melipona rufiventris* to find floral resources. These were highly rewarding food sites, and the scent marks elicited an attraction (Nieh et al. 2004). Reader et al. (2005) reported that bumblebees and honeybees avoid the flowers visited by hoverflies. In general, little is known about the use of scent marks amongst tribes or families other than honeybees and bumblebees. Gawleta et al. (2005) reported that the wool-carder bee, *Anthidium manicatum*, was able to discriminate amongst flowers recently visited by conspecifics and bumblebees. These results suggest that the use of scent marks is possible amongst different bee families. However, it is not known whether other bee species such as sweat bees (Halictidae) deposit scent marks and can detect marks left by other bee species. In this study, we focused on the behavior of the sweat bee *Halictus aerarius* Smith (Halictidae). The bee has a social structure similar to honeybees (Sasaki 1985) and visits many plant species as a generalist forager. We test whether they are able to recognize flowers previously foraged on by conspecifics or other bee species and whether this is via direct detection of nectar levels or through use of indirect cues.

## Materials and methods

The study was conducted between August and September 2004 on a rice field levee (120 m<sup>2</sup>) in Nagaokakyo, Kyoto, Japan. Observations took place between 09:00 and 16:00 hours on days that were clear and sunny. Weather conditions were hot and humid throughout the study. The sweat bee *H. aerarius* (Halictidae) was the most frequent visitor to *Justicia procumbens* var. *leucantha* (Acanthaceae). Other bee species that foraged frequently on *J. procumbens* were the carpenter bee *Xylocopa appendiculata circumvolans* (Apidae), the honeybee *Apis cerana japonica* (Apidae) and the leaf cutter bee *Megachile* sp. (Megachilidae). *H. aerarius* collected both nectar and pollen, while the other three bee species collected only nectar. The anthers of *J. procumbens* are clearly visible, but the nectar is not, at least to the human eye. Each flower opens for just 1 day. Maximum nectar volume of flowers was 0.3 µl (unpublished data). We had previously investigated nectar replenishment and discovered that nectar is not replenished over time in this species (unpublished data).

The experimental design followed Goulson et al. (1998, 2001). The flowers were removed using forceps and offered to a subsequent visitor within 3 min of a previous visitor. To eliminate the possibility that bees were learning the location of unrewarding or rewarding flowers, we showed subsequent visitors the flowers at sites distant from where they were collected. The response of subsequent visitors was observed and classified into three patterns: hovering, landing and probing. In this paper, ‘hovering’ was defined as when a visitor approached within 1 cm of the flowers but did not land on it, ‘landing’ was when a visitor landed on the flower but departed immediately without foraging, and ‘probing’ was when a visitor landed and foraged. We defined the hovering and landing behaviors as rejection responses to flower because bees did not obtain resources in these cases. After each trial, the flower was discarded. As a control (no previous visitors), we used flowers that had been covered in fine netting until they opened. Some foraged flowers were refilled with nectar using a micropipette, immediately after a bee had foraged, and then shown to subsequent visitors, again within 3 min. The nectar used for these refilling experiments was collected from a flower that had been covered in netting before opening.

Individuals that were foraging naturally in the field were used for each test. However, the number of foraging individuals of the three species *X. appendiculata*, *A. cerana* and *Megachile* sp. was smaller than those of *H. aerarius*. It is possible that some individuals were used more than once because we did not mark the individual bees. In an attempt to minimize this, we used only a small sample number for each investigation on each day. Moreover, observations were taken over as wide an area as possible (see also Stout and Goulson 2001; Reader et al. 2005). Comparisons of the proportions of bees rejecting flowers in different treatments were made using  $\chi^2$  tests. We used the Fisher’s exact probability tests in the comparison between the unvisited flowers (control) and refilled flowers.

## Results

When we compared the rejection rate of unvisited control flowers with that of the refilled (visited) flowers, *H. aerarius* showed a high rejection rate to flowers that had previously been visited (Table 1). Insufficient data were collected on the re-visitation rates to refilled flowers that had previously been visited by *Megachile* sp., as numbers of the species declined sharply during the study period, preventing us from collecting adequate data. There was no detectable difference between flowers that were artificially refilled with nectar and flowers that remained empty after visits by conspecifics ( $\chi^2=3.11$ ,  $df=1$ ,  $P=0.0779$ ), *A. cerana* ( $\chi^2=3.67$ ,  $df=1$ ,  $P=0.0554$ ) or *X. appendiculata* ( $\chi^2=1.65$ ,  $df=1$ ,  $P=0.1985$ ; see Fig. 1).

**Table 1** A comparison of the rates of rejection (hovering and landing patterns) to flowers foraged by previous visitors and then artificially replenished with nectar vs control flowers that had never been visited

Previous visitor	Probed (%)	Rejected (%)		<i>P</i> value
		Hovering	Landing	
<i>H. aerarius</i>	58.5	24.4	17.1	<0.0001
<i>A. cerana</i>	31.8	63.6	4.5	<0.0001
<i>X. appendiculata</i>	20.8	75	4.2	<0.0001
<i>Megachile</i> sp.	–	–	–	–

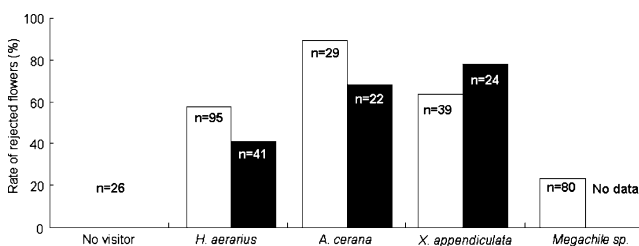
Fisher's exact probability tests are used.

There were significant differences in the responses of *H. aerarius* depending on the species of bee that had previously visited the flower. In particular, the rate of rejection of flowers previously visited by *Megachile* sp. was significantly lower than that of flowers foraged on by *X. appendiculata* ( $\chi^2=14.65$ ,  $df=1$   $P<0.0001$ ), *A. cerana* ( $\chi^2=36.15$ ,  $df=1$   $P<0.0001$ ) and conspecifics ( $\chi^2=16.65$ ,  $df=1$   $P<0.0001$ ). Moreover, the rate of rejection of flowers previously visited by *Megachile* sp. was significantly lower than that of refilled flowers foraged on by *X. appendiculata* ( $\chi^2=20.82$ ,  $df=1$   $P<0.0001$ ) and *A. cerana* ( $\chi^2=11.99$ ,  $df=1$   $P<0.0005$ ).

When we compared the rejection responses, the rate of rejection at 'hovering' was larger than at 'landing,' for flowers foraged on by previous visitors and subsequently refilled with nectar (Table 1).

## Discussion

If individual bees are able to recognize and avoid flowers that have been visited by heterospecifics, then it will lead to increased foraging efficiency because they can focus on probing rewarding flowers (Schmitt and Bertsch 1990; Giurfa and Núñez 1993; Stout and Goulson 2001). Our results indicate that *H. aerarius* possesses this ability. Rejections by *H. aerarius* tended to occur after an approach to within 1 cm of a flower, so it is likely that the bee recognized chemical marks left by previous visitors. The



**Fig. 1** Rate of the flowers rejected by *H. aerarius*, depending on whether they were foraged by interspecific (white bars) or refilled with nectar after being foraged by previous visitors (black bars). Sample size of the bars refers to the number of trials

strong repellent responses occurred irrespective of the nectar volume in the flowers because *H. aerarius* rejected foraged flowers that had been replenished with nectar. Because we cannot eliminate the possibility that bees are responding to a visual physical cue left behind by previous foragers, it would be valuable to investigate this further. If bees are responding to chemical cues (as seems most likely), the response of bees to unvisited flowers that have chemicals added to them should decline over time (e.g. Schmitt et al. 1991; Goulson et al. 2000), which could be readily tested.

Our findings support those of most previous studies into heterospecific scent mark detection (Stout and Goulson 2001; Gawleta et al. 2005; Reader et al. 2005). *H. aerarius* demonstrated a rejection response to flowers foraged by several other bee species. The hydrocarbons found on insect cuticle tend to be similar across diverse insect taxa (Lockey 1980), although the precise blend varies even amongst closely related species (Goulson et al. 2000; Eltz 2006). It is likely that *H. aerarius* exhibits a generalized avoidance responses to flowers contaminated with hydrocarbons commonly found on insect cuticles and hence can avoid flowers visited by a range of different bee species.

The gland that produces the repellent compounds is different amongst bee species. Repellent scent marks of honeybees are thought to be 2-heptanone, secreted from mandibular glands (Giurfa 1993). Less volatile compounds are secreted from the dufour gland in the carpenter bee *Xylocopa virginica* (Frankie and Vinson 1977). Bumblebees are able to recognize a mixture of long-chain hydrocarbons secreted from tarsal glands (Schmitt et al. 1991; Goulson et al. 2000; Eltz 2006; but see Jarau et al. 2005). Goulson et al. (2000) demonstrated that bumblebees respond to flowers previously foraged on by congeners that produce scent marks with different compositions. *H. aerarius* is similarly able to detect scent marks left by several bee species.

It is interesting to note that the rejection rate of flowers by *H. aerarius* was higher for both foraged flowers and refilled flowers after being visited by *X. appendiculata* and *A. cerana* compared to those foraged on by *Megachile* sp. Why should sweat bees show different repellent rates amongst bees? The frequency of a repellent effect might depend on the amount of chemical substances left by each bee. Of particular interest, the repellency of flowers foraged by *A. cerana* and *X. appendiculata* tends to be higher than the flowers foraged by conspecifics. The amount of secretion left by these bees might be larger than that left by *H. aerarius*, which is the smallest of the bee species included in this study. We did not examine the responses by *X. appendiculata*, *A. cerana* and *Megachile* sp. to flowers visited by *H. aerarius*. It would be interesting to investigate further whether size of a flower visitor influences the

strength of scent mark they deposit and the subsequent response of heterospecifics.

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