

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

2

3 Tomoyuki Yokoi, Dave Goulson and Kenji Fujisaki

4

5 Laboratory of Insect Ecology, Graduate School of Agriculture,

6 Kyoto University, Japan

7

8 Author for correspondence

9 T. Yokoi · K. Fujisaki

10 Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University,

11 Kitashirakawa, Sakyo 606-8502, Kyoto, Japan

12 Tel: +81-75-753-6147; Fax: +81-75-753-6474

13 e-mail: tomoyoko@kais.kyoto-u.ac.jp

14

15 D. Goulson

16 School of Biological & Environmental Sciences, University of Stirling,

17 Stirling, FK9 4LA, Scotland

18

19 Key words

20 sweat bee, scent marks, heterospecifics, foraging, floral resources

21

22

Abstract

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

39

40

Introduction

41 Foraging bees can improve their foraging efficiency when foraging amongst flowers
42 that have previously been visited by other insects by using chemical cues such as scent

43 marks to assess the availability of resources. It has been shown that honeybees and
44 bumblebees detect attractant or repellent marks left by conspecifics (or by themselves)
45 and utilize them to assess flower resources (e.g. Free and Williams 1979, 1983; Schmitt
46 and Bertsch 1990; Giurfa et al. 1994; Goulson et al. 1998). Although it depends on the
47 plant species and the experimental situation, revisitation rate to the flowers foraged by
48 previous visitors increased after between 20-60 minutes and 24 hours in the field
49 (Williams 1998; Stout and Goulson 2001, 2002). Moreover, several studies have found
50 evidence for use of scent marks by solitary bees (Frankie and Vinson 1977; Gilbert et al.
51 2001). The studies on stingless bees used artificial food sources and found attracting
52 scent marks, although their responses to scent marks on natural flowers have not been
53 examined (e.g. Aguilar and Sommeijer 2001; Nieh et al. 2003; Jarau et al. 2004;
54 Schmidt et al. 2005). Attractant scent marks have also been found in bumblebees, but
55 again only in laboratory experiments (Cameron 1981; Schmitt and Bertsch 1990;
56 Schmitt et al. 1991; Williams 1998; Goulson et al. 2000). With regard to the role of scent
57 marks, Saleh and Chittka (2006) showed that the scent marks left by bumblebees could
58 be interpreted as attractive and repellent signals based on the reward level of the
59 resource and the context in which they were presented.

60 It is not known how widespread interspecific interactions via floral scent marks are
61 (Goulson et al. 2000). Bumblebees avoid flowers visited by congeners (Goulson et al.
62 1998). Stout and Goulson (2001) also found that honeybees and bumblebees were able
63 to detect marks left by one another. However, in a different floral system Williams

64 (1998) reported that bumblebees and honeybees showed no interspecific repellent
65 responses.

66 The stingless bee, *Trigona spinipes* utilizes the scent mark of *Melipona rufiventris* to
67 find floral resources. These were highly rewarding food sites and the scent marks
68 elicited an attraction (Nieh et al. 2004). Reader et al. (2005) reported that bumblebees
69 and honeybees avoid the flowers visited by hoverflies. In general, little is known about
70 the use of scent marks among tribes or families other than honeybees and bumblebees.
71 Gawleta et al. (2005) reported that the leaf cutter bee [are you sure this is a leafcutter
72 bee? They are generally in the genus *Megachile* – maybe safer to remove the English
73 name], *Anthidium manicatum*, was able to discriminate amongst flowers recently visited
74 by conspecifics and bumblebees. These results suggest that the use of scent marks is
75 possible among different bee families. However, it is not known whether other bee
76 species such as sweat bees (Halictidae) deposit scent marks and can detect marks left by
77 other bee species. In this study, we focused on the behavior of the sweat bee *Halictus*
78 *aerarius* Smith (Halictidae). The bee has a social structure similar to honeybees (Sasaki
79 1985) and visits many plant species as a generalist forager. We test whether they are
80 able to recognize flowers previously foraged on by conspecifics or other bee species,
81 and whether this is via direct detection of nectar levels or through use of indirect cues.

82

83 Materials and Methods

84 The study was conducted between August and September 2004 on a rice field levee

85 (120 m²) in Nagaokakyo, Kyoto, Japan. Observations took place between 0900 and
86 1600 h on days which were clear and sunny. Weather conditions were hot and humid
87 throughout the study. The sweat bee *Halictus aerarius* (Halictidae) was the most
88 frequent visitor to *Justicia procumbens* var. *leucantha* (Acanthaceae). Other bee species
89 that foraged frequently on *J. procumbens* were the carpenter bee *Xylocopa*
90 *appendiculata circumvolans* (Apidae), the honeybee *Apis cerana japonica* (Apidae) and
91 the leaf cutter bee *Megachile* sp. (Megachilidae). *H. aerarius* collected both nectar and
92 pollen, while the other three bee species collected only nectar. The anthers of *J.*
93 *procumbens* are clearly visible but the nectar is not, at least to the human eye. Each
94 flower opens for just one day. Maximum nectar volume of flowers was 0.3µl
95 (unpublished data). We had previously investigated nectar replenishment, and discovered
96 that nectar is not replenished over time in this species (unpublished data).

97 The experimental design followed Goulson et al. (1998, 2001). The flowers were
98 removed using forceps and offered to a subsequent visitor within 3 minutes of a
99 previous visitor. To eliminate the possibility that bees were learning the location of
100 unrewarding or rewarding flowers, we showed subsequent visitors the flowers at sites
101 distant from where they were collected. The response of subsequent visitors was
102 observed and classified into three patterns: hovering, landing and probing. Here,
103 “hovering” was defined as when a visitor approached within 1 cm of the flowers but did
104 not land on it; “landing” was when a visitor landed on the flower but departed
105 immediately without foraging, and “probing” was when a visitor landed and foraged.

106 We defined the hovering and landing behaviors as rejection responses to flower, because
107 bees did not obtain resources in these cases. After each trial the flower was discarded.
108 As a control (no previous visitors), we used flowers that had been covered in fine
109 netting until they opened. Some foraged flowers were refilled with nectar using a
110 micropipette, immediately after a bee had foraged, and then shown to subsequent
111 visitors, again within 3 minutes. The nectar used for these refilling experiments was
112 collected from a flower that had been covered in netting prior to opening.

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

123

124 Results

125 When we compared the rejection rate of unvisited control flowers with that of the
126 refilled (visited) flowers, *H. aerarius* showed a high rejection rate to flowers that had

127 previously been visited (Table 1). Insufficient data were collected on the re-visitation
128 rates to refilled flowers that had previously been visited by *Megachile* sp. as numbers of
129 the species declined sharply during the study period, preventing us from collecting
130 adequate data. There was no detectable difference between flowers that were artificially
131 refilled with nectar and flowers that remained empty after visits by conspecifics
132 ($\chi^2=3.11$, $df=1$ $P=0.0779$), *A. cerana* ($\chi^2=3.67$, $df=1$, $P=0.0554$) or *X. appendiculata*
133 ($\chi^2=1.65$, $df=1$, $P=0.1985$ see Fig.1).

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

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

145

146

Discussion

147 If individual bees are able to recognize and avoid flowers that have been visited by

148 heterospecifics, then it will lead to increased foraging efficiency because they can focus
149 on probing rewarding flowers (Schmitt and Bertsch 1990; Giurfa and Núñez 1993; Stout
150 and Goulson 2001). Our results indicate that *H. aerarius* possesses this ability.
151 Rejections by *H. aerarius* tended to occur following an approach to within 1cm of a
152 flower, so it is likely that the bee recognized chemical marks left by previous visitors.
153 The strong repellent responses occurred irrespective of the nectar volume in the flowers,
154 because *H. aerarius* rejected foraged flowers that had been replenished with nectar.
155 Since we cannot eliminate the possibility that bees are responding to a visual physical
156 cue left behind by previous foragers, it would be valuable to investigate this further. If
157 bees are responding to chemical cues (as seems most likely), the response of bees to
158 unvisited flowers which have chemicals added to them should decline over time (e.g.
159 Schmitt et al. 1991, Goulson et al. 2000), which could be readily tested.

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

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

178 Interestingly, the rejection rate of flowers by *H. aerarius* was higher for both
179 foraged flowers and refilled flowers after being visited by *X. appendiculata* and *A.*
180 *cerana* compared to those foraged on by *Megachile* sp.. Why should sweat bees show
181 different repellent rates among bees? The frequency of a repellent effect might depend
182 on the amount of chemical substances left by each bee. Of particular interest, the
183 repellency of flowers foraged by *A. cerana* and *X. appendiculata* tends to be higher than
184 the flowers foraged by conspecifics. The amount of secretion left by these bees might be
185 larger than that left by *H. aerarius*, which is the smallest of the bee species included in
186 this study. We did not examine the responses by *X. appendiculata*, *A. cerana* and
187 *Megachile* sp. to flowers visited by *H. aerarius*. It would be interesting to investigate
188 further whether size of a flower visitor influences the strength of scent mark they
189 deposit and the subsequent response of heterospecifics.

190

191

Acknowledgements

192 We are grateful to B. Darvill, T. Nishida and members of our laboratory for helpful
193 comments and criticisms to the manuscript. This work was supported in part by the 21st
194 century COE program for Innovative Food and Environmental Studies Pioneered by
195 Entomomimetic Sciences, from the Ministry of Education, Culture, Sports, Science and
196 Technology of Japan.

197

198

References

- 199 Aguilar I, Sommeijer M (2001) The deposition of anal excretions by *Melipona favosa*
200 foragers (Apidae: Meliponinae): behavioural observations concerning the location of
201 food sources. *Apidologie* 32: 37-48
- 202 Cameron SA (1981) Chemical signal in bumble bee foraging. *Behav Ecol Sociobiol* 9:
203 257-260.
- 204 Eltz T (2006) Tracing pollinator footprints on natural flowers. *J Chem Ecol* 32: 907-915
- 205 Frankie G, Vinson SB (1977) Scent marking of passion flowers in Texas by females of
206 *Xylocopa virginica texana* (Hymenoptera: Anthophoridae). *J Kans Entomol Soc* 50:
207 613-625
- 208 Free JB, Williams IH (1979) Communication by pheromones and other means in *Apis*
209 *florae* colonies. *J Apic Res* 18: 16-25
- 210 Free JB, Williams IH (1983) Scent-marking of flowers by honeybees. *J Apic Res* 22:

211 86-90

212 Gawleta N, Zimmermann Y, Eltz T (2005) Repellent foraging recognition across bee
 213 families. *Apidologie*, 36: 325-330

214 Gilbert F, Azmeh S, Barnard C, Behnke J, Collins SA, Hurst J, Shuker D, the
 215 behavioural ecology field course. (2001) Individually recognizable scent marks on
 216 flowers made by a solitary bee. *Anim Behav* 61: 217-229

217 Giurfa M (1993) The repellent scent-mark of the honeybee *Apis mellifera* ligustica and
 218 its role as communication cue during foraging. *Insect Soc* 40: 59-67

219 Giurfa M, Núñez JA (1993) Visual modulation of a scent-marking activity in the
 220 honeybee, *Apis mellifera* L. *Naturwissenschaften*, 80: 376-379

221 Giurfa NM, Núñez JA, Backhaus W (1994) Odour and colour information in the
 222 honeybee, *Apis mellifera* L. *J comp Physiol* 175: 773-77

223 Goulson D, Hawson SA, Stout JC (1998) Foraging bumblebees avoid flowers already
 224 visited by conspecifics or by other bumblebee species. *Anim Behav* 55: 199-206

225 Goulson D, Stout JC, Langley J, Hughes WOH (2000) Identity and function of scent
 226 marks deposited by foraging bumblebees. *J Chem Ecol* 26: 2897-2911

227 Goulson D, Chapman JW, Hughes WOH (2001) Discrimination of unrewarding flowers
 228 by bees: direct detection of rewards and use of repellent scent marks. *J Insect Behav*
 229 14: 669-677

230 Jarau S, Hrncir M, Ayasse M, Schulz C, Francke W, Zucchi R, Barth FG (2004) A
 231 stingless bee (*Melipona seminigra*) marks food sources with a pheromone from its

232 claw retractor tendons. J Chem Ecol 30: 793-804

233 Jarau S, Hrncir M, Zucchi R, Barth FG (2005) Morphology and structure of the tarsal
234 glands of the stingless bee *Melipona seminigra*. Naturwissenschaften 92: 147-150.

235 Lockey KH (1980) Insect cuticular hydrocarbons. Comp Biochem Physiol B 65
236 457-462

237 Newman DA, Thomson JD (2005) Effects of nectar robbing on nectar dynamics and
238 bumblebee foraging strategies in *Linaria vulgaris* (Scrophulariaceae). OIKOS 110:
239 309-320

240 Nieh JC, Ramírez S, Nogueira-Neto P (2003) Multi-source odor-marking of food by a
241 stingless bee, *Melipona mandacaia*. Behav Ecol Sociobiol 54: 578-586

242 Nieh JC, Barreto LS, Contrera FAL, Imperatriz-Fonseca VL (2004) Olfactory
243 eavesdropping by a competitively foraging stingless bee, *Trigona spinipes*. Proc R
244 Soc B 271: 1633-1640

245 Reader T, MacLeod I, Elliott PT, Robinson OJ, Manica A (2005) Inter-order
246 interactions between flower-visiting insects: Foraging bees avoid flowers previously
247 visited by hoverflies. J Insect Behav 18: 51-57

248 Saleh N, Chittka L (2006) The importance of experience in the interpretation of
249 conspecific chemical signals. Behav Ecol Sociobiol 61: 215-220.

250 Sasaki Y (1985) Studies on the social structure of *Halictus (Seladonia) aerarius* (Smith)
251 (Hymenoptera, Apoidae). Dissertation. Tokyo University of Agriculture, Tokyo,
252 Japan

- 253 Schmidt VM, Zucchi R, Barth FG (2005) Scent marks left by *Nannotrigona*
254 *testaceicornis* at the feeding site: cues rather than signals. *Apidologie* 36: 285-291
- 255 Schmitt U, Bertsch A (1990) Do foraging bumblebees scent-mark food sources and does
256 it matter? *Oecologia* 82: 137-144
- 257 Schmitt U, Lübke G, Francke W (1991) Tarsal secretion marks food sources in
258 bumblebees (Hymenoptera: Apidae). *Chemoecology* 2: 35-40.
- 259 Seeley TD (1985) *Honeybee Ecology: a study of adaptation in social life*. Princeton,
260 University Press, Princeton
- 261 Stout JC, Goulson D (2001) The use of conspecific and interspecific scent marks by
262 foraging bumblebees and honeybees. *Anim Behav* 62: 183-189
- 263 Stout JC, Goulson D (2002) The influence of nectar secretion rates on the responses of
264 bumblebees (*Bombus* spp.) to previously visited flowers. *Behav Ecol Sociobiol* 52:
265 239-246
- 266 Stout JC, Goulson D, Allen JA (1998) Repellent scent-marking of flowers by a guild of
267 foraging bumblebees (*Bombus* spp.). *Behav Ecol Sociobiol* 43: 317-326
- 268 Williams CS (1998) The identity of the previous visitor influences flower rejection by
269 nectar-collecting bees. *Anim Behav* 56: 673-681