Behav Ecol Sociobiol (1998) 43: 317–326

Original article

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Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.)

Received: 24 November 1997 / Accepted after revision: 8 March 1998

Abstract We have found that foraging bumblebees (*Bombus hortorum*, *B. pascuorum*, *B. pratorum* and *B. terrestris*) not only avoid flowers of *Symphytum officinale* that have recently been visited by conspecifics but also those that have been recently visited by heterospecifics. We propose that the decision whether to reject or accept a flower is influenced by a chemical odour that is left on the corolla by a forager, which temporarily repels subsequent foragers. Honeybees and carpenter bees have previously been shown to use similar repellent forage-marking scents. We found that flowers were repellent to other bumblebee foragers for approximately 20 min and also that after this time nectar levels in *S. officinale* flowers had largely replenished. Thus bumblebees could forage more efficiently by avoiding flowers with low rewards. Flowers to which extracts of tarsal components were applied were more often rejected by wild *B. terrestris* workers than flowers that had head extracts applied, which in turn were more often rejected than flowers that had body extracts applied. Extracts from four *Bombus* species were equally repellent to foragers. The sites of production of the repellent scent and its evolutionary origins are discussed.

Key words Tarsal secretion · Floral rewards · Pheromone · *Symphytum officinale*

Introduction

Nectivorous social bees (Hymenoptera: Apidae) must forage in environments which exhibit unpredictable spatial patchiness of rewards. The concurrent activity of many bee species together with variation in floral rewards produce environments which are highly heterogeneous in terms of reward distribution (Heinrich 1979; Pleasants and Zimmerman 1983; Zimmerman 1988). Research has shown that honeybees (*Apis mellifera*, Apidae) and bumblebees can select and preferentially visit the most rewarding flowers in a patch (Morse 1980; Corbet et al. 1984; Wetherwax 1986; Kato 1988) and avoid revisiting flowers whilst foraging (Schmid-Hempel et al. 1985; Pyke and Carter 1992; Giurfa and Núñez 1993). This is achieved partly by selective rejection of recently visited flowers and partly by systematic searching (Giurfa and Núñez 1992, 1993; Goulson et al. 1998a).

The selection of rewarding flowers and the avoidance of recently visited ones can be explained by several proximate mechanisms, including visual assessment of rewards (Thorp et al. 1975, 1976; Kevan 1976), olfactory assessment (nectar: Heinrich 1979; pollen: Dobson et al. 1996) or a combination of both (Marden 1984). However, nectar is often concealed in the base of flower corollas, making direct assessment of rewards difficult. It has been suggested that foragers may use other cues to detect nectar levels in flowers including the metabolites from yeasts in the nectar (Crane 1975; Williams et al. 1981) and intrafloral humidity gradients (Corbet et al. 1979), although there is little field evidence to support these theories.

Bumblebees avoid revisiting previously probed flowers by using systematic patterns of movement among plants in a patch (Bell 1991; Thomson et al. 1997), among inflorescences on a plant (Goulson et al. 1998a) and among florets on an inflorescence (Corbet et al. 1981). However, since the foraging environment is complex and the activity of other individuals affects the distribution of rewards, systematic search patterns alone are not sufficient for bumblebees to avoid visiting depleted flowers. The most convincing explanation for the rejection of recently visited flowers is that honeybees and

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bumblebees are detecting scent marks left by previous foragers (Kato 1988; Giurfa and Núñez 1992; Giurfa 1993; Goulson et al. 1998a).

The literature concerning the use of forage-marking pheromones is complex, but basically two types of forage-marking scents have been investigated. The first type is an attractant scent which is left on flower corollas by foraging bees. These scents encourage other bees to alight and probe for nectar on particularly rewarding flowers and have been identified in honeybees (Ribbands 1955; Butler et al. 1969; Ferguson and Free 1979; Free et al. 1982) and bumblebees (Cameron 1981; Schmitt and Bertsch 1990; Schmitt et al. 1991). Such attractants have considerable persistence due to their chemical structure and low volatility (Schmitt and Bertsch 1990). Ribbands (1955) showed that after landing briefly, without exposure of the scent organ, honeybees deposit an attractive scent on surfaces. Nasanov secretions and (*Z*)-11-eicosen-1-ol from the sting apparatus of honeybees have both been shown to attract foragers (Free and Williams 1972; Free et al. 1982). Attractants deposited at the entrance to honeybee hives facilitate the orientation of homecoming foragers and are leaked from an articular slit in the tarsi and so are deposited on all the surfaces the honeybee has walked on as a 'footprint' (Butler et al. 1969; Lensky et al. 1985). The attractant scent used by bumblebees for forage marking is a secretion from the tarsi and consists mainly of alkenes and alkanes (Schmitt et al. 1991). The mechanism of nest entrance marking by bumblebees is unknown (Cederberg 1977; Pouvreau 1996).

The second type of forage-marking scent has been found in honeybees and is a short-lived repellent scent (Giurfa and Núñez 1992). These scents mark flowers which yield no reward (Free and Williams 1983; Giurfa and Núñez 1993) and rewarding flowers which have been depleted by the forager (Giurfa 1993). When deposited on flower corollas, they discourage other individuals from landing and probing for nectar. It is most likely that 2-heptanone, secreted from mandibular glands, causes the repellent effect (Vallet et al. 1991). Frankie and Vinson (1977) found that there was also strong evidence for the use of repellent scent marks by foraging carpenter bees (*Xylocopa virginica texana*, Anthophoridae). Similar repellent scents and their site of production have not been extensively investigated in bumblebees but Corbet et al. (1984) suggested that a time-dependent cue placed on depleted flowers could account for bumblebees rejecting flowers visited 1–2 min previously, but accepting flowers probed much earlier.

A recent preliminary field study by Goulson et al. (1998a) found that individual *Bombus terrestris* and *B. pascuorum* will reject flowers they have recently visited. They found that bumblebees will also reject flowers which have recently been visited by either conspecifics or bumblebees from the other species. Furthermore, bumblebees were unable to discriminate between flowers with nectar removed and those full of nectar, suggesting that it was not detection of rewards which was causing rejection but some other mechanism, probably repellent scent-marking (Goulson et al. 1998a).

The aim of this study was to investigate four aspects of repellent scent-marking by foraging bumblebees.

(a) The incidence of rejection of flowers recently visited by conspecifics or heterospecifics for a guild of four bumblebee species sharing a common resource, comfrey (*Symphytum officinale*, Boraginaceae).
(b) Whether the response of foragers to previously visited flowers is affected by (1) the species of the recent visitor and the recipient test bumblebee, (2) the time between the visit of the recent forager and the recipient and (3) the food collection method of both foragers. Bumblebees collect nectar from *S. officinale* in a conventional manner (probing for nectar from the tubular opening of the corolla), or they rob nectar (collecting nectar through a hole bitten in the base of the flower corolla; Inouye 1983) or they collect pollen [which requires sonicating (buzzing) the anthers to release pollen; King 1993].
(c) The relationship between nectar build-up in *S. officinale* and the duration of the repellent effect of previously visited flowers.
(d) The origin of the bumblebee repellent scent mark. This could be either the tarsi, where the bumblebee attractant scent mark originates (Schmitt et al. 1991) or, alternatively, the head, where the honeybee repellent scent mark originates (Vallet et al. 1991), which also touches the flower corolla as bumblebees forage.

Methods

Most of the study was carried out at the Itchen Valley Country Park (near Southampton, Hampshire, UK) in June and July 1997. Workers from four bumblebee species (*B. hortorum*, *B. pascuorum*, *B. pratorum* and *B. terrestris*) were observed foraging on a large patch (100 × 30 m) containing approximately 70 plants of *S. officinale*. All four species collect nectar and pollen from *S. officinale*. Observations were made between 0900 and 1100 and between 1300 and 1600 hours (BST) on days when the temperature was 19–24 °C, there was a light wind, patchy sun and no rain. When conditions were outside these limits, no observations were made.

Reaction of bumblebees to previously visited flowers

This experiment was designed to test whether the response of bumblebees to flowers is affected by previous bumblebee visits. Bumblebees were presented with flowers which had been visited by either (a) a conspecific individual, (b) a heterospecific individual, (c) no previous individuals or (d) had an unknown previous history. For (a) and (b), flowers were picked from wild *S. officinale* plants immediately after a bumblebees visit. For (c), flowers were picked from wild plants which had been covered with a fine netting for at least 1 h to exclude insects. It was assumed that in this time period short-lived repellent marks would have evaporated (this was later tested). For (d), flowers were randomly picked from wild plants.

Flowers were presented by picking inflorescences with a short stem (any open flowers on the inflorescence not visited by the most recent forager were removed) and holding the inflorescence by hand adjacent to the flower on which the test bumblebee was

feeding. Since bumblebees tend to depart from a flower in the same direction as they arrive (Pyke and Carter 1992), it was possible to anticipate the direction of departure and place the test flower in their path. If the flowers were approached and then not landed upon, or were alighted upon only briefly, the visit was recorded as a 'rejection'. If the bumblebee landed on the flower and then probed for nectar or collected pollen, the visit was recorded as an 'acceptance'. If the test flower was not approached by the bumblebee, the presentation was not recorded. Each flower was only used once. Flowers were never offered to the original forager. The time between the original forager's visit and the presentation of the test flower to the recipient bumblebee was recorded. Presentations of flowers were not made more than 3.5 min after the original forager's visit.

We were not able to catch and mark each individual bumblebee without disrupting their foraging. Scents from paints or glue (for attaching tags) may have affected olfactory behaviour. We also considered it unethical to capture and kill all bumblebees used. Therefore it was not possible to guarantee that individuals were tested only once, although each flower had a different visitation history and bumblebees were never offered the same flower more than once. Casual observation of the sample area suggested that there were high numbers of all bumblebee species, and Laverty (1994) mentioned that bumblebees which were caught and marked were seldom seen again in the same area. We believe, therefore, that an insignificant number of the replicates are in fact pseudoreplicates (Hurlbert 1984) in the sense that the same individual may have been tested for its response to a flower more than once.

Data were analysed using a χ^2 test with Yates' correction. The frequencies of rejection by recipient bumblebees of flowers which had been previously visited were compared in a series of pairwise comparisons with the frequencies of rejection of flowers which had not been visited. This tested whether the response to the picked flower was caused by the presence of the previous forager. Similarly, the frequencies of rejection of flowers known to have been previously visited were compared with the frequencies of rejection of randomly picked flowers. This was to test whether recipient bumblebees were reacting to scents left by the original test forager or whether they were reacting to scents left by an unknown visitor prior to the original test forager. Because of the large number of tests carried out (32), significance values were adjusted using a sequential Bonferroni procedure (Rice 1989). We also compared the response of bumblebees to flowers which had no recent visitors with their response to randomly picked flowers.

Factors affecting the reaction of bumblebees to previously visited flowers

Five factors were recorded in tests (a) and (b) above: the species of the original and the recipient bumblebee, the time between picking and presenting flowers and the food collection method of each bumblebee. Bumblebees were classed as 'conventional nectar feeders', 'nectar robbers', 'pollen collectors' or 'both'. The 'both' category included individuals which collected both pollen and nectar from the same flower (regardless of the nectar collection method). The method of collection was easily observed without disturbing the foraging bees.

The frequencies of rejection of flowers were analysed with binomial errors in GLIM with a logit link (Crawley 1993) according to original bumblebee species, recipient bumblebee species, time between original and recipient bumblebee visits and food collection method of both bumblebees (plus all interactions). Factors which did not contribute significantly to the model were removed in a stepwise manner.

Longevity of the repellent effect of previously visited flowers

To exclude other bumblebees, *S. officinale* inflorescences were covered after foragers' visits. After ≤3, 5, 10, 20, 60, 240 min and 24 h, the visited flowers were picked and presented to bumblebees of the same species as the original forager. Only *B. terrestris* and *B. pascuorum* were used for this experiment. Flowers which had never been visited were also presented to bumblebees as controls. To ensure these flowers had not been previously visited, we removed all the open flowers from several plants and covered them to exclude bumblebees and allow the opening of new flowers. After 24–48 h, flowers were uncovered, picked immediately and presented to bumblebees within 3 min of picking. All flowers were used once only, and were of a similar floral phase.

The frequencies of rejection of flowers at time intervals after they were visited were compared (by χ^2) with the frequencies of rejection of flowers which had never been visited. All comparisons were pairwise. Because of the large number of tests (14), the sequential Bonferroni technique was again used to adjust significance levels and test for table-wide significance.

Nectar replenishment in flowers

All the nectar was removed from flowers on the inflorescences of ten *S. officinale* plants at 1000 hours on 16 June 1997 (temperature 21 °C, cloud cover 60%). These plants were then isolated from insects with fine netting. At intervals of 5, 10, 20, 30, 40, 50, 60 and 70 min after nectar removal, ten randomly picked flowers were tested for their nectar content. Nectar was drawn out with a glass microcapillary tube and blotted onto filter paper (Whatman type 1). The resulting spread-out blot of nectar was immediately drawn around with a pencil. A regression equation fitted through the origin was calculated using known volumes of sucrose solution pipetted onto filter paper [volume of nectar (ml) = 102.22/area of circle (cm^2), $R^2 = 0.90$]. This was used to convert the area of the nectar blot on the filter paper into a volume.

Origin of the proposed repellent scent mark

We hypothesised that the origin of the scent mark is probably in the lower tarsi or in the head. To test this prediction, 15 workers of *B. pascuorum*, *B. terrestris*, *B. hortorum* and *B. lapidarius* were captured and freeze killed in liquid nitrogen. The lower three tarsal segments were removed from each leg of each bumblebee. These segments were immediately crushed whilst still frozen in liquid nitrogen and stored in pentane. Pentane is a solvent for lipid-type compounds (Cameron 1981) and has been used in investigations into the production of attractant scent marks (Schmitt 1990). Legs from five individuals of the same species were combined in approximately 2 ml pentane. The heads and, separately, the remainder of the bumblebees' bodies were then washed in pentane and these extracts were also stored. Again, extracts from five individuals were combined.

During July 1997, *Phacelia tanacetifolia* (Hydrophyllaceae) plants in the research gardens at the University of Southampton Chilworth Research Centre (near Southampton, Hampshire, UK) were used to test the response of wild bumblebees to flowers with extracts applied to the corolla. *P. tanacetifolia* plants were used as they attracted many bumblebees and the *S. officinale* plants were no longer attracting as many foragers. Ten *P. tanacetifolia* plants were covered with fine netting to exclude insects for at least 24 h. Each flower of this species is open and receptive for less than 24 h (J.C. Stout, personal observation). Thus after being covered for 24 h, the only open flowers on these plants had never been visited. Five microlitres of each of the extracts was applied to open flower corollas and these flowers were presented to wild foraging *B. terrestris*. Pentane is highly volatile and evaporates quickly, leaving less volatile components of the extracts on the flower corollas. Flowers were presented to bumblebees within 2 min of applying the extract. This test was repeated at least 13 times for each sample. Flowers which had 5 µl of pentane applied to the corolla were also offered to foraging bumblebees as a control.

The variations in the frequencies of rejection of flowers treated with each solvent extract (and the pure pentane) were analysed with binomial errors in GLIM according to bumblebee species, body

part and rejection frequencies (plus interactions). Factors which did not contribute significantly to the model were removed in a stepwise manner. As the ratio of the residual deviance to the residual degrees of freedom exceeded 1.5, the test statistics given are *F*-values (Crawley 1993).

Results

Reaction of bumblebees to previously visited flowers

The frequencies of bumblebees rejecting flowers which had recently been visited were consistently higher than both the frequencies of bumblebees rejecting flowers which had not been visited within the preceding hour and the frequencies of rejection of randomly picked flowers (Fig. 1). In all cases these differences were statistically significant ($P < 0.05$; Table 1). Flowers with no recent visitors were rejected at a similar rate to flowers which were randomly picked (Table 1). Only *B. hortorum* rejected more randomly picked flowers than flowers with no recent visitors and this difference was not significant ($\chi^2 = 3.12$, $df = 1$, $P > 0.05$, n.s.).

Factors affecting the reaction of bumblebees to previously visited flowers

There were no interactions between any of the factors which significantly affected the frequency of rejection of flowers. When the factors were treated independently, the species of the original forager had no significant effect on the frequency of rejection of recipient bumblebees ($\chi^2 = 3.82$, $df = 3$, n.s.). There were also no significant effects of the time between picking and presenting flowers ($\chi^2 = 1.44$, $df = 1$, n.s.) and the food collection method of the original forager ($\chi^2 = 1.58$, $df = 3$, n.s.) on the frequency of rejection of flowers. However the species of recipient test bumblebee ($\chi^2 = 25.33$, $df = 3$, $P < 0.005$) and the food collection method of the recipient test bumblebee ($\chi^2 = 33.33$, $df = 2$, $P < 0.005$) showed a significant influence on the frequency of rejection of flowers. *B. pascuorum* individuals rejected fewer flowers than the other species (Table 2) and pollen collectors were also inclined to reject fewer flowers.

Longevity of the repellent effect of previously visited flowers

The frequency of rejection of flowers which had been visited by conspecifics declined over time after the original forager's visit (Fig. 2). The frequencies of rejection of flowers which had been visited by conspecifics were significantly higher than frequencies of rejection of flowers which had never been visited for 20 min after the original forager's visit ($P \ll 0.01$ for both *B. pascuorum* and *B. terrestris*; using the sequential Bonferroni technique to adjust table-wide significance levels, all significant values remain significant $P < 0.05$). Sixty minutes and more after the original forager's visit, the frequencies of rejection were not significantly different to the rejection of flowers which had never been visited ($\chi^2 < 2.83$, $df = 1$, n.s.).

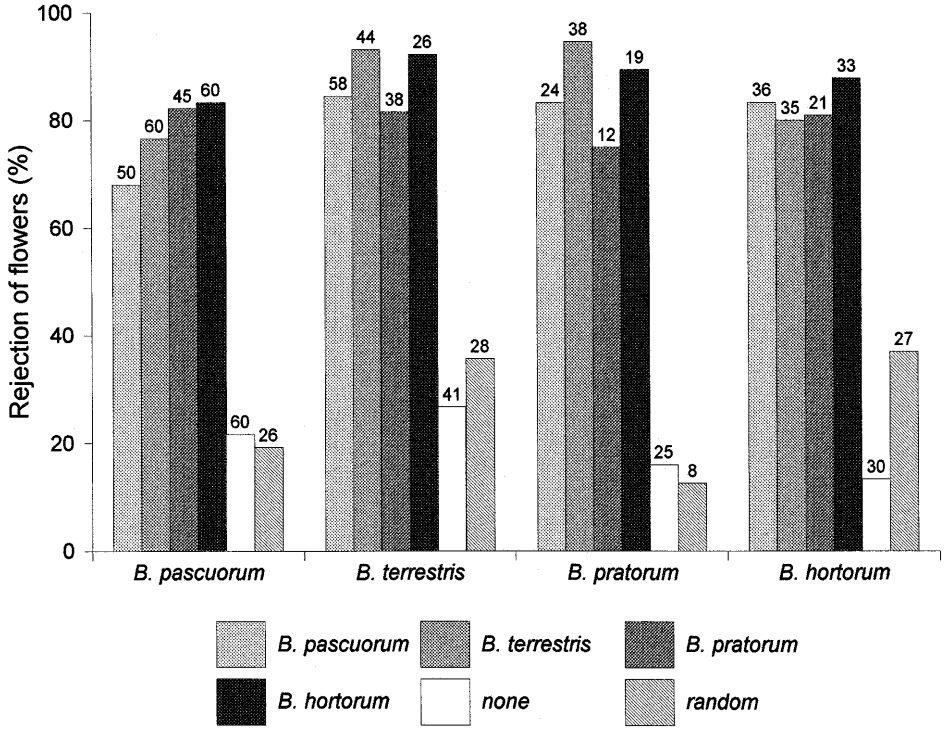

Fig. 1 The frequencies of rejection of flowers by each of the four recipient bee species. *Bars* represent the following from *left* to *right*: flowers which had been visited in the previous 3 min by *Bombus pascuorum*, *B. terrestris*, *B. pratorum* or *B. hortorum*, flowers which had not been previously visited (*none*) and flowers which had been randomly picked (*random*). Sample sizes are given above the *bars*

Table 1 χ^2 values (calculated with Yates' correction) for frequencies of rejection of flowers which had recently been visited compared with the frequencies of rejection of flowers which had no recent visitors (*none*) and the frequencies of rejection of randomly picked flowers (*random*), and for frequencies of rejection of flowers which had no recent visitors compared with the frequencies of rejection of randomly picked flowers. Using the sequential Bonferroni technique to adjust table-wide significance levels, all significant values remain significant ($P < 0.05$)

Previous visitor	Recipient forager			
	B. pascuorum	*B. terrestris*	*B. pratorum*	*B. hortorum*
B. pascuorum vs none	22.07***	31.91***	19.6***	28.83***
B. pascuorum vs random	14.39***	18.65***	10.3**	12.34***
B. terrestris vs none	34.71***	33.88***	37.01***	26.12***
B. terrestris vs random	22.47***	24.64***	23.41***	10.12**
B. pratorum vs none	35.14***	21.59***	9.93**	19.53***
B. pratorum vs random	24.52***	12.53***	5.21*	6.93**
B. hortorum vs none	41.14***	24.78***	19.6***	28.48***
B. hortorum vs random	29.61***	16.17***	11.1***	12.4***
None vs random	0.001 n.s.	0.27 n.s.	0.11 n.s.	3.12 n.s.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 2 The proportion of flowers rejected by recipient foragers according to their food collection strategy, and the mean proportion of flowers rejected by each recipient species and by each food collection strategy. Collection of nectar from the conventional direction is listed as the *Nectar* food collection method. Sample sizes are given in parentheses

Recipient bumblebee food collection method	Recipient bumblebee species				
	B. pascuorum	*B. terrestris*	*B. pratorum*	*B. hortorum*	Mean
Nectar	0.82 (176)	1.0 (4)	1.0 (1)	0.94 (80)	0.86
Pollen	0.55 (31)	0.84 (106)	0.75 (4)	0.57 (37)	0.73
Robbing	1.0 (3)	0.93 (40)	0.89 (88)	1.0 (8)	0.91
Both	0.60 (5)	0.94 (16)	1.0 (2)	1.0 (8)	0.90
Mean	0.78	0.88	0.89	0.84	

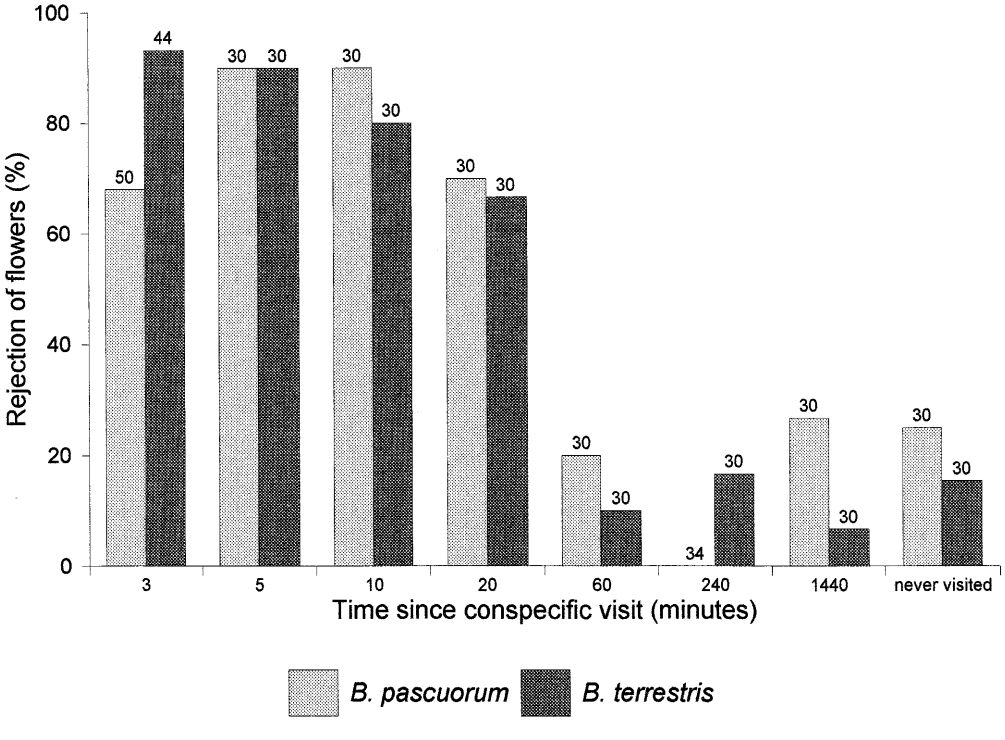

Fig. 2 The frequency of rejection of flowers by *B. terrestris* and *B. pascuorum* within time intervals after the original conspecific foragers' visit. Sample sizes are given above the *bars*

Nectar replenishment in flowers

One hour after removal, nectar build-up in flowers appears to have peaked (Fig. 3). More than half the standing crop of nectar is replenished within the first 20 min.

Origin of the proposed repellent scent mark

The species of bumblebee from which the extracts were taken did not significantly affect the frequencies of rejection of flowers by *B. terrestris* ($F_{3,31} = 0.3$, n.s.). The part of the body from which the extract was taken did, however, influence rejection frequencies. Tarsal extracts induced higher frequencies of rejection than did head extracts, which in turn induced higher frequencies of rejection than body extracts (Fig. 4) ($F_{2,33} = 16.14$, $P < 0.005$). In pairwise comparisons, there was a significant difference between the effect of the leg extracts versus the pentane controls ($F_{1,12} = 13.3$, $P < 0.01$), and the head extracts versus pentane ($F_{1,12} = 12.8$, $P < 0.01$), but not between the body extracts and pentane ($F_{1,12} = 0.43$, n.s.). When the leg and the head extracts were compared, there was a significant difference between the frequencies of rejection of these flowers ($F_{1,22} = 4.8$, $P < 0.05$).

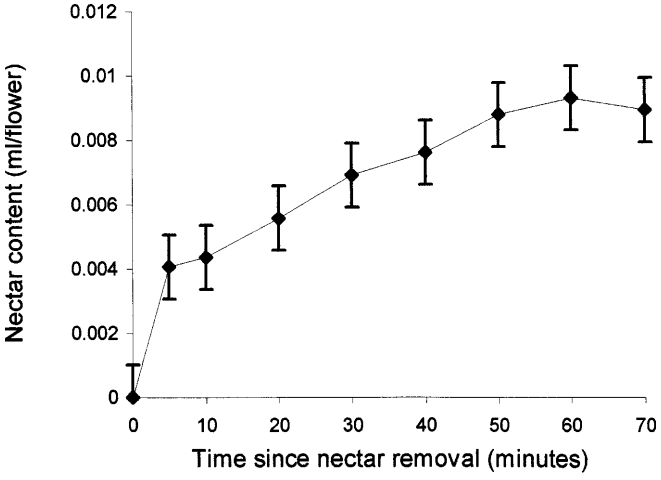

Fig. 3 Nectar build-up rate in *Symphytum officinale* (mean ± SE). Sample size is ten at each time point

Discussion

The biological action of the repellent scent mark

Bumblebees rejected flowers recently visited by other bumblebees more frequently than flowers which had not been visited during the previous hour. They also rejected a higher frequency of previously visited flowers than randomly picked flowers. The possibility that pseudoreplication may invalidate these results is minimal since the significance values were very high and we estimate that there was little chance of testing the same bumblebee more than once. Therefore, these results suggest that the recipient forager was responding to a cue left by the original bumblebee rather than to other cues left by previous visitors. The rejection of recently visited flowers presumably increases foraging efficiency by reducing the

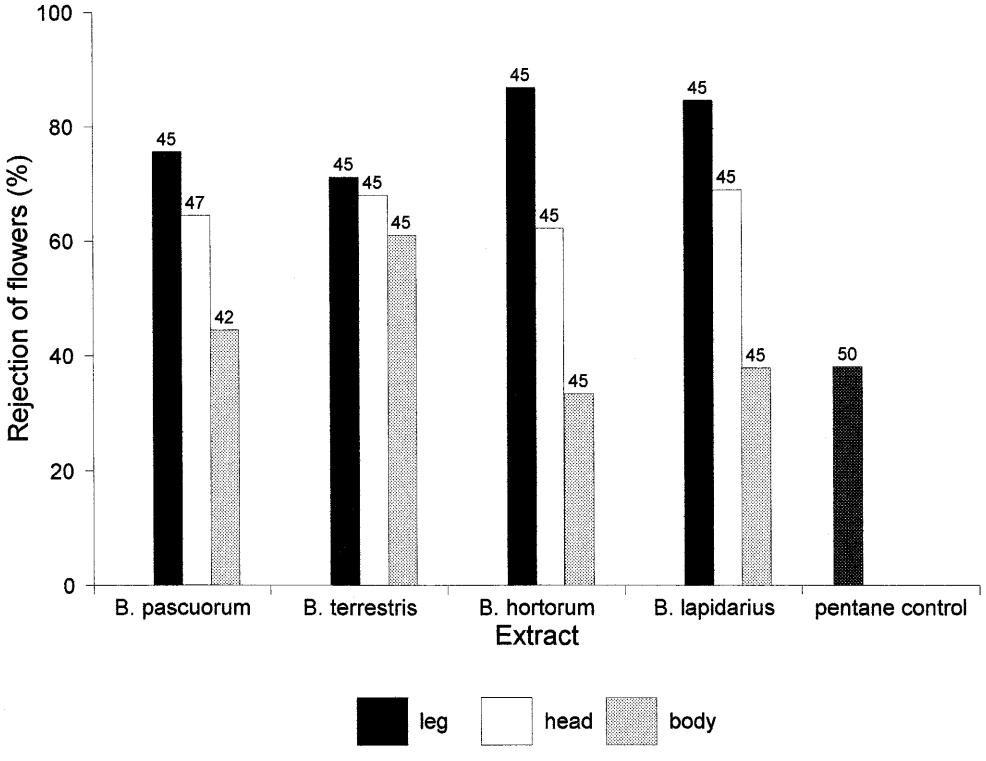

Fig. 4 Frequencies of rejection of flowers by *B. terrestris* according to the type of extract applied to the flower corolla. Extracts were from four bumblebee species (*B. pascuorum*, *B. terrestris*, *B. hortorum* and *B. lapidarius*) or were pure pentane. Sample sizes are given above the *bars*

amount of time bumblebees waste probing recently visited empty flowers (Giurfa and Núñez 1993).

The species of the original forager had no effect on the frequency of rejection of previously visited flowers. Flowers previously visited by heterospecifics were rejected as often as flowers visited by conspecifics for all species. Lower frequencies of rejection were observed for recipient *B. pascuorum* than for the other species. Pollen collectors also rejected fewer flowers than nectar gatherers. The slightly lower frequency of rejection by *B. pascuorum* may in part be explained by the fact that *B. pascuorum* is generally less conservative in its flower choice (J. Ollerton, personal communication). Pollen collectors may be more inclined to accept recently visited flowers. When anthers are sonicated for pollen, only a small proportion of the total pollen available is dispensed per forager (King and Buchmann 1996). This not only increases the pollen dispersal potential for the flower, but means that a pollen collector may visit a flower which has been recently visited and still elicit a reward. Nectar takes time to replenish, which may account for the slightly higher frequencies of rejection amongst the nectar collectors.

The repellent effect of visited flowers did not fade within 200 s after the test forager. It lasted between 20 and 60 min after the original forager's visit. This approximates to the time taken for nectar to replenish in flowers. The longevity of the repellent effect may vary between plant species with different rates of nectar replenishment. Nectar build-up in *Borago officinalis* (Boraginaceae) is faster than in *S. officinale*: 3–5 min is sufficient for nectar to build up to levels found in unvisited flowers (J.C. Stout, personal observation). Corbet et al. (1984) found that individual *B. officinalis* flowers were visited by bumblebees on average once every 3.25 min; again this approximates to the nectar build-up rate. There are two possibilities which may account for the change in the longevity of repellency of visited flowers of different plant species. Firstly bumblebees may be able to regulate the amount of repellent chemical applied to a flower, and adjust it to the nectar refill rate. This implies a controlled application of the repellent scent, with more scent being applied when refill rates are longer. Alternatively, bumblebees might simply learn to adjust their threshold for rejection of scent-marked flowers depending on the perceived nectar refill rate of that species. Hence bumblebees may choose to ignore faint (i.e. old) scent marks on flowers with rapid rates of nectar production. This would require experience, learning and memory retention so that individuals know when to ignore a repellent scent and when to accept a flower. Indeed, the learning abilities of bees are quite considerable (Menzel 1985; Dukas and Real 1993a,b; Hammer and Menzel 1995). Repellency may also depend on the density of foragers, the availability of floral rewards and whether bumblebees drain flowers totally of their resources. Some nectar is often left in the flowers by foragers (Hodges and Wolf 1981) and if resources are scarce and foragers are common then bumblebees may be more willing to revisit flowers which have recently been emptied.

The site of production of the repellent scent mark

The tarsi, head and thorax of bumblebees all touch the flower corolla when bumblebees are collecting pollen or nectar from the conventional direction, but only the tarsi and the head (primarily the mandibles) of the nectar robbers touch the corolla (J.C. Stout, personal observation). Since we observed similar frequencies of rejection of flowers recently visited by conventional nectar collectors, pollen collectors and nectar robbers, the repellent effect must be associated with the body part which contacts the corolla in all cases; either the tarsi or the head. When flowers were offered to wild foraging *B. terrestris*, we found that those with tarsal components or head extracts applied to the corollas were rejected more often than flowers treated with other body components. This confirms that the element which causes the repellent effect is produced in the tarsi or somewhere on the head.

It has been previously demonstrated that honeybee repellent scents are secreted from mandibular glands, and bumblebee attractant scents from tarsal glands (Vallet et al. 1991; Schmitt et al. 1991). Since we demonstrated that the tarsal extracts from bumblebees produced the strongest repellent effect, it may be concluded that the tarsi are the most likely candidates for the site of production. However, the repellent effect of the head extracts cannot be ignored. The tarsal extracts were made from crushed tarsi, whilst the heads were only washed over with solvent. This could have affected the response of foragers, since extracts of crushed tarsi could contain more of the repellent chemical than head washes. Assuming that the higher frequency of rejection of tarsal extracts was not an artefact of crushing or washing, there are two options for the site of repellent scent mark production in the bumblebee. The head and the tarsi may both be important in producing the repellent scent mark. The mandibular glands and the tarsal glands of queen honeybees secrete pheromones which when secreted together have an inhibitory effect on workers, preventing them from constructing swarming queen cups and cells (Lensky and Slabezki 1981; Lensky et al. 1985). A similar system has been described by Balderrama et al. (1996) for a honeybee alarm response which requires a combination of scents from glands in the mandibles and the sting chamber. Therefore, it is possible that in bumblebees, glands in the legs and the head may both be needed to produce a repellent scent. However, although we did not investigate the effects of combining the two, high rejection responses were found when extracts of legs and heads were applied separately. Alternatively, the repellent chemicals may be produced in the tarsi alone and wiped over the head and body when the bumblebee grooms pollen off the head and thorax. This could account for the reduced repel-

lency of the head extracts shown in this study, although we might expect body extracts to display a greater repellent effect.

The frequency of rejection by *B. terrestris* of flowers to which pure pentane had been applied (38%) was higher than the frequency of rejection of unadulterated flowers (average 23.5%). The pentane itself may therefore have had a repellent effect on the forager. However, since we were comparing the reaction of foragers to different body parts all dissolved in pentane, the slightly repellent effect of the pentane is irrelevant. The main point is that the leg extracts elicited the greatest rejection response. This was significantly greater than the rejection response caused by the head extracts, which in turn was significantly greater than the rejection responses to body extracts or pure pentane.

The evolutionary origins of the repellent scent mark

Giurfa (1993) proposed that the repellent scent mark used by honeybees was basically a self-use signal which had value for communicating with other foragers. This facilitates efficient foraging among nestmates utilising a common resource. Communicative skills which increase colony efficiency and inclusive fitness are common in eusocial insects such as honeybees (Krebs and Davies 1991) but there is little evidence for forager communication in bumblebees. Here we have shown that not only nestmates but unrelated bumblebees can detect repellent effects on recently visited flowers. In many communities (including the one studied by us), more than one nest and more than one species of bumblebee exist and forage sympatrically. The scent may be primarily deposited for self-use, but detecting scents left by other foragers may confer advantages in terms of increased foraging efficiency.

The fact that all the species of bumblebee studied here could detect the repellent scents suggests that repellent scent-marking has its evolutionary origins in a *Bombus* ancestor. It may even be a trait of the Apidae family, since honeybees also use repellent scents whilst foraging. However, honeybees use a chemical secreted from mandibular glands only, and we have shown here that tarsal glands are also important in producing the repellent scent in bumblebees. It would be interesting to investigate whether other non-social Apidae use repellent scents.

Honeybee workers actively secrete repellent scents from mandibular glands (Vallet et al. 1991), whereas passive secretions from tarsal glands act as attractants to guide homecoming honeybees back to the nest (Lensky et al. 1985). Schmitt and Bertsch (1990) did not determine whether the bumblebee attractant scent mark which originates from the tarsi is actively or passively secreted. Similarly, it is also important to know whether bumblebee repellent scent marks are actively secreted and regulated or are merely 'footprints' left wherever bumblebees walk.

Also unknown is the degree of interaction between the attractive and repellent scent marks. Perhaps there is no interaction and the hydrocarbons which make up the bumblebee attractive scents are distinct from those of the repellent scents. This would then suggest that one or both are actively secreted and controlled. We have demonstrated that in bumblebees, the repellent scents originate (at least in part) from the tarsi, the same site of origin as the attractant scent. An alternative hypothesis therefore is that chemicals from the tarsi are deposited on flowers as bumblebees forage (whether actively secreted or passively leaked) and initially repel other foragers. As volatiles evaporate over time, the change in relative concentrations may affect a forager's behaviour. At a high concentration, the scent mark may repel other foragers and at lower concentrations attract them. This is common in many Diptera responses to chemical scents (J. Chapman, personal communication), and is a possibility which requires further investigation.

Exocrine secretions from the tarsi are not uncommon in arthropods. In honeybees, for example, queen bee tarsal secretions, in conjunction with mandible secretions, are inhibitory, whilst worker bee secretions are used for nest entrance marking (Butler et al. 1969; Lensky and Slabezki 1981; Lensky et al. 1985). Wasps also mark nest entrances with similar secretions (Butler et al. 1969). Some species of ant use secretions from the hind legs for marking pheromone trails (Parry and Morgan 1979) while other ant species secrete a substance used for antennal cleaning from the tarsi (Schonitzer et al. 1996). Ladybirds (Kosaki and Yamaoka 1996) and houseflies (Romoser and Stoffolano 1994) secrete adhesives from glands at the base of tarsal hairs which aid grip on smooth surfaces. It is possible that tarsal secretions used for forage marking in bumblebees evolved from secretions with some other purpose.

Several questions arise from the results we have presented. For example, some bumblebee communities demonstrate competitive exclusion and niche partitioning that result in each bumblebee species utilising separate resources (Heinrich 1976; Inouye 1978; Ranta and Vespsalainen 1981; Pyke 1982). Would detection of heterospecific repellent scents evolve in such communities? Secondly, honeybees and bumblebees are often found foraging on the same floral resources in southern England. Can repellent scents left by foraging honeybees be detected by bumblebees and vice versa? Finally, does repellent scent-marking by bumblebees generate any costs or benefits to the plants on which the bumblebees are foraging? The male reproductive success of the plant may be enhanced because repellent effects may discourage individual bumblebees from visiting all the available flowers on a plant (Iwasa et al. 1995; Goulson et al., in press). Foragers will then visit more plants dispersing pollen more widely.

Acknowledgements This research was funded by a University of Southampton Studentship awarded to J.C. Stout. We are grateful to Kate Allen, Jennifer Drury and Lucy Bristow for help with field

observations and collecting bumblebees. We thank Martin Genner, Lars Chittka and an anonymous referee for helpful comments on the manuscript.

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Communicated by R.F.A. Moritz