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The use of conspecific and interspecific scent marks by foraging bumblebees and honeybees

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Bumblebees (*Bombus* spp.) and honeybees, *Apis mellifera*, both use odour cues deposited on flowers by previous visitors to improve their foraging efficiency. Short-lived repellent scents are used to avoid probing flowers that have recently been depleted of nectar and/or pollen, and longer-term attractant scents to indicate particularly rewarding flowers. Previous research has indicated that bumblebees avoid flowers recently visited by themselves, conspecifics and congeners, while honeybees avoid flowers visited by themselves or conspecifics only. We found that both bumblebees and honeybees also avoided flowers previously visited by each other when foraging on *Melilotus officinalis*, that is, bumblebees avoided flowers recently visited by honeybees and vice versa. Twenty-four hours after a visit, this effect had worn off. Honeybees visited flowers that had been visited 24 h previously more often than flowers that had never been visited. The same was not true for bumblebees, suggesting that foraging honeybees were also using long-term attractant scent marks, whilst bumblebees were not. Flowers previously visited by conspecifics were repellent to bumblebees and honeybees for ca. 40 min. During this time, nectar replenished in flowers. Honeybees were previously thought to use a volatile chemical (2-heptanone) as a repellent forage-marking scent. We suggest that they may be using a less volatile chemical odour to detect whether flowers have recently been visited, possibly in addition to 2-heptanone.

Bumblebees (*Bombus* spp., Apidae) and honeybees, *Apis mellifera* (Apidae) have frequently been used as models to test the predictions of optimal foraging theory (Pyke 1978; Waddington & Holden 1979; Zimmerman 1982; Wells & Wells 1983). They are considered to be ideal subjects because workers are free from the constraints of finding mates and nest sites, they collect food for the whole colony and are thus never satiated, and they have few predators (Pyke et al. 1977; Pyke 1978; Heinrich 1979; Best & Bierzychudek 1982). However, both honeybees and bumblebees forage in unpredictable heterogeneous environments (Heinrich 1979; Pleasants & Zimmerman 1983; Zimmerman 1988), and use complex systems of learning, memory and communication to improve their foraging efficiency (Hammer & Menzel 1995; Chittka et al. 1999). Although it has been long known that honeybees use a highly developed olfactory communication system both in the nest and at their food source, it is only relatively recently that similar talents have been recognized in bumblebees (Schmitt & Bertsch 1990; Tengo et al. 1991; Valterova & Urbanova 1997; Goulson et al. 1998, 2000; Stout et al. 1998; Bloch & Hefetz 1999; Dornhaus & Chittka 1999).

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Both bumblebees and honeybees use scent marks whilst foraging. When collecting nectar and/or pollen, they deposit short-lived repellent odours on the flower corolla. Subsequent flower visitors avoid these depleted flowers (Giurfa & Núñez 1992; Giurfa 1993; Goulson et al. 1998; Williams 1998). Over time, as nectar (and, in some cases, pollen) replenishes in the flower, the scent mark fades until eventually flowers are revisited (Stout et al. 1998). The detection of repellent scent marks is thought to improve foraging efficiency, by reducing the time that is wasted in probing depleted flowers (Williams 1998).

The repellent scent used by honeybees is thought to be 2-heptanone, secreted from mandibular glands (Vallet et al. 1991; Giurfa 1993), whilst bumblebees use mixtures of long-chain hydrocarbons secreted by the tarsal glands (Stout et al. 1998; Goulson et al. 2000). Bumblebees and honeybees also use attractant scent marks (at least in laboratory experiments). These are longer-lasting odours that indicate particularly rewarding flowers to subsequent visitors (Ribbands 1955; Butler et al. 1969; Free & Williams 1972; Ferguson & Free 1979; Cameron 1981; Schmitt & Bertsch 1990; Schmitt et al. 1991). Nasanov

secretions and (*Z*)-11-eicosen-1-ol from the sting apparatus of honeybees (Free et al. 1982; Free & Williams 1983) and tarsal secretions of bumblebees (Schmitt et al. 1991) are thought to be responsible. So far, there is no evidence to suggest that bumblebees and honeybees use attractant scent marks in the field (Williams 1998; Goulson et al. 2000).

Bumblebees of several species detect each other's repellent scents and avoid flowers depleted by themselves, conspecifics and congeners (Stout et al. 1998). Bumblebees and honeybees are often found foraging on the same plant species at the same time, and they would clearly benefit if they could detect flowers that have been visited by each other. Williams (1998) found that bumblebees and honeybees foraging on *Borago officinalis* (Boraginaceae) rejected flowers previously visited by conspecifics or by congeners. However, flowers visited by bumblebees were not repellent to honeybees, and vice versa.

We examined attractant and repellent scent-marking behaviour of wild bumblebees, *Bombus lapidarius*, and honeybees foraging on *Melilotus officinalis* (Fabaceae). Plants of this species support many hundreds of inflorescences. Each inflorescence holds up to 40 flowers, with an average of 12.3 flowers open at any one time. Many bees of several species (*B. lapidarius*, *Bombus terrestris*, *A. mellifera* and *Megachile centuncularis*) forage on these inflorescences. Bees cannot remember all of the individual flowers they have previously visited, but they forage in nonrandom ways and use systematic search patterns to avoid revisiting flowers (Heinrich 1979; Corbet et al. 1981; Best & Bierzychudek 1982; Pyke & Carter 1992; Goulson et al. 2000). However, with so many individuals foraging at the same time, this may not be enough to prevent them from visiting recently depleted inflorescences. Hence, the detection of scent marks deposited by conspecifics and bees of other species would be beneficial.

We tested the following hypotheses. (1) Bumblebees and honeybees will avoid inflorescences recently visited by conspecifics but not inflorescences visited by bees of the other species. (2) Twenty-four hours after a bee visit, inflorescences will be visited at the same rate as inflorescences that have never been visited, regardless of the identity of the previous visitor. (3) The rejection response of both bumblebees and honeybees will fade over time, and this will relate to nectar build-up in florets.

METHODS

We carried out experiments in a patch (consisting of ca. 30 large plants, each holding several hundred inflorescences) of *M. officinalis* at St Catherine's Hill, near Winchester, Hampshire, U.K., from 5 to 13 July 1999, between 1000 and 1700 hours BST. Weather conditions were approximately constant throughout, being clear and sunny with an average temperature of 25–29 °C and little wind. All bees observed were foraging for nectar; some collected pollen in addition to nectar. Whereas some flowers in the Fabaceae are 'tripped' by foraging bees and visited flowers look very different to unvisited ones (for example, in *Cytisus scoparius*, Stout 2000), the petals of *M. officinalis* return to their original position when the foraging insect leaves, and appear no different to unvisited flowers to the human eye (Knuth 1908).

Interspecific Interactions

We tested the response of *B. lapidarius* and *A. mellifera* workers to *M. officinalis* inflorescences that were previously visited by conspecifics or heterospecifics, or inflorescences that had never been previously visited (control inflorescences). The latter were inflorescences that were bagged with fine netting before the flowers opened to prevent bees visiting them.

After a bee visit (honeybee or bumblebee), we either bagged the inflorescence with fine netting and marked the species of the visiting bee on a tag attached to the bag, or immediately picked it and offered it to another bee. To do this, we picked the inflorescence together with a short stem, and held it in the anticipated flight path of test bees (Goulson et al. 1998; Stout et al. 1998). Bees often depart from inflorescences in the same direction as they arrived (Pyke & Carter 1992), and move between adjacent inflorescences. Test inflorescences were therefore held close to the flower the test bee was foraging on (Goulson et al. 1998; Stout et al. 1998). If test bees alighted upon test inflorescences and probed for nectar, we recorded the visit as an 'acceptance'. If, however, test bees approached test inflorescences and then did not land, or landed only briefly and did not probe for nectar, we recorded the visit as a 'rejection'. If test bees did not approach the inflorescences, the visit was not recorded. Bagged inflorescences were left for 24 h, and then offered to a foraging bee, and the response recorded as above. The person holding the inflorescence was not blind to the treatment (we have done blind tests in the past and found no statistical difference in results).

Bees usually visited the majority of the open flowers on an inflorescence, and we removed any individual flowers that were not visited before using the inflorescences in experiments. At least 20 replicate tests were made for each treatment, inflorescences were used only once and if inflorescences were not visited within 3 min of being picked, they were discarded. We offered inflorescences of different treatments to bees in a random order throughout the experiment, so that a variety of tests were carried out on each day. We attempted to use different individual bees for each test, but could not mark them without disturbing their foraging behaviour and so the same individual might have been tested more than once. However, we estimate that because of the large number of bees at the site, the frequency of retesting was low.

Longevity of Scent Marks

We determined the length of time until inflorescences were acceptable to another bee. Inflorescences were bagged after a visit, and the time of the visit was marked on the bag. At specific time intervals after the visit (10, 20, 40 and 60 min), we offered these bagged inflorescences to foraging conspecifics, and noted whether they were rejected or accepted. Again, at least 20 replicates were made at each time interval and a variety of

time intervals were tested on each of 2 consecutive days, between 1330 and 1530 hours, to remove possible confounding effects of time of day on rejection rate. Inflorescences were used only once, and were discarded if not visited within 3 min of being picked.

Nectar Build-up in Flowers

To determine the nectar replenishment rate in *M. officinalis*, we bagged inflorescences after a bumblebee visit and, using a 1-µl micropipette, measured the nectar content of these flowers after 10, 20, 40, 60 min and 24 h. Nectar was also measured in flowers that had just been visited and in those that had never been visited. We sampled 15 flowers from different inflorescences for nectar at each time interval.

Data Analysis

For both *B. lapidarius* and *A. mellifera*, we compared, with a two-tailed multinominal exact test, the frequencies of rejection and acceptance of inflorescences that had been recently visited by a conspecific with those of inflorescences that had been recently visited by a heterospecific. The rejection frequencies of inflorescences visited by conspecifics and heterospecifics 24 h previously were similarly compared. Additionally, the frequencies of rejection and acceptance of inflorescences previously visited by conspecifics or heterospecifics were compared with those of control inflorescences.

The relationships between the following pairs of variables were investigated with Spearman rank correlation tests: (1) time since last conspecific visit and frequency of rejection of inflorescences; (2) time since last conspecific visit and mean nectar volume of flowers; and (3) frequency of rejection of inflorescences by bees and mean nectar volume in flowers at corresponding time points.

To determine when inflorescences ceased to be repellent to conspecifics, we compared the frequency of rejection of inflorescences visited by a conspecific at various time intervals with the frequency of rejection of control inflorescences, and of inflorescences visited 24 h previously by a conspecific. To determine when flowers had replenished most of their nectar, we compared, with a two-tailed Student's *t* test, mean nectar volumes in flowers at each time interval with those in control inflorescences. Variances were first compared with *F* tests, and where the variances were found to be significantly different, *t* tests assuming unequal variances were used.

Since several tests were carried out on the same control data, we used sequential Bonferroni procedures to adjust significance levels to control for tablewide type I errors (Rice 1989). This procedure can result in an increase of type II errors, and so we give both the original *P* values and the adjusted values (Cabin & Mitchell 2000).

RESULTS

Interspecific Interactions

The identity of the recent flower visitor did not affect the rejection response of *B. lapidarius*: inflorescences were rejected at an equal rate whether a conspecific or a honeybee had recently visited them (Fig. 1a). *Bombus lapidarius* rejected 98% (*N*=45) of inflorescences recently visited by conspecifics and 90% (*N*=41) of inflorescences recently visited by honeybees (multinomial exact test: *P*=0.351). However, when *A. mellifera* were offered inflorescences that had been visited recently, there was a significant difference in the frequency of rejection according to the identity of the previous visitor (Fig. 1a). More inflorescences were rejected if they had been recently visited by bumblebees (96%, *N*=44) than those recently visited by honeybees (74%, *N*=43; multinomial exact test: *P*=0.024). Bumblebees rejected inflorescences that had been visited in the previous 3 min by either bee species significantly more often than control inflorescences that had never been visited (Table 1). Honeybees rejected significantly more inflorescences that had previously been visited by bumblebees than control inflorescences, but there was no significant difference between the frequency of rejection of inflorescences recently visited by honeybees and control inflorescences (Table 1).

Twenty-four hours after a bee visit, inflorescences previously visited by conspecifics and heterospecifics were rejected at the same rate by both bee species (Fig. 1b).

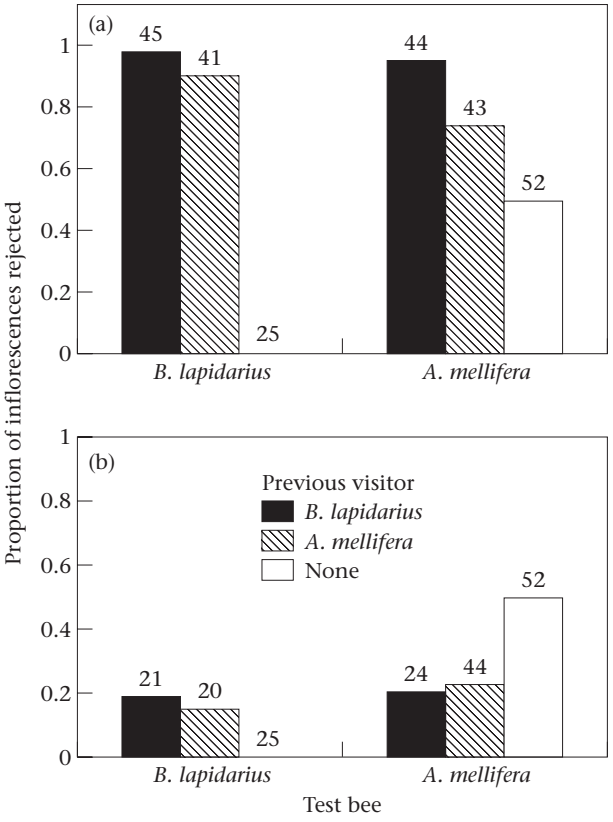

Figure 1. The proportion of inflorescences rejected by *B. lapidarius* and *A. mellifera* according to the identity of the previous visitor (a) <3 min after the first visitor and (b) 24 h after the first visitor. The frequency of rejection of inflorescences that had no previous visitors (control inflorescences) is also shown. Numbers above the bars represent sample sizes.

Table 1. Multinomial exact test two-tailed probabilities for the comparison of the frequency of rejection of recently visited inflorescences (visited within 3 min), or inflorescences visited 24 h previously, with control inflorescences

		<3 min		24 h	
First visitor	Second visitor	P	P_{adj}	P	P_{adj}
B. lapidarius	*B. lapidarius*	<0.001	*	0.065	NS
A. mellifera	*B. lapidarius*	<0.001	*	0.102	NS
B. lapidarius	*A. mellifera*	<0.001	*	0.056	NS
A. mellifera	*A. mellifera*	0.052	NS	0.022	NS

P_{adj}=adjusted probabilities following sequential Bonferroni technique with a significance level of P=0.05: *P<0.05.

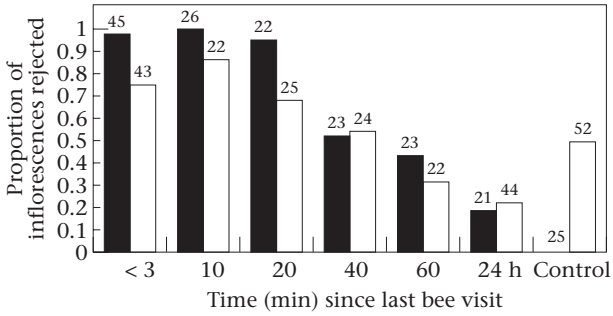

Figure 2. The proportion of inflorescences previously visited by conspecifics rejected by *B. lapidarius* (■) and *A. mellifera* (□) over time. Numbers above the bars represent sample sizes.

Bombus lapidarius rejected 19% (N=21) of inflorescences previously visited by conspecifics and 15% (N=20) of inflorescences previously visited by honeybees (multinomial exact test: P=1.000). Similarly, *A. mellifera* rejected 23% (N=44) of inflorescences previously visited by conspecifics and 21% (N=24) of inflorescences previously visited by bumblebees (multinomial exact test: P=1.000). Bumblebees rejected inflorescences that had been visited 24 h previously by either bee species at the same rate as control inflorescences (Fig. 1b, Table 1). Honeybees rejected inflorescences that had been visited 24 h previously by bumblebees at the same rate as control inflorescences. They rejected more control inflorescences than ones that had been visited 24 h previously by conspecifics (Fig. 1b, Table 1), but this difference was not significant after adjustment of probability thresholds by the sequential Bonferroni procedure.

Longevity of Scent Mark

The frequency of rejection of inflorescences previously visited by conspecifics decreased over time for both *B. lapidarius* and *A. mellifera* (Fig. 2). There was a significant relationship between time interval and proportion of inflorescences rejected for both species (*B. lapidarius*: $r_S = -0.96$, N=7, P<0.05; *A. mellifera*: $r_S = -0.86$, N=7, P<0.05). Forty minutes or more after a conspecific bee visit, bumblebees and honeybees rejected inflorescences at the same rate as inflorescences that had been visited 24 h previously (Table 2). *Bombus lapidarius* rejected none of the control inflorescences whilst *A. mellifera* rejected 50% of control inflorescences. Hence, for *B. lapidarius* there was a significant difference in the rejection of control inflorescences at all time intervals except 24 h, but *A. mellifera* rejected control inflorescences at the same rate as previously visited inflorescences regardless of the time interval (Table 2).

Nectar Build-up in Flowers

After a bee visit, nectar built up in individual flowers (Fig. 3) and the relationship between time since the previous bee visit and nectar volume was statistically significant (r_S=1, N=7, P<0.001). As nectar levels increased, the frequency of rejection of inflorescences by both bee species decreased (*B. lapidarius*: $r_S = -0.96$, N=7, P<0.05; *A. mellifera*: $r_S = -0.86$, N=7, P<0.05). Forty minutes after a bee visit, nectar had replenished to a level that was not different to that in control flowers (Table 3).

DISCUSSION

Bumblebees and honeybees both avoided inflorescences recently visited by bees of the other species at least as

Table 2. Multinomial exact test two-tailed probabilities for the comparison of the frequency of rejection of inflorescences that were previously visited by conspecifics at each time interval with control inflorescences and inflorescences that had been visited 24 h previously

	B. lapidarius				*A. mellifera*			
Time since last bee visit (min)	Control		24 h		Control		24 h	
	P	P_{adj}	P	P_{adj}	P	P_{adj}	P	P_{adj}
<3	<0.001	*	<0.001	*	0.052	NS	<0.001	*
10	<0.001	*	<0.001	*	0.012	NS	<0.001	*
20	<0.001	*	<0.001	*	0.319	NS	<0.001	*
40	<0.001	*	0.072	NS	0.948	NS	0.032	NS
60	0.002	*	0.217	NS	0.354	NS	0.771	NS
24 h	0.065	NS	—	—	0.022	NS	—	—
Control (never visited)	—	—	0.065	NS	—	—	0.022	NS

P_{adj}=adjusted probabilities following sequential Bonferroni technique with a significance level of P=0.05: *P<0.05.

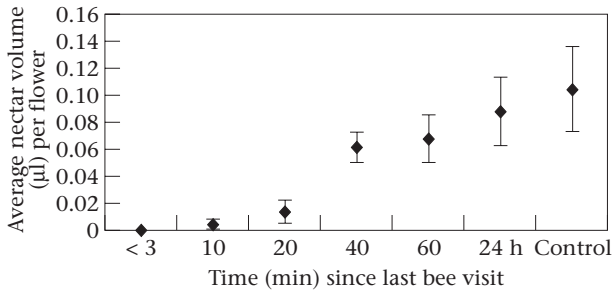

Figure 3. The average nectar volume±SE in individual *M. officinalis* flowers over time since a bee visit.

Table 3. Comparison of the average nectar volume per flower at each time point since a bee visit with the average nectar volume per flower in control flowers

Time since last bee visit (min)	*F* test significance	*t*	*df*	*P*	P_{adj}
<3	0.001	3.29	14	0.005	*
10	0.002	3.14	14	0.007	*
20	0.007	2.76	16	0.014	NS
40	0.030	1.30	17	0.213	NS
60	0.223	1.00	28	0.325	NS
24 h	0.788	0.42	28	0.678	NS

Variances were compared using *F* tests, then two-tailed Student *t* tests were used to compare averages. If a significant *F* test was found, *t* tests assuming unequal variances were used. P_{adj}=adjusted probabilities following sequential Bonferroni technique with a significance level of *P*=0.05: *P<0.05.

often as inflorescences recently visited by conspecifics. We propose that both species can detect the repellent scent of the other species. However, the repellent forage-marking scents of honeybees and bumblebees are reported to be very different: 2-heptanone for honeybees (Vallet et al. 1991; Giurfa 1993) and straight-chain hydrocarbons for bumblebees (Goulson et al. 2000). The molecular weight of 2-heptanone is 114, and it is a very volatile substance, whereas bumblebee tarsal hydrocarbons have a molecular weight of ca. 300–400, and are less volatile (Goulson et al. 2000). It is possible that 2-heptanone is not the only repellent forage-marking scent used by honeybees. In the past, worker honeybees were thought to use it as an alarm pheromone (Shearer & Boch 1965) and to release stinging behaviour (Free & Simpson 1968). However, since levels of 2-heptanone are higher in foragers than in guard bees it may be important in repellent scent marking (Giurfa 1993). Previous investigations into repellent scent marking in honeybees have found that after a bee visit, flowers were initially avoided by subsequent bees, but were repellent for a very short time (<1 min), which is consistent with the use of a highly volatile repellent odour (Giurfa & Núñez 1992; Williams 1998). However, we found that both bumblebees and honeybees were repelled by *M. officinalis* inflorescences for 40–60 min. This suggests that both species were detecting a less volatile repellent scent mark than 2-heptanone. Perhaps honeybees are repelled by 2-heptanone in the very short term or when foraging on flower species with a rapid nectar build-up rate (for example in Giurfa & Núñez's 1992 and Williams' 1998 experiments: see below), but use a different odour on flower species that secrete nectar more slowly.

Bumblebees can detect the tarsal hydrocarbons of several other species of bumblebee even though tarsal secretions differ slightly in composition between species (Stout et al. 1998; Goulson et al. 2000). They may also detect honeybee tarsal secretions. Worker honeybees deposit footprint substances at the entrance of bee hives to facilitate the orientation and homecoming of foragers (Butler et al. 1969). Hence, footprint secretions may also be deposited on flowers, which may induce bumblebees to reject them. To test this hypothesis, washes from honeybees could be applied to flowers in the field and offered to bumblebees (as in Stout et al. 1998; Goulson et al. 2000).

Honeybees rejected inflorescences recently visited by bumblebees, which suggests they can detect the repellent tarsal hydrocarbons of bumblebees. They rejected inflorescences that had previously been visited by bumblebees more than those previously been visited by honeybees, which were rejected at a rate not significantly different to the control inflorescences.

The stronger response to the *Bombus* repellent scent deserves further investigation. It could be that because bumblebees have longer tongues (average tongue length of *B. lapidarius*=8.1 mm, Prys-Jones 1982) they may drain flowers to a greater degree than honeybees, which have shorter tongues (average tongue length of *A. mellifera*= 6.6 mm, Alpatov 1929). Hence, inflorescences previously visited by honeybees may be more likely to contain nectar than those previously visited by bumblebees. Williams (1998) found that honeybees and bumblebees drained some *B. officinalis* flowers, but were equally likely to leave nectar in other probed flowers. However, this may not be the case when bees are foraging on *M. officinalis* flowers because of differences in flower structure and corolla length: the flowers are relatively shallow and produce less nectar than those of *B. officinalis* (J. C. Stout, unpublished data), and could be easily drained by both bumblebees and honeybees. Hence variation in nectar left in flowers may not explain the differential responses by honeybees to flowers previously visited by honeybees and bumblebees. This requires further investigation.

The difference in the odour of bumblebee tarsal hydrocarbons and honeybee repellent scents may allow honeybees to determine the identity of the previous visitor, causing them to reject flowers visited by bumblebees more often than those visited by honeybees. Alternatively, if bumblebees and honeybees are both using tarsal secretions as repellent scents, bumblebees, being larger than honeybees, may deposit larger quantities. This may cause a higher frequency of flower rejection because of stronger repellent odours. The source of the honeybee repellent scent clearly needs to be established.

Bumblebees and honeybees both rejected less than 25% of inflorescences that were visited 24 h previously. However, whereas bumblebees rejected inflorescences visited

24 h earlier at the same rate as inflorescences that had never been visited, honeybees rejected inflorescences visited 24 h earlier significantly less than inflorescences that had never been visited. Indeed, honeybees rejected half of all control inflorescences. This suggests that honeybees prefer to visit inflorescences that have already been visited, sampling only half of the inflorescences they encounter that have had no previous visitors. This is consistent with the findings that honeybees use attractant scent marks to mark rewarding flowers (Ribbands 1955; Ferguson & Free 1979; Free & Williams 1983). Again, we found no evidence for bumblebees using attractant scent marks in the field, although they are reported to use them in the laboratory (Schmitt & Bertsch 1990).

For both bumblebees and honeybees, the rejection response faded gradually over time, and 40 min after a bee visit, inflorescences were rejected at the same rate as those visited 24 h previously. This is just after flowers had replenished most of their nectar: there was no significant difference between nectar volume in flowers 40 min after a bee visit and in control flowers that had never been visited, and after adjustment of probability thresholds, with the sequential Bonferroni technique, this time dropped to 20 min. Nectar continued to accumulate in flowers after this time, although not in significant volumes. Hence, bees appear to begin revisiting inflorescences after an appropriate time interval. Forty minutes is much longer than the time interval between visits found by Williams (1998) when bumblebees and honeybees were foraging on *B. officinalis* ('half life' of repellency=37 s) and by Giurfa & Núñez (1992) when honeybees were foraging from an artificial feeder ('endurance' of 45 s). The shorter repellent effect in these studies might have been because nectar was continuously replenishing in Giurfa & Núñez's (1992) artificial flowers, *B. officinalis* secretes nectar at a much faster rate than *M. officinalis* (J. C. Stout, unpublished data), and nectar was sometimes left in *B. officinalis* flowers. This suggests that bees can adapt the length of time before they revisit flowers according to the plant species they are foraging on. Since flower species secrete nectar at different rates, this would appear to be a sensible strategy. If flowers were revisited too soon, bees would receive a suboptimal reward, and if flowers were left for longer, the extra nectar gain would not compensate for the time wasted in avoiding nearly full flowers.

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