ORIGINAL ARTICLE

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The influence of nectar secretion rates on the responses of bumblebees (*Bombus* spp.) to previously visited flowers

Abstract Bumblebees can avoid recently depleted flowers by responding to repellent scent-marks deposited on flower corollas by previous visitors. It has previously been suggested that avoidance of visited flowers for a fixed period would be a poor strategy, since different plant species vary greatly in the rate at which they replenish floral rewards. In this study, we examined the duration of flower repellency after an initial bumblebee visit, using wild bumblebees (*Bombus lapidarius*, *B. pascuorum* and *B. terrestris*) foraging on four different plant species (*Lotus corniculatus*, *Melilotus officinalis*, *Phacelia tanacetifolia* and *Symphytum officinale*). We constructed a model to predict flower visitation following an initial visit, based on the nectar secretion pattern of the different plant species, the insect visitation rate per flower, and the search and handling times of bumblebees foraging on the plant species in question. The model predicts an optimal duration of flower avoidance which maximises the rate of reward acquisition for all bees. However, this optimum may be open to cheating. For two plant species, the evolutionary stable strategy (ESS) is a shorter duration of flower avoidance than the optimum. We found the duration of flower avoidance was markedly different among flower species and was inversely related to nectar secretion rates. The predicted ESSs for each plant species were close to those observed, suggesting that the key parameters influencing bumblebee behaviour are those included in the model. We discuss how bees may alter the duration of their response to repellent scents, and other factors that affect flower re-visitation.

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Introduction

It has become apparent that many bee species are able to detect and avoid flowers that were recently visited by themselves or other bees. This improves foraging efficiency (Williams 1998) and has been observed in bumblebees (Kato 1988; Goulson et al. 1998; Stout et al. 1998; Williams 1998), honeybees (Núñez 1967; Free and Williams 1983; Giurfa and Núñez 1992; Williams 1998), stingless bees (Goulson et al. 2001) and some solitary bee species (Gilbert et al. 2001; Goulson et al. 2001). Bumblebees avoid recently visited flowers by responding to chemical 'footprints' made up of long-chain tarsal hydrocarbons which are deposited on flower corollas by previous bee visitors (Stout 1999; Goulson et al. 2000). As chemicals in the footprints evaporate, flowers lose their repellent effects and are visited again by other bumblebees (Stout et al. 1998; Stout and Goulson 2001). There is some evidence for bumblebees using tarsal footprints to mark rewarding artificial flowers in the laboratory (Schmitt and Bertsch 1990; Schmitt et al. 1991), but this has not been demonstrated in the field (Williams 1998; Goulson et al. 2000; Stout and Goulson 2001). Although the chemical components of the footprint vary between bumblebee species (Goulson et al. 2000), repellency appears to be induced across species boundaries, at least within bumblebees and between bumblebees and honeybees (Goulson et al. 1998; Stout et al. 1998; Stout and Goulson 2001).

It seems that the duration of the repellent effect varies according to the plant species being visited. Wild bumblebees (*Bombus terrestris* and *B. pascuorum*) foraging on *Symphytum officinale* (Boraginaceae) avoided flowers for 20–60 min after an initial visit (Stout et al. 1998). Similar patterns were found for *B. lapidarius* foraging on *Melilotus officinalis* (Fabaceae) (Stout and Goulson 2001). However, Williams (1998) found that for *B. lapi-*

Table 1 Regression equations of nectar build-up over time in the plant species studied (µl/min). For *Symphytum officinale*, the best fit was obtained using a quadratic regression, but for the other plant species a logistic regression was more appropriate

Plant species	Regression equation	r^2	F	df	P
Lotus corniculatus	$y=0.090\ln(x)-0.265$	0.944	67.39	1,4	0.001
Melilotus officinalis	$y=0.014\ln(x)-0.014$	0.751	18.13	1,6	0.005
Phacelia tanacetifolia	$y=0.068\ln(x)-0.128$	0.734	13.80	1,5	0.014
Symphytum officinale	$y=0.0067x-3\times10^{-6}x^2$	0.761	183	2,115	0.001

darius foraging on *Borago officinalis* (Boraginaceae), the "half-life" of repellency was only 37 s. It would not make sense for bumblebees to use a fixed rejection period across all plant species, for plants vary greatly in the rate at which they secrete nectar (Goulson et al. 2000). This would lead to premature acceptance of flowers with low secretion rates, or rejection of rewarding flowers when visiting species with high secretion rates. For example, *B. officinalis* secretes nectar at a greater rate than *S. officinale* or *M. officinalis* (Stout, unpublished data), and it seems likely that this may explain the shorter duration of repellency observed on *B. officinalis*. Other factors may also influence the optimal duration of repellency. If flowers are scarce, have a low handling time, or competitors are abundant, then it seems intuitively likely that bumblebees will be less selective (MacArthur and Pianka 1966).

The aim of this study was to determine whether the duration of the repellent effect really does vary when bumblebees visit different flower species, and whether this variation can be explained in terms of the nectar secretion rates, search and handling times, and the abundance of bees. We develop a model to predict the optimal duration of repellency based on observed parameter estimates, and compare these predictions with observations on six flower/bumblebee systems.

The model

Let S be the time taken to locate a flower and decide on rejection or acceptance (search time), H the handling time of flower and p_a the proportion of flowers that are acceptable; then we can approximate the pattern of nectar build-up in flowers (N) with the equation:

$$N = bt + ct^2 \quad \text{or} \quad N = b\ln(t) + c \tag{1}$$

where t is time and b and c are constants. The quadratic equation gives a high r^2 value for the relationship between nectar build-up and time in *S. officinale* for the time span 0–24 h. The logistic equation gives high r^2 values for the other plant species tested (*Lotus corniculatus*, *M. officinalis*, *Phacelia tanacetifolia*) for the time span 0–24 h (Table 1, Fig. 1). For simplicity the model is developed using a quadratic equation, but can readily be modified when other equations better describe nectar build-up.

The average reward received per flower encountered will be $n_a p_a$ where n_a is the average reward provided by an acceptable flower.
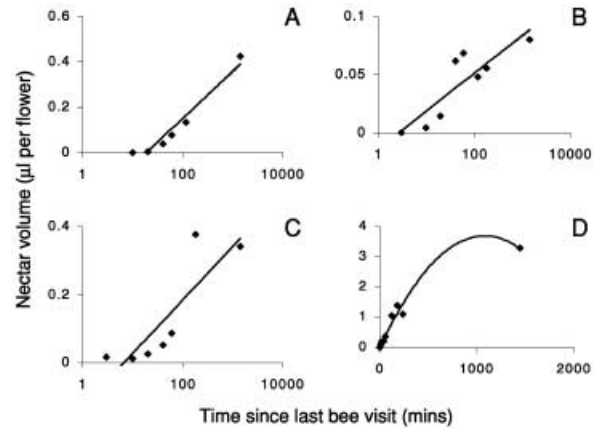

Fig. 1 Patterns of nectar build-up in flowers of four different species (**A** *Lotus corniculatus*; **B** *Melilotus officinalis*; **C** *Phacelia tanacetifolia*; **D** *Symphytum officinale*). Flowers were drained by bees at time 0. Equations of regression lines are given in Table 1

The average time taken to locate a flower, and handle it or reject it will be given by:

$$p_a(S+H) + S(1-p_a) \tag{2}$$

Therefore the expected rate at which rewards are gathered is:

$$E = \frac{n_a p_a}{p_a(S+H) + S(1-p_a)} \tag{3}$$

We now make the simplifying assumption (later to be abandoned) that all bees adopt the same threshold for rejection of flowers. The average time between visits to flowers will be:

$$t_a = t_c + \frac{1}{V} \tag{4}$$

where t_a is the average time between probing visits to flowers, and t_c is the time taken for flowers to reach the threshold for acceptance. V is the rate at which individual flowers are encountered by insects (including rejections and acceptances). Following a probing visit, flowers are rejected at time intervals less that t_c. Once they pass t_c, they are accepted by the first bee to encounter them.

Combining Eqs. 1 and 3 we obtain:

$$E_r = \frac{p_a(bt_a + ct_a^2)}{p_a(S+H) + S(1-p_a)} \tag{5}$$

The proportion of plants that have acceptable nectar levels is given by:

$$p_a = \frac{\frac{1}{V}}{t_a} = \frac{1}{V t_a} \tag{6}$$

Combining Eqs. 5 and 6 we obtain:

$$E_r = \frac{\frac{1}{V t_a}\left(b t_a + c t_a^2\right)}{\frac{1}{V t_a}(S+H) + S\left(1 - \frac{1}{V t_a}\right)} \tag{7}$$

This simplifies to:

$$E_r = \frac{b t_a + c t_a^2}{H + V t_a S} \tag{8}$$

The constants b and c are readily determined. Values for H, V and S are likely to vary between flower species, bee species, and locations, but are all readily measured. Thus the optimum value of t_a that maximises E can be found. Since the top line of the equation is simply the nectar accumulation curve, a logistic equation can be readily substituted where appropriate.

This model predicts optimum values of t_a that represent the best rates of return if all bees use the same strategy. If all bees are from a single nest, and foraging for the common good, then this would represent the true optimum. However, this optimum may be open to cheating.

If, within a large population of bees that have adopted t_a, a single bee foraged indiscriminately (without rejecting any flowers), its expected rate of reward would be approximated by:

$$E_i = \frac{p_a n_a + (1 - p_a)\left(b t_c / 2 + (c t_c / 2)^2\right)}{H + S} \tag{9}$$

Using parameter values derived from field observations (see below), it seems that for some plant species, E_i is substantially greater than E_r. Thus the predicted optimum is not necessarily an ESS. The ESS can be found by equating (8) and (9), and solving numerically for t_c. At this point, no single bee can improve its reward by adopting a different strategy. But the overall gain per bee may then be less than could be obtained if all co-operated.

Model assumptions

1. For simplicity we have not allowed handling time to vary according to the reward provided by the flower, yet in reality we may expect handling times to be longer when extracting larger amounts of nectar. This would lead us to slightly overestimate the true optimum time between visits.
2. We also assume that bumblebees remove all nectar when visiting a flower. Using micropipettes, we were unable to detect nectar in flowers that had just been visited by bees, so this assumption would appear to be valid.
3. Differences in the energetic costs of flight versus handling of flowers are likely to be negligible and are ignored (Heinrich 1979a).

4. We assume that bumblebees are able to accurately assess the time that has elapsed since a flower was last visited. This could be either through detection of a decaying scent mark (Stout et al. 1998; Goulson et al. 2000) or by direct assessment of nectar levels (Crane 1975; Thorp et al. 1975; Corbet et al. 1979; Williams et al. 1981; Marden 1984).
5. To calculate the reward that a single bumblebee would obtain by visiting flowers indiscriminately, we ignore the influence that this bumblebee would have on nectar availability. As long as the number of flowers and foraging bumblebees is large, this assumption is reasonable.

Methods

Quantification of nectar build-up

Four plant species were used in this study: *L. corniculatus*, *M. officinalis*, (Fabaceae), *P. tanacetifolia* (Hydrophyliaceae) and *S. officinale* (Boraginaceae). These four plant species were selected because they have similar flowering phenology, they produce measurable quantities of nectar, are attractive to bumblebees and were locally abundant. Three of the species occurred in wild populations (*L. corniculatus* at Nutley Farm, Broughton; *M. officinalis* at St. Catherine's Hill, Winchester; *S. officinale* at the Itchen Valley Country Park, Southampton); the fourth was planted in experimental plots (*P. tanacetifolia* at the University of Southampton Research Centre, Chilworth). All sites were located in Hampshire in the south of England.

To measure the build-up of nectar in flowers following depletion by a bumblebee, flowers were bagged with a fine netting after a bumblebee visit and the time of the visit was marked on the bag. After set time intervals (0, 10, 20, 40, 60, 120, 180 min and 24 h), the volume of nectar in the flowers was measured using a micropipette (Drummond "Microcaps", Drummond Scientific, USA). Nectar measurements were taken from 25 May to 27 July 1999 between 0845 and 1700 hours BST, at the same time as observations of bee behaviour were made (see below). An appropriate equation for nectar build-up over time was obtained for each plant species using regression analysis.

Observations of bee behaviour

Wild worker bumblebees were observed foraging on the four different flower species from 25 May to 27 July 1999 between 0845 and 1700 hours BST. *B. lapidarius* was observed foraging on *L. corniculatus* and *M. officinalis*; *B. pascuorum* was observed on *S. officinale*; and *B. terrestris* on *L. corniculatus*, *S. officinale* and *P. tanacetifolia*.

After approaching flowers, bumblebees either land and probe for nectar (henceforth 'accepting' flowers) or depart after touching the corolla with their antennae or landing briefly but not probing for nectar or collecting pollen (henceforth 'rejecting' flowers). The time taken for bees to search for (S) and handle (H) flowers was measured. Search times were measured as the time it took individual bumblebees to fly between flowers, and on encountering a flower, the time taken to decide whether to reject or accept that particular flower. Handling times were measured as the time taken to extract nectar from flowers, i.e. the time from accepting a flower to departing from it. At least 14 individuals of each bumblebee species foraging on each plant species were observed. Bumblebees made between 3 and 45 flower visits, any that made <3 visits were not included in the calculations. The mean S and H for each individual bumblebee was calculated, then mean values for each bee species on each plant species were calculated. Only

Table 2 Search (*S*) and handling (*H*) times of each bee species (s)

Plant species	Bee species	*H*			*S*		
		Mean	SE	*n*	Mean	SE	*n*
L. corniculatus	*B. lapidarius*	0.038	0.002	14	0.016	0.001	37
L. corniculatus	*B. terrestris*	0.042	0.004	17	0.014	0.001	24
M. officinalis	*B. lapidarius*	0.026	0.008	19	0.016	0.002	18
P. tanacetifolia	*B. terrestris*	0.026	0.002	14	0.021	0.001	22
S. officinale	*B. pascuorum*	0.043	0.004	15	0.025	0.004	22
S. officinale	*B. terrestris*	0.054	0.005	17	0.024	0.002	35

Table 3 Multinomial exact test two-tailed probabilities for the comparison of the frequencies of flowers accepted and rejected at each time point with frequencies of control flowers accepted and rejected. The predicted time interval (t_a) between visits to flowers is also shown. The optimal value is that which collectively gives the greatest rate of return. The evolutionary stable strategy (ESS) is a strategy which is not open to 'cheating'

Plant species	Bee species	Time since last bee visit (min)							Predictions	
		3	10	20	40	60	120	1440	Optimal	ESS
L. corniculatus	*B. lapidarius*	<0.001*	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	0.040*	300	300
L. corniculatus	*B. terrestris*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.002*	0.001*	348	348
M. officinale	*B. lapidarius*	<0.001*	<0.001*	<0.001*	<0.001*	0.002*	0.001*	0.065	55	55
P. tanacetifolia	*B. terrestris*	<0.001*	0.009*	0.023	0.659	1.000	n.t.[a]	1.000	22	18
S. officinalis	*B. pascuorum*	<0.001*	0.002*	0.061	0.585	1.000	n.t.[a]	0.867	124	14
S. officinalis	*B. terrestris*	<0.001*	0.041	0.510	0.510	0.228	n.t.[a]	0.809	142	17

*Significant following sequential Bonferroni procedure with a significance level of $P<0.05$
[a] No tests were made at this time point

nectar-collecting visits were used. Mean *S* and *H* values were compared between bee species foraging on the same plant species (using *t*-tests). Data for the different bumblebee species on the same plant species were then pooled and *S* and *H* values compared between plant species using a Kruskall-Wallis non-parametric analysis of variance (non-parametric methods were used because there was significant heterogeneity of variance: Sokal and Rholf 1995).

Visitation rates per flower per minute (*V*) were also measured. Any bee approaching a focal flower (regardless of whether the flower was then accepted or rejected) was recorded. Sixty focal flowers were studied for each flower species. *V* was compared between plant species using a Kruskall-Wallis non-parametric analysis of variance.

After a flower had been visited by a nectar-foraging bumblebee, flowers were bagged and tagged to record the bee species and the time of the visit. At a certain time interval after this visit (10, 20, 40, 60, 120 min or 24 h), the bagged flower was picked with a short stem and placed in the path of a different foraging bumblebee of the same species, in the manner described in Stout et al. (1998). We recorded whether the flower was accepted and visited, or rejected and not visited by the second bumblebee. At least 20 tests were carried out at each time interval with each bumblebee species on each of the plant species.

Control flowers (those which had been bagged before they opened, which had never been visited) were also offered to foraging bumblebees, and the acceptance or rejection of the flowers was recorded. Again, at least 20 tests were performed with controls on each bee species on the plant species concerned.

Individual flowers were only used once, and then discarded. We tried to use different individual bumblebees for each test, but did not attempt to mark individual bees because of the adverse effect marking agents may have had on behaviour and scent detection. Since all flowers species used have flowers in inflorescences, any flowers on an inflorescence which were not visited by a bee were removed and discarded before the inflorescence was bagged. Blind trials (with the experimenter ignorant of the recent visitation history of the flower) using *S. officinale* indicated no observer bias (Stout 1999).

Logistic regression (Crawley 1993) was used to investigate the relationship between the time since the last bumblebee visit and the proportion of bumblebees accepting flowers. Multinomial exact tests (two-tailed) were used to compare the frequencies of bumblebees accepting and rejecting flowers at each time point with controls. A sequential Bonferroni procedure was used to control for group-wide type I errors (Holm 1979). This procedure can cause an increase in type II errors, and so we give the original *P* values and those following the Bonferroni procedure (Cabin and Mitchell 2000). We used the time-point at which no significant differences were found between the frequencies of flowers accepted and controls as an estimate of when bees revisit flowers.

Results

Model parameters

Regression equations for nectar build-up versus time (*N*) were statistically significant for all plant species (Table 1, Fig. 1). Insect approaches per flower per minute (*V*) varied significantly with plant species, with *P. tanacetifolia* being the most frequently visited, and *L. corniculatus* flowers visited very rarely (*L. corniculatus*: mean=0.004, SE=0.0015; *M. officinalis*: mean= 0.014, SE=0.0019; *P. tanacetifolia*: mean=0.285, SE= 0.017; *S. officinale*: mean=0.214, SE=0.016; K_3=845.6, $P<0.001$). Search times (*S*) and handling times (*H*) are given in Table 2. Mean search times for *B. pascuorum* and *B. terrestris* foraging on *S. officinale* were not significantly different (t_{25}=0.59, P=0.56, assuming unequal variance), neither were search times for *B. lapidarius* and *B. terrestris* on *L. corniculatus* (t_{59}=1.51, P=0.14, assuming equal variances). There was a significant dif-

Fig. 2 The relationship between the proportion of bees accepting previously visited flowers and time (**A** *Bombus lapidarius* on *L. corniculatus*; **B** *B. terrestris* on *L. corniculatus;* **C** *B. lapidarius* on *M. officinalis;* **D** *B. terrestris* on *P. tanacetifolia;* **E** *B. pascuorum* on *S. officinale;* **F** *B. terrestris* on *S. officinale*). Logistic regression analysis was used to calculate fitted lines. $y=\ln(p/q)$, where *p*=number of bees accepting flowers and *q*=number of bees rejecting flowers (Crawley 1993). *$P<0.05$, **$P<0.01$

Time since last bee visit (mins)
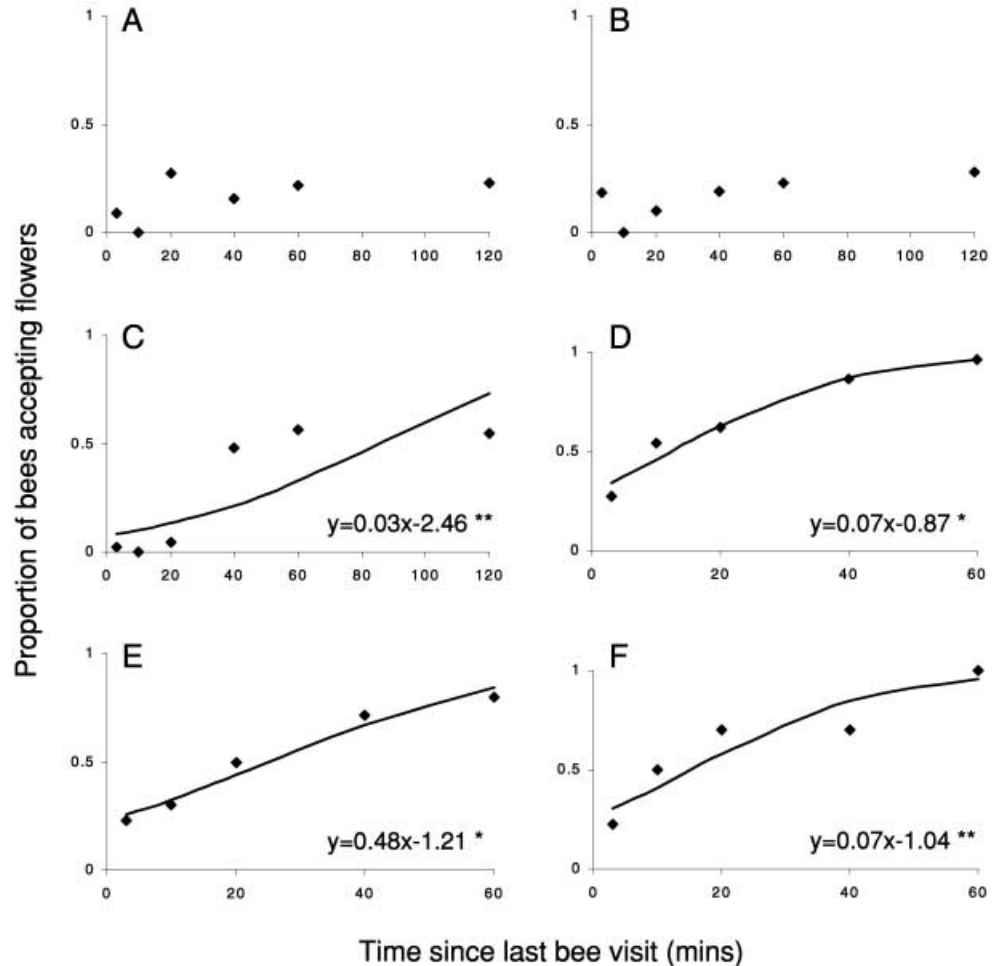
ference in search times between plant species (K_3=508.35, $P<0.001$). Similarly, handling times for *B. pascuorum* and *B. terrestris* on *S. officinale* were not significantly different (t_{30}=1.88, P=0.07, assuming equal variances), neither were the handling times for *B. lapidarius* and *B. terrestris* on *L. corniculatus* (t_{24}=0.856, P=0.40, assuming unequal variances). There was no significant difference in handling times between plant species (K_3=341.58, $P<0.001$).

Observed duration of repellency

The proportion of bumblebees accepting flowers significantly increased as time since the flower was previously visited increased, for all plant species except *L. corniculatus* (Table 3, Fig. 2). After 24 h the frequency of bumblebees accepting previously visited flowers was high, except for *L. corniculatus*, and the frequency of bumblebees accepting control (never-visited) flowers was consistently high (Table 4).

The time at which repellency ceased to operate and the acceptance rate of previously visited flowers rose to that of controls differed markedly between plants species (Table 3). Bumblebees accepted previously-visited

Table 4 Percentage of bees accepting flowers which had been visited 24 h previously and control flowers which had never been previously visited. *n*=sample size

Plant species	Bee species	24 h		Control		*P*
		%	*n*	%	*n*	
L. corniculatus	*B. lapidarius*	47.8	23	85	20	*
L. corniculatus	*B. terrestris*	29.2	24	84	19	*
M. officinale	*B. lapidarius*	81.0	21	100	25	
P. tanacetifolia	*B. terrestris*	95.2	21	95.5	22	
S. officinalis	*B. pascuorum*	78.3	23	85	20	
S. officinalis	*B. terrestris*	77.8	27	85.7	21	

*Significant differences according to multinomial exact tests ($P<0.05$)

S. officinalis flowers after 3–20 min, *P. tanacetifolia* after 10–20 min and *M. officinale* after 2–24 h. *L. corniculatus* were never visited with the same frequencies as control flowers, even after 24 h. There appeared to be no difference between the behaviours of *B. lapidarius* and *B. terrestris* when foraging on *L. corniculatus*, but repellency of *B. terrestris* lasted for a slightly shorter period than in *B. pascuorum* when both were visiting *S. officinale*.
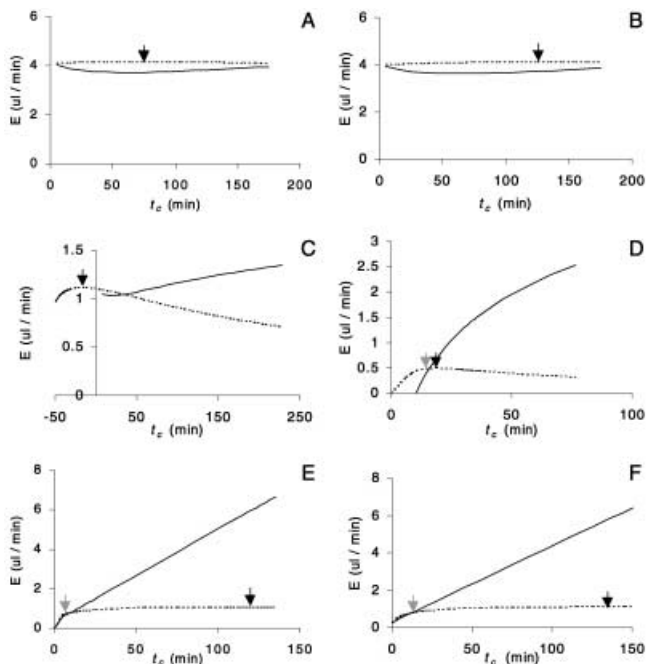
Fig. 3 Predicted energy gains per unit time according to the strategy adopted by bees (**A** *B. lapidarius* on *L. corniculatus*; **B** *B. terrestris* on *L. corniculatus;* **C** *B. lapidarius* on *M. officinalis;* **D** *B. terrestris* on *P. tanacetifolia;* **E** *B. pascuorum* on *S. officinale*; **F** *B. terrestris* on *S. officinale*). If all bees adopt a common threshold for rejection of flowers of t_c min since the flower was previously visited, then the relationship between t_c and reward is shown by the *dashed line*. The reward gained by a single bee that foraged indiscriminately while all other individuals adopted a common t_c is given by the *solid line*. The optimum reward for a population of bees foraging for the common good is marked with a *black arrow*, but in some systems this is open to cheating. In this situation a lower ESS is also marked with a *grey arrow*

Predicted duration of repellency

The model predicts that the optimal time for bees to avoid flowers after an initial visit ranges between 22 and 348 min depending on the bumblebee species and plant species in question. However, when "cheaters" are included in the model, ESS times range between 14 and 348 min (Table 3, Fig. 3).

The rank order of duration of the repellent effect is correctly predicted by the model. Thus the model predicts that repellency should last longest on *L. corniculatus*, be intermediate in *M. officinale* and shortest in *P. tanacetifolia* and *S. officinale*. The actual predicted durations are reasonably close to those that were observed with the exception of *L. corniculatus* in which the model predicts acceptance after 5–6 h, but in reality flowers were still frequently rejected after 24 h.

Discussion

Our data demonstrate clear differences in the duration of rejection responses between four different flower species, ranging from less than 10 min to over 24 h. This

has been suspected (Goulson et al. 2000), but had not been convincingly demonstrated. From our earlier studies it seems likely that repellency is induced by scent marks. When nectar was removed from rewarding *S. officinale* flowers, they were still visited by bumblebees, suggesting that bees were not responding directly to nectar levels (Goulson et al. 1998). Furthermore, we have previously identified the compounds deposited for the three bumblebee species studied here (Goulson et al. 2000), and replicated flower rejection behaviour using both tarsal washes and synthetic components of footprints (Stout et al. 1998; Goulson et al. 2000). Despite this, we cannot rule out the possibility that in some cases bees were assessing nectar levels directly. For example, bees may detect and respond to honey odours, humidity differences or light reflectance from the surface of the nectar (Marden 1984). However, in three out of the four flower species studied, nectar is concealed: in *S. officinale*, it is at the base of the bell-shaped flower, obscured by the anthers; in *L. corniculatus* and *M. officinalis*, it is hidden in typical pea flowers. In the fourth species, *P. tanacetifolia*, flowers are more open and nectar is more exposed. In this case, direct assessment of nectar may be more likely. In addition to using scent marks, some degree of direct assessment may induce more accurate assessment of nectar levels. Indeed, previously visited *P. tanacetifolia* flowers were repellent for short periods (10–20 min) and bees behaved as predicted by the model. Furthermore, we found very high levels (>95%) of acceptance of rewarding flowers (those visited 24 h previously and control flowers) in this plant species.

The duration of repellency appears to be inversely correlated to the rate of nectar secretion, as one might expect. Thus *S. officinale* has the highest rate of nectar secretion, and the shortest repellency response at 3–10 min for *B. terrestris* and 10–20 min for *B. pascuorum*. *M. officinalis* and *L. corniculatus* had comparatively low rates of nectar secretion, and repellency lasted much longer (>2 h and >24 h, respectively).

The observed durations corresponded reasonably well with those predicted from the model. The model predicts the optimum rate of reward acquisition that bumblebees can achieve. For plants with a high rate of nectar secretion the duration of repellency which achieves this optimum is longer than observed in nature. Thus when visiting *S. officinale* the observed durations were 3–20 min (depending on the bee species), but we predict that all bumblebees would obtain a better rate of return if they rejected flowers until over 2 h had elapsed. However, the model also predicts that this optimum is open to cheating. If all other bees adopted a rejection period of 2 h, then a single bumblebee visiting flowers randomly would make large savings in search time, and still obtain a reasonable reward per flower. This strategy would give a higher rate of return. The predicted ESS is much lower, at 14–17 min (depending on bee species), corresponding well with observed values. Although at the ESS all bees are obtaining a lower reward than could be achieved, the strategy is not vulnerable to invasion.

Nectar secretion rate is not the only factor that we would expect to influence the optimal strategy. The abundance of flowers, and the rate at which they are visited by other bees are likely to be important. Thus if flowers are very abundant and competitors rare then a bumblebee can presumably afford to be highly selective, visiting only the most rewarding flowers. By far the most abundant flower in this study was *L. corniculatus*, in which individual flowers were only encountered by bees on average every 227 min. We predicted an optimum duration of repellency of 5–6 h, but in reality bees continued to avoid flowers for up to 24 h, and possibly longer. Our model ignores recruitment of new flowers into the population. If new flowers are highly rewarding, and are sufficiently frequent, then it may pay bees to avoid any flowers that have previously been visited and have traces of scent marks. *L. corniculatus* flowers randomly selected by the authors contained relatively high levels of nectar (Stout, unpublished data), whilst those visited previously had lower levels, suggesting that many of those randomly picked had never previously been visited. It is possible that *L. corniculatus* was being used primarily as a pollen source rather than a nectar source, although bees clearly collected nectar too. If most pollen is removed by the first visitor to a flower, then subsequent visits would yield little or no pollen reward. Hence avoidance of all previously visited flowers would be a sensible strategy. This requires further investigation.

By what mechanism might bumblebees adjust the duration of repellent marks? There are two alternatives. Bees may adjust the amount of scent-marking compounds deposited, or there may be a change in the responsiveness of animals to scent-marks of different ages on different plant species. The latter seems more plausible. It has been shown experimentally that honeybees can not discriminate between two different intensities of the same odour (Pelz et al. 1997). However, as the repellent scent-mark fades, different volatiles evaporate at different rates. Thus the chemical composition of the footprint odour will change over time, being initially dominated by more volatile components and then increasingly by the compounds of lower volatility. Bees may then learn to associate a particular chemical signal with a reward level in different plant species. For example, on highly rewarding flower species, a particular composition of odours (perhaps after 20 min of evaporation) may induce acceptance of a flower. On the other hand, on a less rewarding plant species, the same odour, after the same time interval, may induce rejection. Bees are known to possess formidable powers of learning, particularly when learning involves a reinforcer (Dukas and Real 1993a, b; Chittka 1998; Laloi et al. 1999; Menzel 1999; Menzel and Giurfa 2001). Furthermore, bumblebees are well able to update and modify learned behaviours (Laverty 1980; Dukas 1995) although this may take time (Heinrich 1979b). Bumblebees tend to be flower constant, so that they repeatedly visit flowers of a particular species (Heinrich et al. 1977; Thomson 1981; Waser

1986; Chittka et al. 1997, 1999). Learning an appropriate composition of scent mark for acceptance may be another factor favouring flower constancy. A bumblebee that regularly switched between flower species would not only have to repeatedly recall appropriate handling skills but would also need to retrieve the appropriate relationship between scent-mark compositions and nectar rewards from its long-term memory. Alternatively, learning may not be involved, but instead, on a highly rewarding species, motivational factors may decrease bees' attention to a scent mark, whereas on a less rewarding species, there may be an increase in attention. Bees have been shown to display behavioural flexibility in responding to stimuli associated with nectar content (Marden 1984).

Whichever mechanism is used to adjust the duration of repellency, it is not likely to be completely accurate and is likely to be affected by the bees' experience. Our model predicts a precise threshold, but in reality the rejection response declines gradually (albeit at a different speed on different flower species). The bumblebee population presumably contains individuals with a range of different foraging histories. Some may be naive, and so respond inappropriately. Also, the strength of mark deposited is likely to vary by chance, and so provide a less than perfect cue as to the time that has elapsed since the flower was visited.

In summary, we clearly demonstrate that rejection responses vary in duration between flowers. The mechanism by which the duration is adjusted is not known, but is most probably via learning of an appropriate threshold for rejection. Duration of the rejection response is predicted with encouraging accuracy by a simple model incorporating the pattern of nectar build-up, visitation rates to flowers, and the search and handling times of bees.

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