

Discrimination of Unrewarding Flowers by Bees; Direct Detection of Rewards and Use of Repellent Scent Marks

Dave Goulson,^{1,2} Jason W. Chapman,¹ and William O. H. Hughes¹

Accepted May 8, 2001; revised June 5, 2001

*Bumblebees and honeybees deposit short-lived scent marks on flowers that they visit when foraging. Conspecifics use these marks to distinguish those flowers that have recently been emptied and, so, avoid them. The aim of this study was to assess how widespread this behavior is. Evidence for direct detection of reward levels was found in two bee species: *Agapostemon nasutus* was able to detect directly pollen availability in flowers with exposed anthers, while *Apis mellifera* appeared to be able to detect nectar levels of tubular flowers. A third species, *Trigona fulviventris*, avoided flowers that had recently been visited by conspecifics, regardless of reward levels, probably by using scent marks. Three further bee/flower systems were examined in which there was no detectable discrimination among flowers. We argue that bees probably rely on direct detection of rewards where this is allowed by the structure of the flower and on scent marks when feeding on flowers where the rewards are hidden. However, discrimination does not always occur. We suggest that discrimination may not always make economic sense; when visiting flowers with a low handling time, or flowers that are scarce, it may be more efficient to visit every flower that is encountered.*

KEY WORDS: Foraging; nectar; pollen; efficiency; handling time; Apoidea.

¹Biodiversity and Ecology Division, School of Biological Sciences, University of Southampton, Biomedical Sciences Building, Bassett Crescent East, Southampton SO16 7PX, U.K.

²To whom correspondence should be addressed. Fax: +44-1703-594269. E-mail: dg3@soton.ac.uk.

INTRODUCTION

It has long been known that both bumblebees and honeybees have the ability to distinguish between more and less rewarding flowers of the same species without actually sampling the reward available. These bees often hover in front of a flower, sometimes briefly touching the corolla with their antennae or legs, and then depart without probing into the flower structure. Rejected flowers contain, on average, less nectar than flowers that are probed (Heinrich, 1979; Corbet *et al.*, 1984; Wetherwax, 1986; Kato, 1988; Duffield *et al.*, 1993). A number of mechanisms have been proposed to explain this. Bumblebees may be able to assess the pollen content of open flowers visually (Zimmerman, 1982) and may plausibly be able to determine the nectar content of some flower species in the same way (Thorp *et al.*, 1975, 1976; Kevan, 1976). If they cannot directly see the nectar, then it has been suggested that they may be able to assess nectar volumes from the scent of the nectar or the scent of fermentation products from yeasts in the nectar (Crane, 1975; Heinrich, 1979; Williams *et al.*, 1981). Bees could also be able to detect nectar volumes from humidity gradients surrounding the flower (Corbet *et al.*, 1979).

Thus there are a number of hypotheses as to how bees may distinguish rewarding flowers. However, as yet there is little evidence for any of these. The only proven method by which bees are able to detect and avoid empty flowers is by exploiting the scent left by bees on previous visits (Cameron, 1981; Free and Williams, 1983; Marden, 1984; Kato, 1988; Schmitt and Bertsch, 1990; Giurfa, 1993). It is presumed that the use of scent marks increases foraging efficiency by reducing the time spent handling unrewarding flowers (Kato, 1988; Schmitt and Bertsch, 1990; Goulson *et al.*, 1998). Honeybees, bumblebees, and the solitary carpenter bee *Xylocopa virginica* Cresson leave short-lived scent marks on flowers that repel conspecifics (Núñez, 1967; Frankie and Vinson, 1977; Wetherwax, 1986; Giurfa and Núñez, 1992; Giurfa, 1993; Giurfa *et al.*, 1994; Goulson *et al.*, 1998, Stout *et al.*, 1998). In bumblebees, scent marks consist of mixtures of straight-chain alkanes and alkenes, most of them common cuticular hydrocarbons found in a range of insects (Schmitt *et al.*, 1991; Goulson *et al.*, 2001). In addition to repellent marks, there is also some evidence that honeybees and bumblebees may leave scent marks that act as attractants on particularly rewarding patches of flowers and thus concentrate subsequent foraging bouts on rewarding flowers only (Ferguson and Free, 1979; Kato, 1988; Schmitt and Bertsch, 1990).

The vast majority of work on detection and avoidance of empty flowers has focused on honeybees and bumblebees. Nothing is known about the discriminatory abilities of most of the many thousands of other bee species.

Our knowledge is based on a few detailed studies, each of one bee species visiting one flower species, and in all published studies bees were found to discriminate. Since negative results are likely to be underreported, we do not know whether bees always discriminate among flowers and, if so, whether they always use scent marks. The aim of this study was to examine a range of bee species feeding on different flowers, to determine

- (I) whether bees always discriminate among flowers of a particular species on the basis of reward;
- (II) whether scent marking occurs in bees other than *Apis*, *Bombus*, and *Xylocopa*; and
- (III) whether discrimination also occurs via direct detection of rewards.

METHODS

All experiments were carried out using wild bees foraging on flower species that they naturally visit. Methodology for assessment of discrimination among flowers by foraging insects broadly follows Goulson *et al.* (1998) and Stout *et al.* (1998). In brief, bee responses to flowers that had not recently been visited (containing reward) and to recently visited flowers (with depleted rewards) were compared to determine whether discrimination was occurring. Unvisited flowers were obtained by enclosing flowers in netting for 45 min (or, for *Sida glabra*, by enclosing flower buds in netting until they opened). Visited flowers were flowers from which a bee had just fed. Flowers were observed until they were approached by a bee. For visited flowers, if no bee approached within 2 min, the flower was discarded. Acceptance was recorded when the bee landed on the flower and gathered pollen or probed for nectar. Rejection was recorded if the bee approached to within 1 cm of the flower and then departed without gathering pollen or probing for nectar.

Where rejection behavior was apparent, a third treatment was included to determine the mechanism involved. When bees were gathering pollen, this treatment consisted of unvisited flowers from which the pollen had been removed using a fine paintbrush. When bees were gathering nectar, this treatment consisted of adding nectar to flowers that had just been visited. Nectar was gathered from an unvisited flower with a microcapillary tube. This difference in methodology between flowers used for pollen and flowers used for nectar was necessary because it is not practical to add pollen to anthers, while conversely it is much easier to add nectar than it is to remove all nectar from a flower.

Unless stated otherwise; differences in the frequency of acceptance between treatments were tested using χ^2 with Yates' correction. Six bee/flower

systems were examined according to availability and are summarized in Table I. Details are given below.

- (I) *Agapostemon nasutus* Smith (Hymenoptera: Halictidae) visiting *Sida glabra* (Malvaceae). *S. glabra* is a herbaceous plant growing to a height of 70 cm, with open yellow actinomorphic flowers approximately 8 mm in diameter. Flowers opened at approximately 1430 h and senesced by the following morning. All *A. nasutus* observed were collecting pollen only. The stamens in *S. glabra* are held above the flower and are clearly visible.
- (II) *Trigona fulviventris* Guérin (Hymenoptera: Apidae) foraging for nectar on *Crotalaria cajanifolia* (Fabaceae). *C. cajanifolia* is a shrub bearing racemes of zygomorphic yellow flowers. All of the observed bees were robbing the flowers, as either primary or secondary nectar robbers. These flowers are complex in structure and none of the bees attempted to enter flowers in the legitimate way. Robbing was accomplished by piercing the sepals at the base of the flower. Half of the unvisited flowers had previously been robbed and half were unrobbed.
- (III) *T. fulviventris* foraging for nectar on *Priva mexicana* (Verbenaceae). *P. mexicana* is an annual plant growing to 45 cm and bearing racemes of small zygomorphic tubular purple flowers. On this plant species all visits by *T. fulviventris* were legitimate.
- (IV) *Pereirapis* sp. (Hymenoptera: Apidae) foraging for nectar on *P. mexicana*. While studying *T. fulviventris* visiting *P. mexicana*, bees of a *Pereirapis* sp. were also observed visiting the flowers. The *Pereirapis* sp. were comparatively scarce, so that replication was limited (Table I). Because of the small sample size, differences in the frequency of acceptance between treatments were tested using Fisher's exact test.
- (V) *Trigona fuscipennis* Friese (Hymenoptera: Apidae) foraging for nectar on *Byrsonima crassifolia* (Malpighiaceae). *B. crassifolia* is a tree bearing vertical racemes of zygomorphic yellow flowers. These flowers were simple in structure, with unenclosed nectaries.
- (VI) *Apis mellifera* L. (Hymenoptera: Apidae) foraging for nectar on *Echium vulgare* (Boraginaceae). *E. vulgare* is an herbaceous plant growing to 80 cm which bears vertical cymes of zygomorphic purple tubular flowers grouped into panicles.

System I was studied near Playa del Carmen, Quintana Roo, Mexico, in March 1999. Systems II–V were studied near Tapachula, Chiapas, in southern

Table I. Summary of Bee and Flower Systems Studied, with Numbers of Replicates for Each Treatment Used

Bee species	Plant species	Unvisited	Visited	Visited + reward	Unvisited – reward	Reward type
<i>Agapostemon nasutus</i>	<i>Sida glabra</i>	30	32	—	32	Pollen
<i>Trigona fulviventris</i>	<i>Crotalaria cajanifolia</i>	40	40	—	—	Nectar
<i>Trigona fulviventris</i>	<i>Priva mexicana</i>	42	40	38	—	Nectar
<i>Pereirapis</i> sp.	<i>Priva mexicana</i>	19	17	—	—	Nectar
<i>Trigona fuscipennis</i>	<i>Byrsonima crassifolia</i>	30	30	—	—	Nectar
<i>Apis mellifera</i>	<i>Echium vulgare</i>	40	39	40	—	Nectar

Mexico in October 1999. System VI was studied near Bolonia, Andalucia, in southern Spain in April 1999.

RESULTS

In three of the six systems examined, no detectable discrimination according to reward levels was found. These are described first.

- (II) *T. fulviventris* visiting *C. cajanifolia*: Flowers which had not been visited for 45 min but which had previously been robbed were accepted by 34 of 40 bees (85%). Similarly, flowers which had been visited within the previous 2 min (and thus had all been robbed) were accepted by 36 of 40 bees (90%). Flowers from which bees had been excluded for 45 min, and which had never previously been robbed, were all rejected by bees ($n = 40$). Each test bee would explore the rear of the flower but depart if no robbing hole was found, although on other occasions *T. fulviventris* were observed to be primary robbers. Rejection of unrobbed flowers was significantly higher than that of robbed flowers, regardless of whether bees had been excluded from the robbed flowers for 45 min ($\chi_1^2 = 26.2$, $P < 0.001$) or whether the flower had been visited within the previous 2 min ($\chi_1^2 = 41.3$, $P < 0.001$).
- (IV) *Pereirapis* sp. visiting *P. mexicana*: This species accepted the majority of *P. mexicana* flowers regardless of whether they had been netted for 45 min (18 of 19 flowers; 94.7%) or whether they had

been visited in the previous 2 min by a conspecific (12 of 17 flowers; 70.6%). Although rejection rates were higher when the flower had recently been visited, there was no significant difference between these two treatments (Fisher's exact test, $P = 0.066$). Sample sizes were low in this study so the results should be treated with caution.

- (V) *T. fuscipennis* visiting *B. crassifolia*: Precisely the same proportion of bees accepted flowers in each of the two treatments. Twenty-two of 30 bees (73.3%) accepted flowers that had been bagged for 45 min and, also, flowers that had just been visited.

Evidence for direct detection of reward levels was found in system I, *A. nasutus* visiting *S. glabra*. Flowers that had not previously been visited were accepted by 28 of 30 bees (93.3%), while flowers that had been visited by another bee in the previous 2 min were accepted by only 6 of 32 bees (18.8%). This difference is statistically significant ($\chi_1^2 = 31.5$, $P < 0.001$). Bees accepted unvisited flowers from which the pollen had been artificially removed at an intermediate frequency (14 of 32; 43.8%). This was not significantly different from the acceptance rate of flowers that had just been visited by another bee ($\chi_1^2 = 3.56$, $P < 0.05$). It is a significantly lower frequency of acceptance than was found for flowers that had not been visited and in which the pollen load was intact ($\chi_1^2 = 15.32$, $P < 0.001$).

Evidence for scent marking was found in system III, *T. fulviventris* visiting *P. mexicana*. Bees accepted the majority of flowers which had not been visited for at least 45 min previously (32 of 42; 76.2%). They accepted significantly fewer flowers when they had been visited by another bee within the previous 2 min (16 of 40; 40.0%) ($\chi_1^2 = 9.58$, $P < 0.01$). They also accepted significantly fewer flowers that had just been visited by another bee but to which nectar had been added (10 of 38; 26.3%) ($\chi_1^2 = 17.96$, $P < 0.001$). The addition of nectar to flowers that had just been visited by a bee did not significantly affect the likelihood of acceptance ($\chi_1^2 = 1.08$, $P < 0.05$).

Finally, there appeared to be evidence for both scent marking and direct detection of rewards in system VI, *A. mellifera* visiting *E. vulgare*. Flowers that had not been visited for at least 45 min were accepted at a high frequency (35 of 40; 87.5%). Flowers that had just been visited by another bee were rarely accepted (7 of 39; 17.9%). This difference is statistically significant ($\chi_1^2 = 35.4$, $P < 0.001$). Flowers that had just been visited but had then been refilled with nectar were accepted at an intermediate frequency (18 of 40; 45%). This is significantly lower than the frequency of acceptance of flowers that had not been visited for 45 min ($\chi_1^2 = 14.3$, $P < 0.001$) but is significantly higher than the acceptance rate of flowers that had just been visited ($\chi_1^2 = 5.39$).

DISCUSSION

Various means by which flower visitors could detect those flowers that are most likely to contain rewards have been suggested (Crane, 1975; Thorp *et al.*, 1975, 1976; Kevan, 1976; Corbet *et al.*, 1979; Heinrich, 1979; Williams *et al.*, 1981; Zimmerman, 1982). Until now only the use of repellent scent marks has been convincingly demonstrated, and only in *Apis mellifera*, *Xylocopa virginica*, and three *Bombus* species (Frankie and Vinson, 1977; Giurfa and Núñez, 1992; Goulson *et al.*, 1998; Stout *et al.*, 1998). Our data strongly suggest that scent marks are also used by *T. fulviventris* when foraging legitimately on the tubular flowers of *P. mexicana*, since bees tended to reject flowers that had recently been visited by another bee. The addition of nectar to these flowers did not render them more acceptable, indicating that the bees were not deciding based on a direct assessment of the reward. The most parsimonious explanation is that they are discriminating using the scent of previous visitors (as do other Apidae). The use of repellent scent marks has now been described in species from four distantly related genera, including both solitary and social representatives, and it therefore seems likely that they are widely used by bees when foraging.

Repellent scent marks can be detected and used by other species within the genus *Bombus* (Goulson *et al.*, 1998; Stout *et al.*, 1998). However, it is not known whether repellent scent marks can be detected across broader taxonomic divisions or, indeed, whether different bee species use the same compounds in marks. Since several bee species are commonly observed foraging on the same plant, there is considerable scope for interspecific interactions. This requires investigation.

No apparent discrimination among the flowers was found for three bee/flower combinations (*T. fulviventris* nectar robbing from *C. cajanifolia*, *T. fuscipennis* visiting *B. crassifolia*, and *Pereirapis* spp. visiting *P. Mexicana*). Since *T. fulviventris* appears to use scent marks when foraging on *P. mexicana* but not when robbing *C. cajanifolia*, it seems that some bee species can discriminate using scent marks but do not always do so. It is possible that discriminating among flowers may not always be the best strategy. MacArthur and Pianka (1996) considered the economics of the choice faced by a predator when deciding whether to attack a given prey item or search for a better one. Their predictions are equally applicable to bees visiting flowers: predators should be less choosy if the best prey items (flowers that have not recently been visited) are scarce or if the handling time per prey item is low. Flowers of *B. crassifolia* are simple, and while those of *C. cajanifolia* are complex, once they have been robbed and there is a hole directly into the nectaries, they can be handled quickly by bees. Flowers in which scent marking has been identified are, like *P. mexicana*, generally deep and require the bee

to climb partially inside before the nectar can be reached (Goulson *et al.*, 1998). Presumably such flowers have longer handling times so that investigating flowers which may be empty is costly in time. An elegant test of this hypothesis would be to use artificial flowers in which the handling time and abundance could be manipulated while keeping other factors constant.

Another interesting feature of our results is that *T. fulviventris* appeared to be using scent marks when foraging on *P. mexicana*, but the *Pereirapis* sp. did not. This species was by far the smallest of the bees that we examined, and is also solitary, and hence the economics of foraging decisions may be rather different. Smaller bees have lower metabolic costs, and can profitably forage on flowers providing lower rewards, compared to larger bees (Corbet *et al.*, 1995). Solitary bees also have much lower nectar requirements than social species. Hence tiny amounts of nectar remaining in recently visited flowers may provide for their needs. Alternatively, the *Pereirapis* sp. may have simply not evolved the use of scent marks. With the exception of *Xylocopa virginica*, the bee species that have been demonstrated to use scent marks are all social, while *Pereirapis* spp. are solitary. It may be that the use of scent marks in foraging has evolved from their use in a social context. Data from a far greater range of bee species are required to establish whether this is indeed the case.

Our experiments also provide the first evidence that bees can use direct detection methods to determine which flowers are rewarding. *A. nasutus* are able to detect how much pollen is available in the open flowers of *S. glabra* and avoid flowers that have been depleted by other bees or from which the pollen has been artificially removed. We found no evidence for use of scent marks, and this is not surprising; if pollen availability is directly detectable, then there is no need for an indirect indication of reward levels. We do not know what particular cues are used by the bees in discrimination (for example visual versus olfactory); this would be an interesting area for further research.

We also found some evidence for direct detection of nectar levels by *A. mellifera*, since they were more likely to accept a flower that had been recently visited by another bee if the nectar was artificially replenished. This suggests that they are able to see or smell whether a flower contains a nectar reward. Since they were also more likely to accept unvisited flowers than those which had been visited but to which nectar had then been added, it would appear that they were also using repellent scent marks. Deposition of short-lived repellent scents on flowers has been reported in honeybees (Giurfa and Núñez, 1993; Giurfa, 1993; Williams, 1998). It seems that *A. mellifera* can use both direct detection of rewards and repellent scent marks. Either bees integrate information from scent marks and from their direct perception of rewards to decide whether to visit or to reject each flower

or perhaps the population of foraging bees contains some individuals that are using scent marks and some that are using direct detection. Bees adapt their foraging behavior according to experience (Dukas and Waser, 1994; Menzel and Müller, 1996), and it is quite possible that different bees may learn to associate different cues with the presence or absence of rewards.

ACKNOWLEDGMENTS

We wish to thank Trevor Williams for providing facilities in Tapachula, Chris O' Toole for identification of bees and Sally Corbet for comments on the manuscript.

REFERENCES

- Cameron, S. A. (1981). Chemical signals in bumble bee foraging. *Behav. Ecol. Sociobiol.* **9**: 257–260.
- Corbet, S. A., Willmer, P. G., Beament, J. W. L., Unwin, D. M., and Prys-Jones, O. E. (1979). Post-secretory determinants of sugar concentration in nectar. *Plant Cell Environ.* **2**: 293–308.
- Corbet, S. A., Kerslake, C. J. C., Brown, D., and Morland, N. E. (1984). Can bees select nectar-rich flowers in a patch? *J. Apic. Res.* **23**: 234–242.
- Corbet, S. A., Saville, N. M., Fussell, M., Prys-Jones, O. E., and Unwin, D. M. (1995). The competition box: A graphical aid to forecasting pollinator performance. *J. Appl. Ecol.* **32**: 707–719.
- Crane, E. (1975). *Honey: A Comprehensive Survey*, Heinemann in cooperation with International Bee Research Association, London.
- Duffield, G. E., Gibson, R. C., Gilhooly, P. M., Hesse, A. J., Inkley, C. R., Gillbert, F. S., and Barnard, C. J. (1993). Choice of flowers by foraging honey-bees (*Apis mellifera*)—Possible morphological cues. *Ecol. Entomol.* **18**: 191–197.
- Dukas, R., and Waser, N. M. (1994). Categorization of food types enhances foraging performance of bumblebees. *Anim. Behav.* **48**: 1001–1006.
- Ferguson, A. W., and Free, J. B. (1979). Production of forage-marking pheromone by the honeybee. *J. Apic. Res.* **18**: 128–135.
- Frankie, G. W., and Vinson, S. B. (1977). Scent marking of passion flowers in Texas by females of *Xylocopa virginica texana* (Hymenoptera: Anthophoridae). *J. Kans. Entomol. Soc.* **50**: 613–625.
- Free, J. B., and Williams, I. H. (1983). Scent-marking of flowers by honeybees. *J. Apic. Res.* **18**: 128–135.
- Giurfa, M. (1993). The repellent scent-mark of the honeybee *Apis mellifera ligustica* and its role as communication cue during foraging. *Insects Soc.* **40**: 59–67.
- Giurfa, M., and Núñez, J. A. (1992). Honeybees mark with scent and reject recently visited flowers. *Oecologia* **89**: 113–117.
- Giurfa, M., Núñez, J. A., and Backhaus, W. (1994). Odour and colour information in the foraging choice behavior of the honeybee. *J. Comp. Physiol.* **175**: 773–779.
- Goulson, D., Hawson, S. A., and Stout, J. C. (1998). Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. *Anim. Behav.* **55**: 199–206.
- Goulson, D., Stout, J. C., Langley, G. J., and Hughes, W. O. H. (2001). The identity and function of scent marks deposited by foraging bumblebees. *J. Chem. Ecol.* **27**: 109–124.
- Heinrich, B. (1979). Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia* **40**: 235–245.

- Kato, M. (1988). Bumble bee visits to *Impatiens* spp.: Pattern and efficiency. *Oecologia* **76**: 364–370.
- Kevan, P. G. (1976). Flourescent nectar (technical comment). *Science* **194**: 341–342.
- MacArthur, R. H., and Pianka, E. R. (1966). On optimal use of a patchy environment. *Am. Nat.* **100**: 603–609.
- Marden, J. H. (1984). Remote perception of floral nectar by bumblebee. *Oecologia* **64**: 232–240.
- Menzel, R., and Müller, U. (1996). Learning and memory in honeybees: From behaviour to neural substrates. *Annu. Rev. Neurosci.* **19**: 379–404.
- Núñez, J. A. (1967). Sammelbienen markieren versiegte Futterquellen durch Duft. *Naturwissenschaften* **54**: 322–323.
- Schmitt, U., and Bertsch, A. (1990). Do foraging bumblebees scent-mark food sources and does it matter? *Oecologia* **82**: 137–144.
- Schmitt, U., Lübke, G., and Franke, W. (1991). Tarsal secretion marks food sources in bumblebees (Hymenoptera: Apidae). *Chemoecology* **2**: 35–40.
- Stout, J. C., Goulson, D., and Allen, J. A. (1998). Repellent scent marking of flowers by a guild of foraging bumblebees (*Bombus* spp.). *Behav. Ecol. Sociobiol.* **43**: 317–326.
- Thorp, R. N., Briggs, D. L., Estes, J. R., and Erikson, E. H. (1975). Nectar flourescence under ultraviolet irradiation. *Science* **189**: 476–478.
- Thorp, R. N., Briggs, D. L., Estes, J. R., and Erikson, E. H. (1976). Reply to Kevan. *Science* **194**: 342.
- Wetherwax, P. B. (1986). Why do honeybees reject certain flowers? *Oecologia* **69**: 567–570.
- Williams, A. A., Hollands, T. A., and Tucknott, O. G. (1981). The gas chromatographic-mass spectrometric examination of the volatiles produced by the fermentation of a sucrose solution. *Z. Lebensmittelunters Forsch* **172**: 377–381.
- Williams, C. S. (1998). The identity of the previous visitor influences flower rejection by nectar-collecting bees. *Anim. Behav.* **56**: 673–681.
- Zimmerman, M. (1982). Optimal foraging: Random movement by pollen collecting bumblebees. *Oecologia* **53**: 394–398.