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

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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

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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, revisitation rate to the flowers foraged by

previous visitors increased after between 20-60 minutes and 24 hours 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 regard 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.

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

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 m^2) 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.

5

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 (χ^2=3.11, *df*=1 *P*=0.0779), *A. cerana* (χ^2=3.67, *df* =1, *P*=0.0554) or *X. appendiculata*

133 (χ^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* (χ^2=14.65, *df*=1 *P*<0.0001), *A. cerana*

138 (χ^2=36.15, *df*=1 *P*<0.0001) and conspecifics (χ^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* (χ^2=20.82, *df*=1 *P*<0.0001)

141 and *A. cerana* (χ^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.

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