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IDENTITY AND FUNCTION OF SCENT MARKS DEPOSITED BY FORAGING BUMBLEBEES

DAVE GOULSON,[1,*] JANE C. STOUT,[1] JOHN LANGLEY,[2] and WILLIAM O. H. HUGHES[1]

[1]*Biodiversity and Ecology Division, School of Biological Sciences*
[2]*Department of Chemistry*
University of Southampton
Southampton, UK

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Abstract—Foraging bumblebees can detect scents left on flowers by previous bumblebee visitors and hence avoid flowers that have been depleted of nectar. Tarsal secretions are probably responsible for this repellent effect. The chemical components of the tarsal glands were analyzed by combined gas chromatography–mass spectrometry for three species of bumblebee, *Bombus terrestris*, *B. lapidarius*, and *B. pascuorum*. The hydrocarbons identified were similar for each species, although there were interspecific differences in the relative amounts of each compound present. The tarsal extracts of all three species comprised complex mixtures of long-chain alkanes and alkenes with between 21 and 29 carbon atoms. When *B. terrestris* tarsal extracts were applied to flowers and offered to foraging bumblebees of the three species, each exhibited a similar response; concentrated solutions produced a repellent effect, which decreased as the concentration declined. We bioassayed synthetic tricosane (one of the compounds found in the tarsal extracts) at a range of doses to determine whether it gave a similar response. Doses $\geq 10^{-12}$ ng/flower resulted in rejection by foraging *B. lapidarius*. Only when $\leq 10^{-14}$ ng was applied did the repellent effect fade. We bioassayed four other synthetic compounds found in tarsal extracts and a mixture of all five compounds to determine which were important in inducing a repellent effect in *B. lapidarius* workers. All induced repellency but the strength of the response varied; heneicosane was most repellent while tricosene was least repellent. These findings are discussed in relation to previous studies that found that tarsal scent marks were attractive rather than repellent.

Key Words—*Bombus*, Apidae, Hymenoptera, tarsal gland secretions, foraging behavior, repellency, *n*-heneicosane, *n*-tricosane, (*Z*)-9-tricosene, *n*-pentacosane, *n*-heptacosane.

*To whom correspondence should be addressed; e-mail: dg3@soton.ac.uk

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INTRODUCTION

Both bumblebees and honeybees can distinguish between rewarding and nonrewarding flowers of the same species without sampling the reward available. They often hover in front of a flower, sometimes briefly touching the corolla, and then depart without probing into the flower structure. These 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). Several mechanisms may be in operation. Bees can assess pollen content of open flowers visually (Zimmerman, 1982) and may be able to determine the nectar content of some flower species in the same way (Thorp et al., 1975, 1976; Kevan, 1976). It has been suggested that they may be able to assess nectar volumes from the scent of the nectar itself or the scent of fermentation products from yeasts in the nectar (Crane, 1975; Heinrich, 1979; Williams et al., 1981). They could also plausibly detect nectar volumes from humidity gradients surrounding the flower (Corbet et al., 1979). Although these possibilities have not been excluded, there is now clear evidence that an important cue used by bees to decide whether to probe or reject a flower are scent marks left by bees on previous visits (Cameron, 1981; Free and Williams, 1983; Marden, 1984; Kato, 1988; Schmitt and Bertsch, 1990; Giurfa, 1993; Goulson et al., 1998; Stout et al., 1998). Such marks may increase foraging efficiency by reducing the time spent handling unrewarding flowers (Kato, 1988; Schmitt and Bertsch, 1990; Goulson et al., 1998).

Honeybees, bumblebees, and carpenter bees (Hymenoptera, Anthophoridae *Xylocopa* sp.) leave short-lived repellent marks on flowers that they visit, and conspecifics use these to discriminate between visited and unvisited flowers (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; Williams, 1998). When foraging on artificial flowers, both honeybees and bumblebees can also leave scent marks that are attractive to themselves and to conspecifics and thus concentrate subsequent foraging bouts on rewarding flowers only (Ferguson and Free, 1979; Kato, 1988; Schmitt and Bertsch, 1990).

In honeybees the chemical cue that causes repellency is thought to be secreted from the mandibular glands (Vallet et al., 1991), while Nasanov secretions induce an attractant effect (von Frisch, 1923; Free and Williams, 1972; Free et al., 1982a,b). A Dufour's gland secretion is thought to be responsible for carpenter bees (*Xylocopa virginica texana*, Anthophoridae) avoiding recently visited flowers (Frankie and Vinson, 1977). In bumblebees both attractant and repellent effects appear to be induced by a chemical cue found on the tarsi, and presumed to be secreted by the tarsal gland (Schmitt et al., 1991; Stout et al., 1998). For *Bombus terrestris* the components of both tarsal glands and the deposited scent marks have been identified and are very similar (Schmitt, 1990; Schmitt et al., 1991). Tarsal glands produce primarily straight-chain alkanes and

alkenes of 21–29 carbon atoms, with compounds with odd numbers of carbons predominating. The alkenes are thought to be mostly (*Z*)-9 and (*Z*)-11 configurations (Schmitt, 1990; Schmitt et al., 1991). These compounds are common cuticular hydrocarbons found in a broad range of insects (Lockey, 1980; Blum, 1981, 1987).

Schmitt et al. (1991) found that when dilute synthetic mixtures of the compounds found in the bumblebee tarsi were applied to artificial flowers, foraging bumblebees were attracted to these flowers. However, when synthetics were applied at higher concentrations, bumblebees were repelled from treated flowers. Stout et al. (1998) found that tarsal washes applied to *Phacelia tanacetifolia* (Hydrophyllaceae) induced repellency in the field.

In bumblebees, 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 these species all use the same compounds in their marks, or whether each species is able to recognize a range of different repellent marks left by various species. It is also unclear whether the compounds identified by Schmitt et al. (1991) in attractant marks left by *B. terrestris* on artificial flowers are also responsible for the repellent effects observed in field experiments (Goulson et al., 1998; Stout et al., 1998). The difference could simply be a matter of concentration; an initially repellent mark could turn in to an attractant mark as some of its components evaporate. Conversely the same mark may be either an attractant or a repellent depending on context.

In this study we compare the components of tarsal gland secretions in three *Bombus* species, *B. terrestris* (L.), *B. pascuorum* (L.), and *B. lapidarius* (L.) (Hymenoptera: Apidae). We examine the range of concentrations over which repellency or attraction is produced by applying serial dilutions of washes of *B. terrestris* tarsal glands to flowers in the field and assaying the response of foraging bees. Finally we bioassay synthetic compounds and mixtures of compounds to examine which ones induce behavioral responses.

METHODS AND MATERIALS

Chemical Analysis of Bumblebee Tarsi. In July 1998, 12 worker bumblebees of each of three species (*B. lapidarius*, *B. pascuorum*, and *B. terrestris*) were captured while foraging at sites near Southampton, Hampshire, UK. Bumblebees were captured while visiting flowers by enclosing them in glass scintillation vials, thus minimizing possible contamination. The bees were immediately freeze-killed by placing the vials in Dry Ice. In the laboratory, extracts were prepared by cutting the tarsi and approximately half of the tibia from six individuals of the same species and combining them in 1 ml of hexane. Two replicate samples were made up for each bumblebee species. These samples were used to

optimize gas chromatograph running conditions and to identify the major components of the extracts to be used in bioassays in 1999.

To obtain samples for quantitative analysis, in July and August 1999 a further 20 worker bumblebees of each of the three bumblebee species were captured while foraging and killed as described above. Extracts were prepared by cutting the tarsi and approximately half of the tibia from five individuals of one species and combining them in 0.5 ml of pentane. Four replicate samples were made up for each bumblebee species.

The samples were analyzed with a VG-Analytical 70-250SE mass spectrometer coupled to a Hewlett Packard 5790 gas chromatograph. The column was a BP1 (25 m $\times$ 0.33 mm, with a film thickness of 0.25 μm), and the carrier gas was helium. Temperature programming was as follows: 60°C for 3 min; heating 20°C/min; 300°C for 10 min; 280°C for 12 min. Nonadecane was used as an internal standard to quantify the amounts of compounds present.

Application of Tarsal Extracts to Flowers. Thirty *B. terrestris* workers were captured while foraging and killed as described above in June 1999. The tarsi and lower tibia were removed from all these individuals and washed in 3 ml pentane. The gas chromatographic analysis indicated that in *B. terrestris* one leg provides 514 ng of hydrocarbons. Thus, this stock solution was estimated to contain 30.8 μg of hydrocarbons per milliliter. A serial dilution of the stock solution was made in pentane. Five microliters of an extract at each concentration was applied to each flower in an inflorescence in the field with a Gilson pipet. The amounts of hydrocarbons applied were estimated to be 0.154, 1.54×10^{-4}, 1.54×10^{-7}, 1.54×10^{-8}, 1.54×10^{-9}, and 1.54×10^{-10} μg/flower. Inflorescences were presented to bumblebees in the same manner as described in Goulson et al. (1998).

Inflorescences were scored as accepted by the bee if it landed and probed one of the flowers for nectar and were scored as rejected if the bee approached a flower, perhaps briefly touched it with its antennae or feet, but departed without landing and probing. Inflorescences were covered with fine netting to exclude insect visits for at least 48 hr before use. Each species of bee was presented with a different flower species according to their foraging preferences. Only bees that were foraging on the species of flower to be tested were used. (1) *B. lapidarius* workers were offered *Melilotus officinalis* (Fabaceae) flowers at a site near St. Catherine's Hill, Winchester, Hampshire, UK. (2) *B. pascuorum* workers were offered *Symphytum officinale* (Boraginaceae) flowers in the Itchen Valley Country Park, Southampton, Hampshire UK. (3) Finally, *B. terrestris* were offered *Phacelia tanacetifolia* (Hydrophyllaceae) flowers in the research gardens of the University of Southampton Research Centre at Chilworth, Hampshire, UK.

At least 20 tests were carried out with each concentration of extract for *B. terrestris* and *B. lapidarius*, and at least 13 tests were carried out with each

concentration of extract for *B. pascuorum* (this number is lower due to tests being carried out towards the end of the *S. officinalis* flowering season; hence the number of available flowers was lower).

Inflorescences were used only once, and we also attempted to use individual bumblebees only once, although this was difficult as it was not possible to mark individuals (see Stout et al., 1998, for a discussion of this problem). Bumblebees were also presented with flowers that had 5 μl of pentane applied to the corolla as a control (21, 27, and 27 tests for *B. lapidarius*, *B. pascuorum*, and *B. terrestris*, respectively). The probability of rejection of flowers treated with tarsal extracts was compared with the probability of rejection of flowers treated with pentane for each concentration of tarsal extract using a 2×2 χ^2 test on the original data, with Yates' correction. To examine whether there were overall differences in the rejection rates of different bee species, we also analyzed the entire data set in GLIM using binary errors (reject or accept) according to bee species and the concentration of the extract (see Crawley, 1991).

Application of Synthetic Extracts to Flowers. We obtained synthetic samples of the five most common chemicals found in bumblebee tarsi (from Sigma Chemicals). These were *n*-heneicosane, *n*-tricosane, (*Z*)-9-tricosene, *n*-pentacosane, and *n*-heptacosane. A dilution series of tricosane in pentane was made to give the following; 1000, 10, 1, 10^{-3}, 10^{-6}, 10^{-8}, 10^{-10}, 10^{-12}, 10^{-14}, 10^{-16}, and 10^{-18} μg tricosane/5 μl pentane. Five microliters of all dilutions in the series were applied to *Melilotus officinalis* flowers and offered to *B. lapidarius* as described above. At least 23 tests were made with each dose.

The other four compounds were diluted in pentane to give a concentration of 10^{-12} μg/5 μl. All five chemicals were also combined in equal proportions to give a concentration of 10^{-12} μg of hydrocarbons per 5 μl. Each of the compounds and the mixture were tested by applying them to *M. officinalis* flowers and offering them to *B. lapidarius* as above. At least 28 tests were carried out with each synthetic chemical. Flowers that had not received any treatment were also offered (37 tests).

Inflorescences that had 5 μl pentane applied to each corolla were offered to bumblebees as a control (31 tests). The probability of rejection of flowers treated with each dose of compound was compared with the probability of rejection of flowers treated with pentane using a 2×2 χ^2 test with Yates' correction. Similarly, the probability of rejection of untreated flowers was also compared with the probability of rejection of flowers treated with pentane. Since a large number of tests were carried out (18) a sequential Bonferroni procedure was used to control for group-wide type-1 errors (Holm, 1979). For the dilution series of tricosane, a further analysis was performed to see if there was an overall effect of dose on the probability of rejection. The rejection or acceptance of each flower was analyzed as binary data in GLIM with the log of the tricosane dose used as the explanatory variable.

RESULTS

Analysis of Bumblebee Tarsal Extracts. Chemical analyses of the bumblebee tarsal extracts revealed complex mixtures of long-chain hydrocarbons (alkenes and alkanes) with odd numbers of carbon atoms between 21 and 29, similar to those found by Schmitt (1990) and Schmitt et al. (1991) (Table 1). There were notable differences between the three bee species. *B. lapidarius* samples contained four major compounds: tricosenes, tricosane, pentacosene, and pentacosane. *B. pasuorum* was similar except that tricosenes were present in small amounts only. *B. terrestris* samples contained tricosane, pentacosane, heptacosane, and nonacosene in significant quantities. Smaller amounts of other alkanes and alkenes were variously present (Table 1). All three species contained about 500 ng of hydrocarbons per tarsus.

Application of Tarsal Extracts to Flowers. The majority of control flowers (treated with pentane only) were accepted by foraging bees of all three species (76.2, 70.4, and 66.7% by *B. lapidarius*, *B. pascuorum*, and *B. terrestris*, respectively). The proportion of bumblebees that rejected flowers treated with tarsal extracts decreased as the concentration of the tarsal extract decreased (χ^2_1 = 109.9, $P < 0.001$) (Figure 1). There was also a significant difference between the bumblebee species in the overall likelihood of flowers being rejected (χ^2_2 = 8.4, $P < 0.05$), with *B. pascuorum* exhibiting higher rates of rejection than the other two species. There was no significant interaction between the effect of concentration and that of bumblebee species ($\chi^2_2 = 0.3$, $P > 0.05$). From pairwise comparisons of the frequency of rejection of particular concentrations versus

TABLE 1. COMPOUNDS IN TARSAL WASHES OF THREE BUMBLEBEE SPECIES[a]

Compound (ng/tarus ± SE)	MW	*B. terrestris*	*B. pascuorum*	*B. lapidarius*
Heneicosane	296	12.5 ± 2.41		+
Tricosenes	322	9.38 ± 6.63	5.90 ± 0.95	70.5 ± 15.1
Tricosane	324	110 ± 13.4	99.3 ± 1.21	94.8 ± 8.63
Methyl-tricosane	324			+
Tetracosenes	336		12.5 ± 6.03	+
Tetracosane	338		+	+
Pentacosenes	350	+	174 ± 12.3	155 ± 11.5
Pentacosane	352	114 ± 17.9	106 ± 5.98	170 ± 6.06
Heptacosenes	378		64.5 ± 13.2	+
Heptacosane	380	174.5 ± 28.6	35.5 ± 5.60	+
Nonacosenes	406	102.9 ± 26.1	+	+
Total		514 ± 68.7	491 ± 30.5	490 ± 32.4

[a] Based on four replicate samples per species. MW = molecular weight, + = trace.

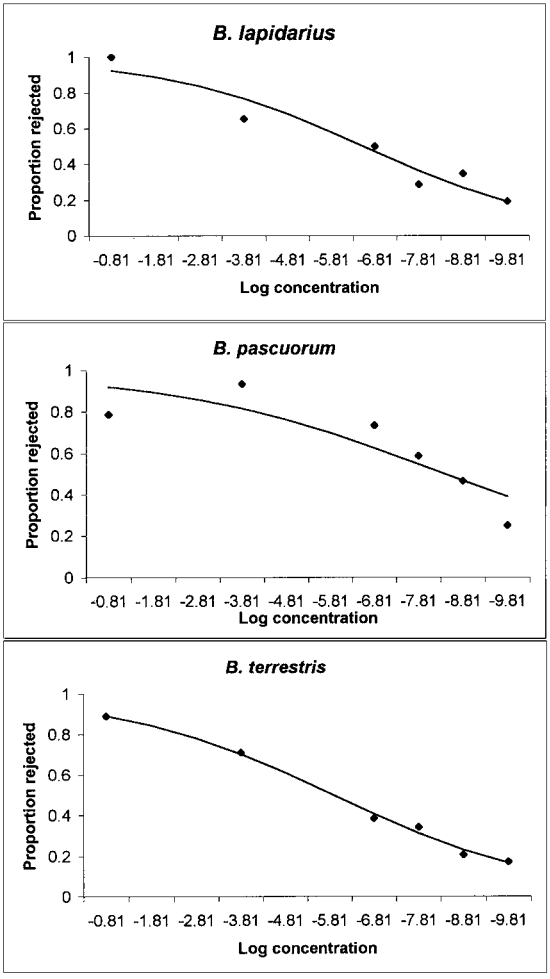

FIG. 1. The proportion of bumblebees rejecting flowers treated with *B. terrestris* tarsal extracts at various concentrations. Flowers were treated with 5 μl of extract, and the concentrations given are milligrams per 5 μl. *B. lapidarius* workers were offered *M. officinalis* flowers, *B. pascuorum* were offered *S. officinalis* flowers, and *B. terrestris* were offered *P. tanacetifolia* flowers. Lines of best fit were calculated in GLIM. For *B. lapidarius* the logistic regression equation is $y = 2.88 + 0.44x$ ($F_{1,4} = 27.3$, $P < 0.01$), for *B. pascuorum* $y = 2.71 + 0.32x$ ($F_{1,4} = 9.05$, $P < 0.05$), and for *B. terrestris* $y = 2.43 + 0.41x$ ($F_{1,4} = 52.2$, $P < 0.001$), where y = ln (number of rejects/number of accepts) and x = log (milligrams per flower).

TABLE 2. PAIRWISE COMPARISONS OF PROBABILITY OF FLOWERS TREATED WITH EACH EXTRACT VS. FLOWERS TREATED WITH PENTANE USING 2×2 χ^2 TESTS WITH YATES CORRECTION[a]

Hydrocarbons (μg/ flower, estimated)	*B. lapidarius*		*B. pascuorum*		*B. terrestris*	
	χ^2	P	χ^2	P	χ^2	P
0.154	24.34	***	7.02	**	15.27	***
1.54×10^{-4}	6.01	**	13.24	***	6.77	**
1.54×10^{-7}	2.13	—	5.78	*	0.027	—
1.54×10^{-8}	0	—	2.57	—	0.025	—
1.54×10^{-9}	0.35	—	0.34	—	0.41	—
1.54×10^{-10}	0.0005	—	0.0006	—	1.39	—

[a] $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, — = not significant, $df = 1$ throughout.

controls, all three species were significantly more likely to reject flowers treated with 0.154 or 1.54×10^{-4} μg/flower. Applying 1.54×10^{-7} μg/flower induced a significant rejection response only in *B. pascuorum*. All further dilutions produced no significant response in any species (Table 2).

Application of Synthetic Extracts to Flowers. The frequency of *B. lapidarius* workers rejecting flowers treated with tricosane was variable but gradually decreased as the amount of compound applied decreased (Figure 2). Compared to control flowers treated with pentane, significant frequencies of rejection were found from 1000 μg/flower down to 10^{-14} μg/flower (except 10^{-5} μg/flower), but not with 1×10^{-16} and 1×10^{-18} μg/flower (Table 3). After adjusting

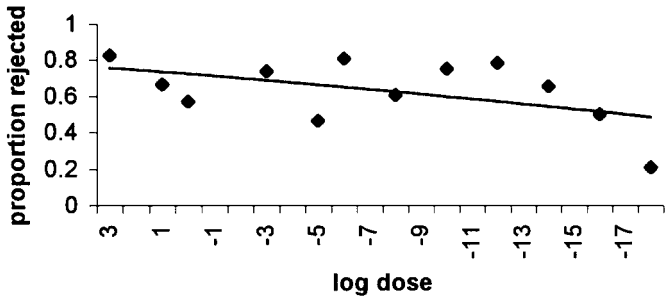

FIG. 2. The proportion of *B. lapidarius* workers rejecting *M. officinalis* flowers treated with different doses of tricosane (milligrams per flower). A logistic regression in GLIM was performed to calculate the line of best fit. Note that the range of doses tested is wider than that used for tarsal extracts.

TABLE 3. PAIRWISE COMPARISONS OF PROBABILITY OF REJECTION BY *B. lapidarius* OF FLOWERS WITH EACH EXTRACT APPLIED VS. FLOWERS WITH PENTANE APPLIED USING χ^2 TESTS WITH YATES' CORRECTION[a]

Compound (μg/flower)	χ^2	P	P (adjusted)
1000 Tricosane	17.31	***	***
10 Tricosane	8.16	**	*
1 Tricosane	4.76	*	—
10^{-3} Tricosane	10.43	**	*
10^{-5} Tricosane	1.91	—	—
10^{-6} Tricosane	14.96	***	**
10^{-8} Tricosane	5.98	*	—
10^{-10} Tricosane	11.23	***	**
10^{-12} Tricosane	15.25	***	**
10^{-14} Tricosane	7.46	**	—
10^{-16} Tricosane	2.84	—	—
10^{-18} Tricosane	0.03	—	—
10^{-12} Pentacosane	16.53	***	***
10^{-12} Heptacosane	8.86	**	*
10^{-12} Heneicosane	17.31	***	***
10^{-12} Tricosene	5.36	*	—
10^{-12} mix	14.45	***	**
No treatment	5.49	*	—

[a] Significance values were adjusted using the Bonferroni procedure $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, — = not significant, $df = 1$ throughout.

significance levels to correct for type-1 errors, the frequencies of rejection of three more doses of tricosane were not significantly different from the frequency of rejection of control flowers (10^{-1}, 10^{-8}, and 10^{-14} μg/flower). Overall, the GLIM analysis revealed that there was a significant positive relationship between tricosane dose and the likelihood of rejection ($\chi^2_1 = 10.88$, $P < 0.001$), with the probability of rejection equal to 0.718 + 0.013 log (μg per flower).

All flowers treated with 10^{-12} μg of synthetic substances were rejected more than control flowers treated with pentane, and untreated flowers were rejected less than flowers treated with pentane (Figure 3). Tricosane, pentacosane, heneicosane, and the mix of all five chemicals were most repellent to foraging bumblebees, with heptacosane producing a weaker response and tricosene (the only alkene tested) producing the weakest response of all. After the data are adjusted for type-1 errors, the reaction of bumblebees to flowers that had been treated with tricosene and untreated flowers is not significantly different from the reaction of bumblebees to flowers which had just been treated with pentane (Table 3).

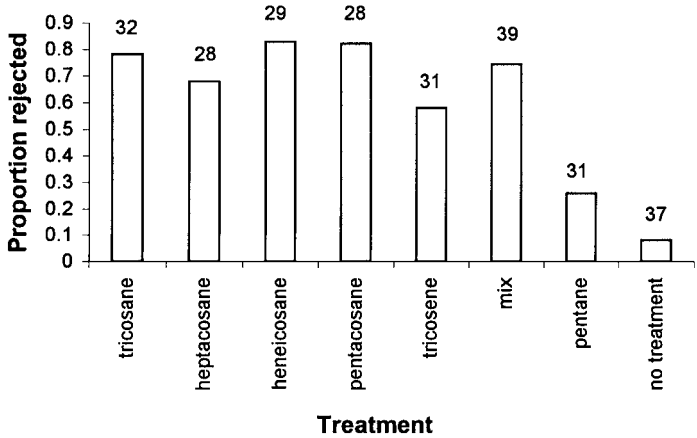

FIG. 3. The proportion of *B. lapidarius* workers rejecting *M. officinalis* flowers treated with 1×10^{-12} μg of different compounds diluted in 5 μl of pentane, with 5 μl of pentane alone, or with nothing. Doses given are milligrams per flower. Numbers above the bars represent sample sizes.

DISCUSSION

GC-MS analysis of extracts from bumblebee tarsi was largely in accordance with previous studies; the main compounds identified were straight-chain alkanes and alkenes with odd numbers of carbon atoms between 21 and 29. These hydrocarbons commonly occur in the cuticle of a broad range of insects (Lockey, 1980).

Species specificity has previously been discovered in the composition of labial gland secretions of male bumblebees (Bergstrom et al., 1981) and in Dufour's gland secretions of bumblebees (Tengö et al., 1991). Oldham et al. (1994) analyzed cuticular hydrocarbons on the same bumblebee species that we studied, and also compared *B. terrestris terrestris*, and *B. terrestris audax*. Although they did not examine tarsal glands, they concluded that the mix of cuticular hydrocarbons was constant across different body parts, but that species and the two *B. terrestris* subspecies differed in the relative quantities of different compounds. This is in close accordance with our findings; the three species were broadly similar but there were a few notable differences. For example pentacosene was a major component of tarsal extracts in *B. lapidarius* and *B. pascuorum*, but was present in only tiny amounts in *B. terrestris*. The composition of tarsal extracts closely follows that for cuticular hydrocarbons found over the rest of the body, described by Oldham et al. (1994). During foraging many parts of the bumblebee body, not just the tarsi, may come into contact with the corolla,

depending upon the shape of the flower. Thus it seems probable that scent marks are not exclusively placed by the feet.

We have found previously that these three species of bumblebee are able to use scent marks left by each of the other species (Stout et al., 1998). We have also found that tarsal extracts from *B. terrestris* mimic the repellency of natural scent marks when applied to flowers and bioassayed with *B. terrestris* (Stout et al., 1998). It is thus not surprising that tarsal extracts applied to flowers are effective at repelling the other two species. Interestingly, the species differed in their overall sensitivity to extracts, with *B. pascuorum* being generally more likely to reject treated flowers than the other two species. The difference may be because we studied the three species when foraging on different flowers. We found no differences in the responses of these three species to natural scent marks when they were all foraging on *S. officinale* (Goulson et al., 1998; Stout et al., 1998), and there were no differences in the response of *B. terrestris* to flowers of *P. tanacetifolia* treated with tarsal extracts from *B. terrestris*, *B. pascuorum*, *B. lapidarius*, or *B. hortorum*. (Stout et al., 1998). We have previously argued that bees may learn to use an appropriate concentration of scent mark as the threshold for rejection depending on the circumstances (Stout et al., 1998). A fixed threshold would be suboptimal, since flowers differ in the rate at which they secrete nectar, so that the optimum interval between visits will be shorter on some species than others. Furthermore, if visitation rates are high or flowers are scarce, we would predict that bees should be less choosy and more likely to accept flowers that were visited quite recently. It is known that bumblebees do sample available floral rewards and modify their behaviour accordingly (Dukas and Real, 1993). If bumblebees can estimate the time since the last bumblebee visit according to how strong the scent mark is [as suggested by Schmitt et al. (1991) and Stout et al. (1998)], then it would be possible for them to learn what concentration of scent corresponds to an appropriate threshold for acceptance of a flower. This is an area that deserves further study.

Of the five synthetic chemicals applied to flowers and offered to *B. lapidarius*, all resulted in a higher degree of rejection than controls. There were differences in the strength of the response, with heneicosane and pentacosane producing the greatest repellent effect and tricosene the least. It is perhaps not surprising that bees respond to all of the compounds tested. They respond to scent marks left by other *Bombus* species, and the species produce scent marks with different compositions. If a bee is to be able to detect scent marks left by a range of species it must be sensitive to the range of compounds that they leave behind. Since these compounds are common to most insects, not just *Bombus* spp., it is possible that bumblebees may be able to detect flowers that have been visited by other insects. This too requires further investigation.

According to our estimates of the quantities of hydrocarbons present in tarsal extracts, rejection responses were induced by synthetic compounds at

much lower doses than by tarsal extracts. There are several possible explanations for this. Bees normally encounter complex mixes of hydrocarbons on flowers; synthetic compounds provide an unfamiliar stimulus and so may induce repellency at lower doses. The tarsal samples used to quantify components in the GC were taken at a different time than those used in the bioassays, and it is possible that the amounts of volatile cuticular hydrocarbons present differed between occasions (perhaps in response to the ambient weather conditions).

There is a prominent anomaly in recent studies of scent marking in bumblebees that requires an explanation. Schmitt et al. (1991) found that scent marks were used to mark rewarding flowers, and so had an attractant effect, while more recent studies have only found repellent effects, whether using natural marks, tarsal extracts, or synthetic compounds (Goulson et al., 1998; Stout et al., 1998; Williams, 1998). We previously postulated that scent marks might be initially repellent, but as the volatiles evaporate they may become attractants (Stout et al., 1998). This, in part, stimulated the current study; use of dilution series should reveal whether repellency or attraction is determined by the strength of the scent mark. However, we found no evidence for attractive marks at any concentration, with rejection responses weakening to levels found in controls at the lowest concentrations tested. For the dilution series of synthetic tricosane, the lowest dose tested contained less than one molecule per flower, so it cannot be argued that an attractive response may have been detected at still lower doses. However, this argument could be used for the dilution series of tarsal extracts, which did not span such a great range of concentrations.

An alternative possibility is that the more volatile compounds produce repellency, and the less volatile ones attraction. Since all of the compounds tested induced repellency, this seems unlikely. It is possible that the changing composition of a scent mark over time as the more volatile components of the natural mixture evaporate could result in attractive marks. However, we have previously found that bumblebees tend to reject flowers of *S. officinale* for about 40 min following a visit, but that flowers visited 1, 4, or 24 hr previously have acceptance rates equal to flowers that have never been visited. At no point were flowers that had previously been visited found to be more attractive than controls. In the present study, unvisited (and unmarked) flowers receive very high rates of acceptance, so there was little scope for a scent mark to increase attractiveness of flowers. Overall, we consider it to be unlikely that attractant marks are operating in the systems we have studied.

Close examination of the experimental design used by Schmitt et al. (1991) suggests another explanation. Their study used artificial flowers that were either always rewarding (regardless of whether they had been visited or not) or were never rewarding. In this circumstance bees would inevitably spend longer feeding on the rewarding flowers, so that rewarding flowers would become covered in cuticular hydrocarbons. Given that bees are readily able to learn associations

between sensory cues and rewards (reviewed in Menzel and Müller, 1996), it is likely that they may have learned to visit the marked flowers preferentially, since these were the rewarding ones. It is less easy to conceive how short-range attractant marks could operate with real flowers that never provide unlimited rewards. It is possible that attractant marks may be used to indicate plants with unusually high nectar secretion rates; studies to date have not explicitly examined whether differences in reward rates between patches influence how bees interpret scent marks.

It has only recently become apparent that the use of scent marks by bees when choosing which flowers they are going to visit is not confined to honeybees. As yet we do not know how widespread this phenomenon is. Do solitary species, bees not belonging to the Apidae, or other flower-visiting insects use scent marks? If so, are interspecific interactions mediated by scent marks? Does the use of scent marks influence the reproductive success of the plants that are visited? Far more research is needed to address these questions.

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