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Behavior of Inexperienced Bumble Bees toward Spatial Clumping of Nectar (Hymenoptera: Apidae)

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Taneyhill D E & Thomson J D [Montfort Coll, Chiang Mai, Thailand; Dept Zool, Univ Toronto, 25 Harbord St, ON M5S-3G5, Canada]: **Behavior of Inexperienced Bumble Bees toward Spatial Clumping of Nectar (Hymenoptera: Apidae) –** Entomol Gener **29**(-): 000–000; Stuttgart 2007-XX – – – [Article]

Patch departure was studied in experimentally naive, laboratory-reared bumble bees, *Bombus impatiens* (Cresson 1863) foraging from artificial umbels (rings of eight wells in blocks of Plexiglas, each well containing 0–4 *μL* of 30% sucrose solution). Bees from three colonies probed an average of about ten wells (all available wells plus two revisits to emptied wells) before departing. On the empty rings, bees probed significantly more wells before departing if they had previously visited a filled ring. The numbers of wells probed on filled rings varied with the volume of nectar, with the maximum associated with 2 *μL*, and fewer on average associated with both 1 *μL* and 4 *μL*. In tests with triplets of rings containing one empty ring and two others filled with sucrose solution, the numbers of wells probed on the empty rings depended on both the volumes of nectar in previous rings and their order of encounter. The results did not support the threshold departure rules (e.g, 'depart after finding two empty flowers') that have been proposed as the proximal mechanism of bee patch departure. Instead, the mechanism by which the bumble bees decided to depart patches seemed to be a complex function of experience in recently encountered patches.

Key words: *Bombus impatiens* (Cresson 1863) – departure rules – food patch – foraging

Taneyhill D E & Thomson J D [Montfort Coll, Chiang Mai, Thailand; Dept Zool, Univ Toronto, 25 Harbord St, ON M5S-3G5, Canada]: **Le Comportement des Bourdons Naifs vers les Volumes du Nectar Artificiel. –** Entomol Gener **29**( ): 000–000; Stuttgart 2007-XX. – – – [Article]

Nous avons étudié le comportment à chercher nourriture chez les bourdons naïfs, *Bombus impatiens* (Cresson 1863). Les bourdons cherchaient du nectar artificiel dans une cage à voler, et ils ont obtenu le nectar des fleurs artificielles qui ressemblaient aux ombelles des fleurs naturelles de l'espèce *Aralia hispida*. Les ombelles artificielles étaient étalées en paires, et étaient en forme des cercles qui presentaient huit petits puits dans chacun des cercles. Les fleurs artificielles étaient remplis d' une solution de 30 pourcents sucrose, 0–4 *μL*. Dans le premier expérience, les bourdons ont visité plus de fleurs dans une ombelle artificielle vide de nectar avant de partir, s'ils avaient visité précédemment une ombelle pleine de nectar dans chacun des puits. Pour les expériences qui suivaient, nous avons utilisé des triangles de ombelles artificielles. Le nombre de puits visité par les bourdons dans les ombelles vide de nectar dependait des volumes de nectar trouvés dans les ombelles qui les bourdons avaient visité avant de rencontrir l'ombelle vide. Les resultats n'ont convenu pas des modèles du comportment à chercher nourriture chez les bourdons, les modèles qui se sont appelés '*threshold departure rules'* (c'est à dire, un bourdon devrait partir d'une ombelle si le volume de nectar trouvé dans une fleur est moins d' un montant spécifique). Au contraire, le mecanisme à partir semble être une fonction des montants de nectar trouvés dans les fleurs qui les bourdons ont visitées auparavant.

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Mots clefs: *Bombus impatiens* (Cresson 1863) – bourdon – comportement de partir – nectar – ombelle artificielle

1 Introduction

Studying pollinator behavior from an evolutionary view involves asking questions about adaptation, mechanism, and ontogeny. These are elements of TINBERGEN's [1963] classic program for studying animal behavior. The adaptationist approach dominated the study of foraging behavior for years [DAWKINS 1990], but more recently there has been a shift in emphasis, as researchers have realized that mechanisms themselves are important in directing behavioral evolution [KREBS & DAVIES 1991, HUNTINGFORD 1993]. In this paper, the ontogeny and mechanism of patch departure in bumble bees are examined, where patches are defined as multiflowered plants from which the bees gather nectar and pollen. Patch departure has long been a centerpiece of foraging theory [STEPHENS & KREBS 1986], and studies with bumble bees played a major role in tests of the early optimal foraging models [PLOWRIGHT & LAVERTY 1984]. More recently, bumble bees have been the subjects of experiments aimed at studying the impact of cognitive constraints on foraging behavior [DUKAS & REAL 1993]. The goal is to add to this framework by observing the behavior of experimentally naive bees toward spatial patterns of nectar standing crop.

Mechanistic descriptions should include details of ontogeny: are foragers born knowing how to exploit patches efficiently, or do they acquire such knowledge through learning? Optimal patch use depends on the distribution of prey among patches [IWASA, HIGASHI & YAMAMURA 1981]. If prey distributions do not vary appreciably within and between generations, then natural selection might favor hardwiring of the rules for patch departure. Innate behavior might also be selected for in animals with limited neural capacity. Alternatively, species that forage in unpredictable or changing environments might benefit from retaining the flexibility to learn about reward distributions and adjust their behavior accordingly [STEPHENS 1993, KREBS & INMAN 1994]. However, since most empirical studies have used experienced subjects to test optimality or proximal models, comparatively little is known about patch departure in animals with little or no foraging experience (but see KEVAN & GRECO [2001], HILL, BURROWS & HUGHES [2002]).

The experiments reported in this paper were motivated by observations of nectar-foraging bumble bees visiting *Aralia hispida* (Vent), a perennial plant found in disturbed sites in eastern North America. This plant's flowers, which may be either male or hermaphroditic, are borne in umbels [THOMSON & BARRETT 1981]. Typically, an umbel presents a set of open flowers in a ring. THOMSON, MADDISON & PLOWRIGHT [1982] gave bumble bees 'paired-umbel choice tests', wherein bees were simultaneously offered two picked umbels, each with 12 flowers. One of the umbels had all of its flowers enriched with 0.5 μL sucrose solution, while the other had all of its flowers drained of nectar. Bees that chose the full umbel first probed an average of 14 flowers before departing (all available flowers, then two revisits to emptied flowers); bees that visited the empty umbel first probed an average of two flowers before departing. When bees chose the filled umbel first, they often then moved to the empty umbel; interestingly, the bees then probed an average of over five flowers before departing.

The bees' behavior on the filled and empty umbels when they were encountered first was consistent with ideas from subsequently proposed mechanisms for bee patch departure [HODGES 1985; PLEASANTS 1989, CRESSWELL 1990, KADMON & SHMIDA 1992], models that may be called threshold departure rules: leave an inflorescence when the amount of nectar in either the last flower visited [HODGES 1985, PLEASANTS 1989] or last two flowers [KADMON & SHMIDA 1992] is below a given threshold. In those cases, the bees might have been following a rule such as 'depart after two empties'. However, the bees' behavior on empty umbels immediately after visiting a filled umbel was not consistent with this [PLOWRIGHT et al 1984]; instead, their departure decisions were strongly influenced by experience on the previous umbel. The bees acted as if they expected the standing crop of nectar to be spatially clumped or autocorrelated.

Thomson et al [1982] argued that the standing crops of nectar probably are highly patchy in *A hispida*. If so, then the bees' behavior could be considered optimal, loosely speaking. However, since the subjects in the choice experiments were wild foraging bumble bees of unknown experience, it was not possible to know to what extent this pattern was learned or innate.

In the present study, the departure behavior of experimentally naïve bumble bees is examined, using laboratory experiments designed to simulate the field tests with *A hispida*. Young worker bees that were inexperienced are used, having never encountered either real flowers or patchiness in nectar.

Second, having established during the first experiment that the laboratory bees behaved similarly to those in the field, the methods for testing further hypotheses about the departure mechanism were extended. In all experiments, bees foraged from circular rings of artificial flowers (wells drilled in blocks of Plexiglas). The bees usually searched such patches systematically, moving around the ring without skipping wells. Due to the ring construction, however, they could not necessarily tell when they had returned to the starting point, and indeed, they often revisited previously emptied flowers. Rings were always either filled (all artificial flowers contained nectar) or empty (no nectar in any flowers). Assuming that the bees keep a constant speed as they move around the rings, the well-known Marginal Value Theorem [CHARNOV 1976] predicts that the optimal behavior (of an omniscient bee) is simply to depart filled rings after visiting each artificial flower once, and to never enter empty rings. Allowing for the single constraint that the bee does not know the state of an artificial flower before probing it, the optimal policy would be to always depart a ring after encountering one empty artificial flower, as this event contains all the necessary information about the patch. Observing different patterns could thus allow us to infer aspects of the actual departure mechanism.

2 Material and methods

2.1 Experiment 1: Naive bees on pairs of umbel rings

A colony of *B impatiens* (Cresson 1863) was obtained from Bees Under Glass Ltd of Cantley, Quebec, Canada. Prior to the experiments the bees were kept within their nest box and were fed honey solution, and provided with pollen *ad lib*. The colony was kept in a mesh flight cage within a greenhouse growth chamber. To entice the bees to forage within the flight cage, small plastic Petri dishes filled with 30% sucrose solution were placed in the cage. After the first bees had begun to leave the colony box to forage, the dishes were removed and substituted experimental arrays of artificial umbel pairs. These were rectangles of 5mm-thick Plexiglas, 12.7 cm x 3.3 cm, with 2.0 cm rings of eight wells, each 2 mm in diameter and 3mm deep, drilled near both ends. The edges of the two rings were 4.5 cm apart. To provide visual cues to the location of the wells, a computer graphics program was used to generate radial, daisy-like blue patterns on a black background; these were placed underneath the artificial umbels so that each of the 16 flower centers was underneath a drilled well. A Rainin® digital dispensing pipette was used to place reagent grade 30% sucrose solution (g solute/100 ml solvent) into the wells. In experimental trials, a single worker bee was allowed to enter the cage; if the bee did not begin foraging on the umbel rings within 10 min, the run was terminated. In each trial the focal bee was offered six pairs of umbel rings, with sucrose solution amounts according to the following design: two pairs full–full (all eight wells of both rings of the pair filled with 2 μL sucrose solution), two pairs full–empty, and two pairs empty–empty. This design ensures that all conditional probabilities of ring type encounters (for example, finding a full after an empty ring or vice versa) are equal to one half.

The pairs were set in a 3 x 2 rectangular array with a distance of 25 cm between adjacent pairs. The positions of each pair and the left–right orientation of the full–empty pairs were randomized for each run (die rolls).

Bees were allowed to visit as many pairs as they wished during the trials. Tthe numbers of wells that the bees probed on each ring was recorded. Probing was defined as the bee placing the tongue into the well for a time long enough to be detected by eye. After bees had finished foraging, they were placed back in the colony nest box. Individual bees were marked with small spots of enamel paint on the pile of the upper thorax, in order to prevent repeat visits. Data from 32 worker bees were used.

2.2 Experiment 2: Behavior on triplets of rings

The following experiments were performed with two new colonies of *B impatiens,* kept in an indoor flight room, with the bees foraging within a 1m x 1.5m x 0.5m wooden flight box fitted with a Plexiglas roof. In these experiments, equilateral triangles of Plexiglas containing three of the umbel rings were used, with between–ring and ring diameter measurements identical to those from Experiment 1. The rationale for using triplets of umbel rings was to break the inherent symmetry and concomitant correlation of encountered ring number and interpatch travel distance involved in using pairs (see **Results** and the descriptions below for more details).

2.2.1 Experiment 2A: All rings filled with 4 microliters

In Experiment 1 with pairs of rings (above), a decline in the numbers of wells probed was noted on the second rings of Full–Full pairs (see **Results**); this was a situation not considered in the original field study with *A hispida*. Using triplets allowed us to test whether such an effect would continue for a third ring. During each trial, bees were presented with all three rings of wells in three triplets filled with 4 μL sucrose solution. Data were obtained from ten individual workers.

2.2.2 Experiment 2B: 4 and 2 microliters in filled rings

This experiment tested the effect of two different volumes of sucrose solution on the pattern of visitation in filled rings. During each trial, all three rings of each triplet were filled with either 2 or 4 μL sucrose solution. Ten marked individual workers were allowed to make visits to setups with both volumes. For each individual trial, the volume of sucrose was assigned as 2 or 4 μL at random before each run.

2.2.3 Experiments 2C and 2D: Effect of artificial nectar volume on departure from empty rings

The final two series tested for effects of nectar volume on the number of wells probed on subsequently visited empty rings. In Experiment 2C, the bees were given 3 triplets, each of which had one ring with 4 μL in all wells, one with 2 μL in all wells, and one with no sucrose (but with 3 μL water as a visual stimulus) in all wells. In experiment 2D, all bees were given 3 triplets, each with one ring filled with 2 μL , one with 1 μL, and one with 0 (1.5 μL water as a visual stimulus). The positions of the varied amounts within the triplets were assigned at random before each run in both experiments.

2.3 Handling times associated with artificial nectar volume

The handling times associated with each of the amounts of sucrose solution were measured by recording individual marked workers on videotape. The times were read from the videotape by slow motion replay on a Panasonic V–1656 videocassette recorder, using 0.02–second resolution.

2.4 Analysis of data

For Experiment 1, the numbers of wells probed on empty umbel rings chosen first was compared to the number probed on empty rings after visiting a filled umbel, as in Thomson et al [1982]. This was an *a priori* contrast; an *a posteriori* contrast of the numbers of wells probed in filled rings chosen first versus filled rings visited as the second of a full–full pair was also made.

Experiments 2A and 2B were analyzed via repeated measures ANOVA with ring order as the repeated effect, with the number of wells probed per ring as the response variable. Since bees did not always visit a second triplet, making the overall designs unbalanced, data from the first two triplets were analyzed separately. Individual bees were treated as a class variable, as was volume of sucrose (2 and 4 μL) in 2B. In both experiments, only data from the first two triplets were used in the analysis, because bees often returned to the colony box to unload their gathered nectar before moving to a third triplet. Original counts of probed wells were transformed to square roots prior to analysis.

For experiments 2C and 2D, the numbers of wells probed on empty rings given five possible situations of prior experience on that ring were compared (for example, as the first ring visited, after a $2\mu L$ ring, after a $1\mu L$ ring, after a 2 then a 1 and vice versa for Experiment 2D). Analyses were performed on pooled data for all bees. Comparisons were made by ANOVA followed by multiple comparisons via the GT2 test [Sokal & Rohlf 1981]. Nonparametric rank sum tests were used to examine the numbers of wells probed on the full rings in both experiments. For ANOVAs, the original counts of probed wells were transformed to square roots.

Handling times were analyzed by regressing mean handling time on nectar volume, and by inspection of individual plots of cumulative time as a function of the number of wells visited [Haccou & Meelis 1992]. For the latter, each plot was fitted to the power function $y = x$; if bees tended to slow as they moved around the ring then β would be > 1, making the gain function (energy gained as a function of patch time) decelerating.

3 Results

3.1 Experiment 1: Naive bees on pairs of umbel rings

For the main question of interest, bees probed an average of more than one extra well on empty rings if they had first visited a full ring (**Fig 1**; means 3.4 for empty visited after full versus 2.2 for empty visited first; $N = 28, 84$; $p < 0.001$, Wilcoxon Rank Sum Test). On filled rings, the bees behaved similarly to the wild bees on *A hispida*; they visited just over 10 wells (ie all the wells plus two revisits) before departing ($\bar{Y} = 10.1$, $N = 122$). Interestingly, the bees probed significantly fewer wells on the second rings of full–full pairs ($\bar{Y} = 8.5$, $N = 55$, $p < 0.05$, binomial test for paired responses). The bees also probed fewer wells on filled rings after first visiting an empty ring ($\bar{Y} = 7.2$), although this is based on only 10 observations (bees usually departed full–empty pairs if they visited the empty ring first).

3.2 Experiment 2: Behavior on triplets of rings

3.2.1 Experiment 2A: All rings filled with 4 microliters

In Experiment 1, the bees had probed fewer wells on the second of a full-full pair; here the same trend was seen, and on third rings of triplets the decline continued ($p < 0.0001$, repeated measures ANOVA). The proportional decline in the number of wells probed on successive rings was about 0.8 the preceding number (**Fig 2**).

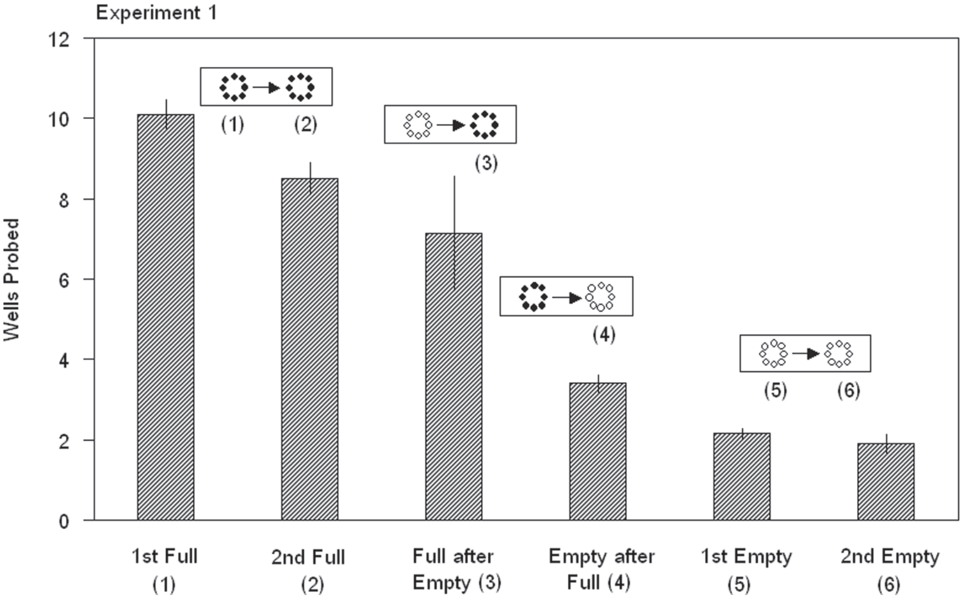


Fig 1: Representative plots of cumulative handling time as a function of the total number of wells probed, shown for individual bees, *Bombus impatiens* (Cresson 1863) – [Hymenoptera: Apidae] for each of the amounts of sucrose solution used in the experiments. The distribution of fitted exponents β for the function $y = x^{\beta}$ for cumulative handling time as a function of total wells is shown in 2j.

On second-visited triplets, bees probed more wells on the first ring than in the last ring of their first triplet, but fewer than on the first ring of the first triplet (**Fig 2**). The number of wells probed again declined for the next two rings ($p < 0.05$, repeated measures ANOVA).

3.2.2 Experiment 2B: 4 And 2 microliters in filled rings

Within the 2*μL* triplets, bees behaved similarly to those from the first colony in Experiment 1: they visited more than 10 wells on the first umbel ring and an average of more than 8 wells on the second ring (**Fig 3**). On the third rings of 2 *μL* triplets bees probed fewer still, as in Experiment 2A ($p < 0.0001$, repeated measures ANOVA). Bees also visited fewer wells on average in the 4*μL* rings than in the 2*μL* rings ($p < 0.001$, between subjects effect of volume, repeated measures ANOVA). There was a significant ring order × volume interaction ($p < 0.05$), suggesting that the pattern of decline differed between 2 and 4 *μL* triplets.

Fig 3: Mean numbers of wells probed (±SE) by bees, *Bombus impatiens* (Cresson 1863) – [Hymenoptera: Apidae] in Experiment 2A, where all rings within wells were filled with four *μL* sucrose solution. Only data from the first two rings of triplets are shown, since bees usually returned to the colony before foraging from a third triplet. Pooled results from ten individual worker bees.

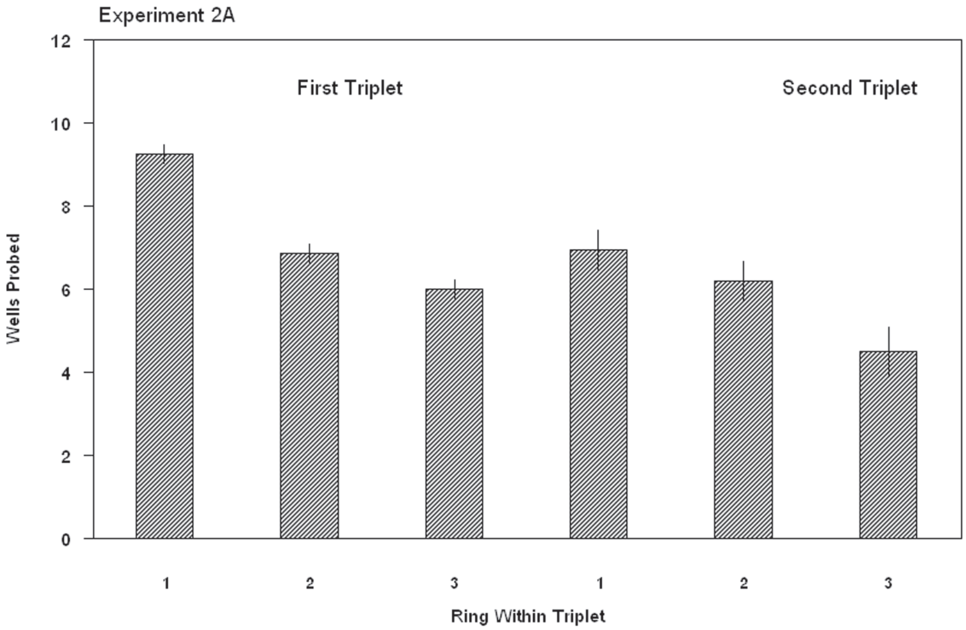
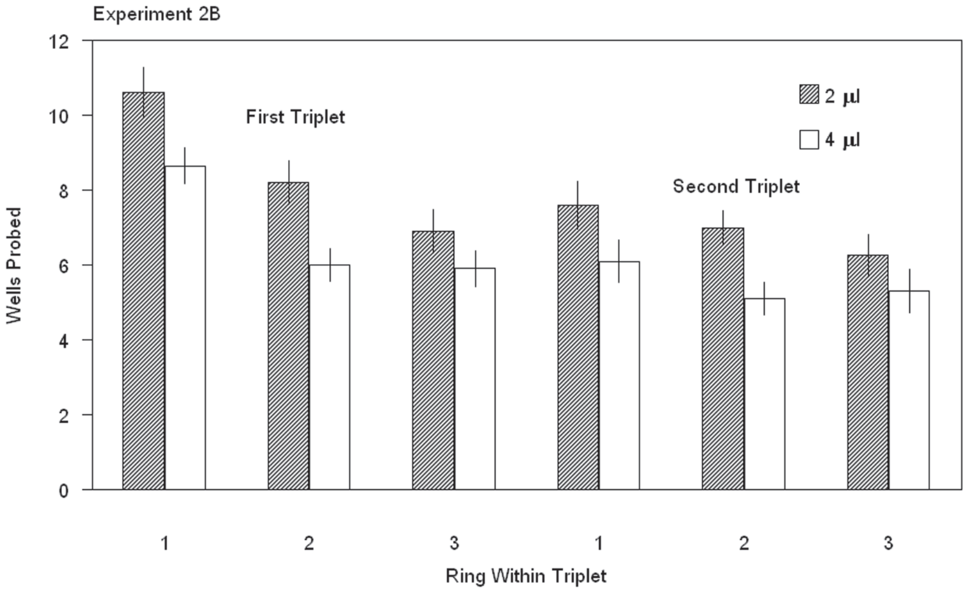


Fig 2: Mean numbers of wells (± SE) probed by bees, *Bombus impatiens* (Cresson 1863) – [Hymenoptera: Apidae] on umbel ring pairs, for all of the possible encounter situations in Experiment 1. The notation for encounter situation is: Full means a filled ring visited as the first of a pair (possible in both full–full pairs and full–empty pairs); Full After Full means the number of wells probed on the second of full–full pairs, Full After Empty means the number probed on full rings of full–empty pairs when the full ring was visited after the empty, Empty After Full denotes the number of wells probed in Empty rings as the second of a Full–Empty pair, etc. Data for Full are pooled for all bees that visited a filled ring first, whether that was the first of a full-full pair or the first of a full-empty pair.



3.2.3 Experiments 2C and 2D: Effect of artificial nectar volume on departure from empty rings

There were five possible encounter situations for the empty ring in both experiments. In 2C (**Fig 4**), bees probed more wells on average in empty rings if they had first visited one or two filled rings (ANOVA; $p < 0.0001$). The mean numbers of wells probed on 2 and 4 *μL* rings were in accord with the results from the two previous experiments. Bees visited slightly fewer wells on average on 4 *μL* rings than on 2 *μL* (respective means 8.8 and 9.4, $N = 72, 84$), although the difference was not statistically significant ($p > 0.25$, Wilcoxon Rank Sum Test). For both amounts, the number of wells probed was greatest when the ring was encountered first ($\bar{Y} = 10.4$, $N = 38$ for 2 *μL*; $\bar{Y} = 10.25$, $N = 28$ for 4 *μL*).

For experiment 2D, the mean numbers of wells probed on the empty rings had a somewhat different pattern (**Fig 5**). As in Experiment 2C, bees always probed more wells in empty rings if they had first visited one or two filled rings ($p < 0.0001$, ANOVA). Among the other four possible encounter situations there were no further groupings detected by the multiple comparisons test. As in Experiment 2C, bees probed the greatest number of wells when the ring was visited first; the mean for 2 *μL* ($\bar{Y} = 10.3$, $N = 28$) compares well with the averages from Experiments 1 and 2C (respective means 10.1 and 10.4).

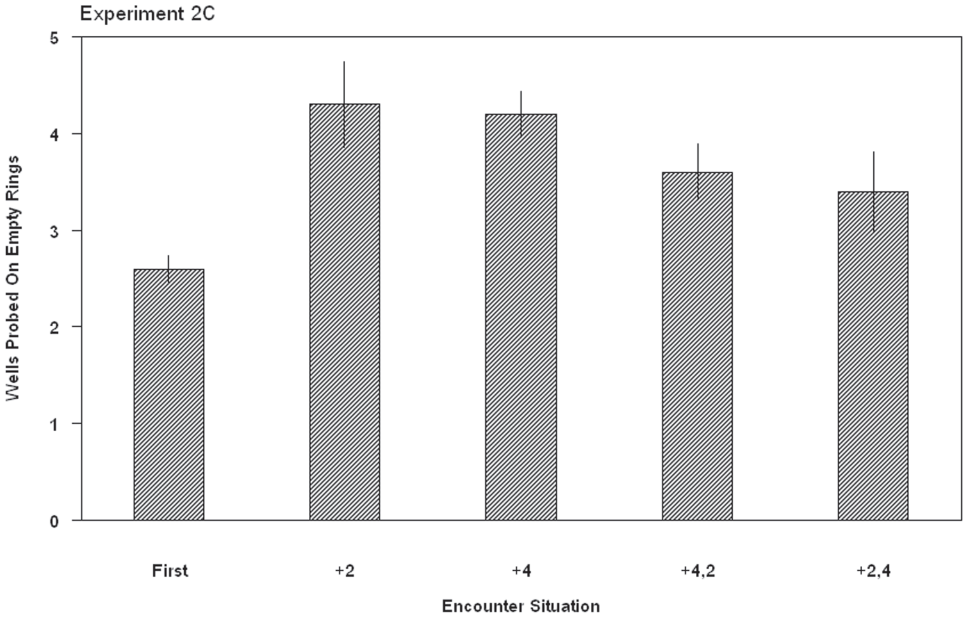


Fig 4: Mean numbers of wells probed (± SE) by bees, *Bombus impatiens* (Cresson 1863) – [Hymenoptera: Apidae] in Experiment 2B, where all rings within wells were filled with two *μL* sucrose solution. Only data from the first two rings of triplets are shown, since bees usually returned to the colony before foraging from a third triplet. Pooled results from ten individual worker bees.

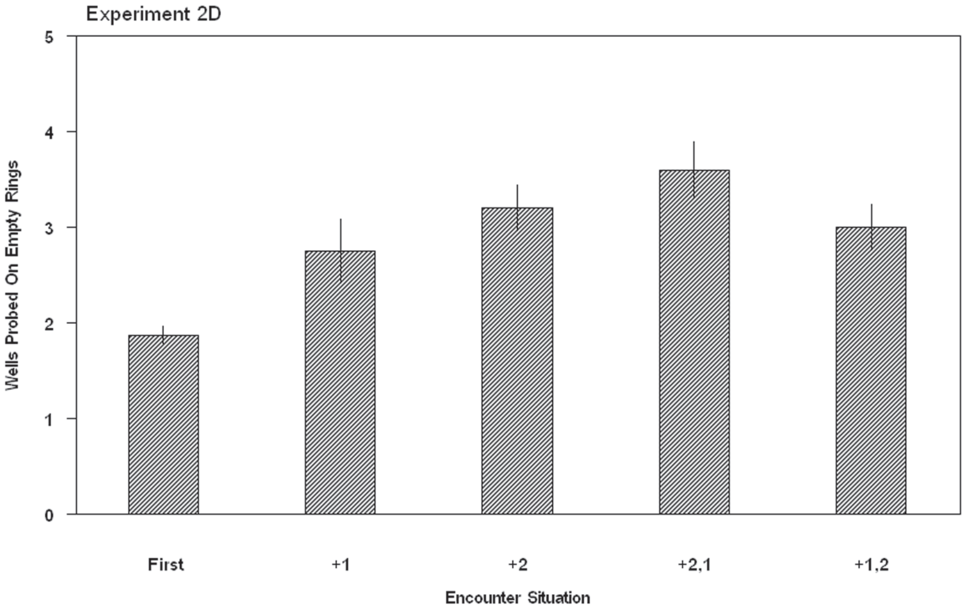


Fig 5: (Top) Mean numbers of wells probed by bees, *Bombus impatiens* (Cresson 1863) – [Hymenoptera: Apidae] on empty rings (±SE) in Experiment 2C. For the encounter situation, First means the mean number when bees visited the empty ring as the first of a triplet, +4 means the empty ring was visited second after the bee had first visited a 4 μL triplet, +2 means that the empty ring was visited second after the bee had first visited a two μL ring, +4,2 means that the empty ring was visited third after the bee had first visited a four and then a two μL ring, and +2,4 means that the empty ring was visited third after the bee had first visited a two and then a four μL ring.

3.3 Handling times associated with volumes of artificial nectar

The mean handling time per well increased linearly with volume ($Y = 1.1 + 0.78X$; $r^2 = 0.41$, units = seconds, microliters). Addition of a quadratic term produced no significant improvement in the fit. Inspection of plots of cumulative handling times for individual bees (**Fig 6**) for each of the amounts of sucrose solution showed that average handling time per well varied among individuals: note, for example, that bee 78 red (**Fig 6a**) worked nearly twice as fast as bee 56 blue (**Fig 6b**) on visits to 2 μL rings. Occasional instances of departure from a linear gain function were seen (by eye), as for bee 25 yellow (e) in a visit to rings containing 4μL. Overall, bees tended to work at a constant rate for all of the amounts of sucrose used in the experiments; of 48 combined plots for 1, 2, and 4 μL, the exponent for the fitted power function was < 1 in 23 and > 1 in 25 (**Fig 6j**).

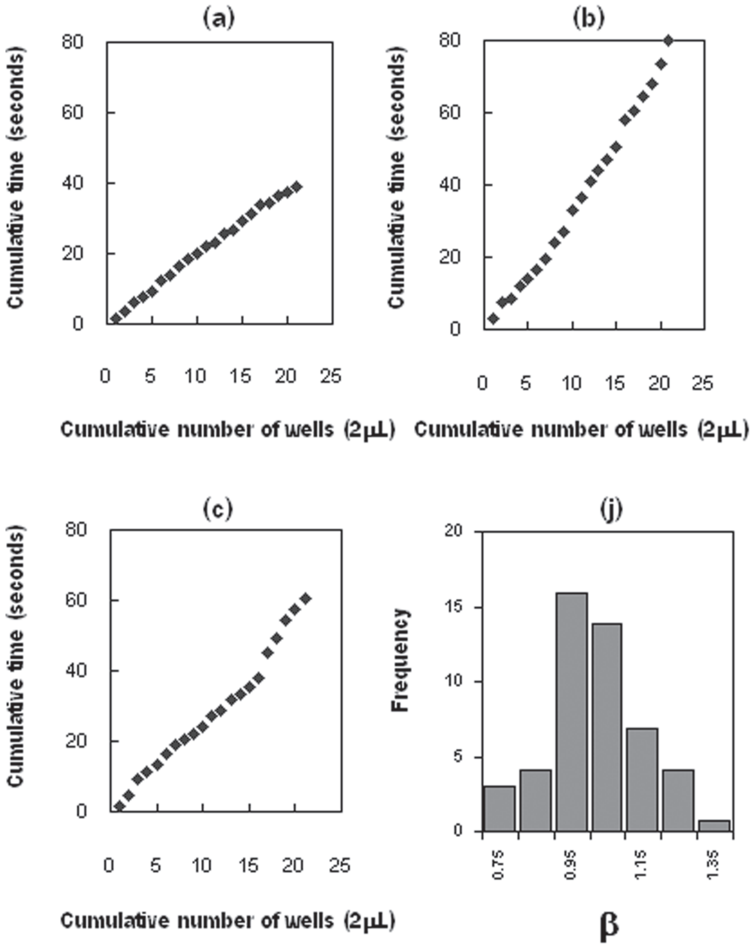
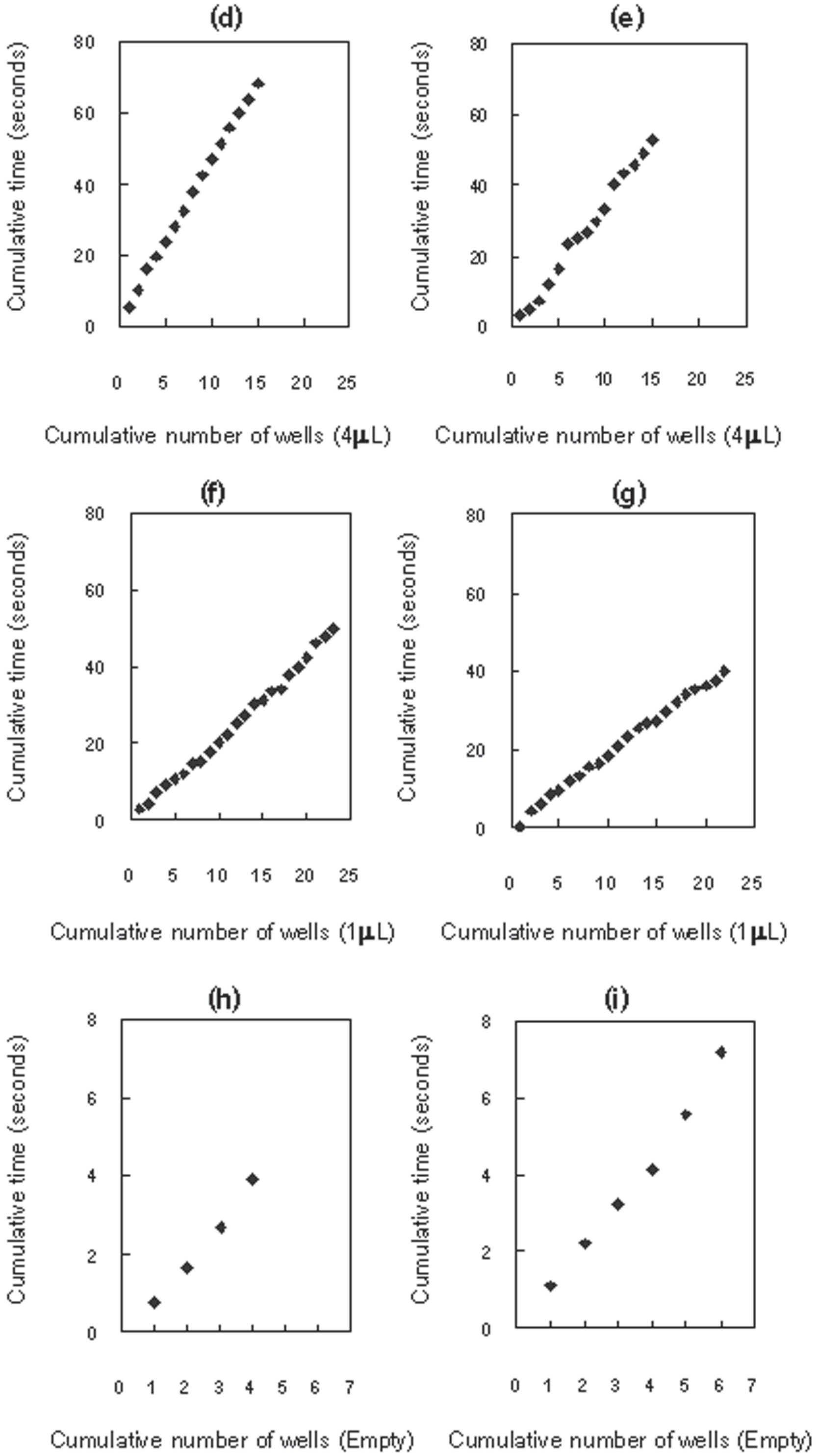


Fig 6: (Top) Mean numbers of wells probed on empty rings (±SE) in Experiment 2D (data pooled for all bees, *Bombus impatiens* (Cresson 1863) – [Hymenoptera: Apidae]. For the encounter situation, First means the mean number when bees visited the empty ring as the first of a triplet, +1 means the empty ring was visited second after the bee had first visited a 1 *μL* triplet, +2 means that the empty ring was visited second after the bee had first visited a two *μL* ring, +1,2 means that the empty ring was visited third after the bee had first visited a one and then a two *μL* ring, and +2,1 means that the empty ring was visited third after the bee had first visited a two and then a one *μL* ring.


(d)
(e)
Cumulative time (seconds)
80
60
40
20
0
0
5
10
15
20
25
Cumulative number of wells (4µL)
(f)
(g)
Cumulative time (seconds)
80
60
40
20
0
0
5
10
15
20
25
Cumulative number of wells (1µL)
(h)
(i)
Cumulative time (seconds)
8
6
4
2
0
0
1
2
3
4
5
6
7
Cumulative number of wells (Empty)

4 Discussion

Two central questions are considered: (1) how did relatively inexperienced bees behave toward patchiness in nectar, and (2) what sorts of departure mechanisms are consistent with the bees' responses to the experimental designs used here? First, it is noted that in Experiment 1, the lab bees behaved much as did bees in the wild [Thomson et al 1982], giving up after visiting two flowers of an empty artificial umbel and revisiting an extra two flowers of a filled artificial umbel. This suggests that laboratory effects were not distorting their normal departure decisions. Many studies designed to investigate proximal mechanisms have used birds in cages [Brunner, Kacelnik & Gibbon 1992] or Skinner boxes [Kacelnik & Todd 1992], or fish in tanks [Wildhaber, Green & Crowder 1994]; often it is not known how the animals' behavior compares to that in the field. Although the bees in this study were not in their natural setting, they were foraging for the same reasons that they would have been in the wild: to obtain energy with which to maintain the colony and raise juveniles.

The experimentally naive bees, like the wild bees, stayed longer within an empty umbel ring if they had first visited a filled ring. Thus it is concluded that bumble bees' apparent expectation of spatial clumping in the distribution of nectar is at least partly innate. It is possible, of course, that aspects of this behavior are modified by learning: for example, the bees in this study visited fewer wells on empty rings after a filled ring than the bees in the *A hispida* study. The numerical difference might be due to features of the distribution of nectar in *A hispida* umbels that bees in the field had learned, or it might be due to any of the many differences in the stimuli that the bees were exposed to in the two studies (amounts of nectar used, physical appearance of the experimental flowers, olfactory cues, or the presence of pollen in *A hispida*). Note, however, that the proportional mean numbers of flowers visited on empty umbels after visiting a filled umbel were very similar (5.56/12 = 0.46 [Thomson et al 1982] vs 3.4/8 = 0.43 [this study]).

The second question of interest concerns the mechanism of patch departure. The behavior of the bees in treatments not considered in the original *A hispida* field experiments showed some unexpected results that, combined with those from the first experiment, provide some insight into how prior experience affects the bees'decisions to depart from flowers.

Bees from all three colonies consistently probed only about eight wells on second umbel rings that contained 2 μL, whether they were the second rings of a filled pair or triplet, or were encountered after an empty ring. A tempting explanation would be that the bees learned the number of wells per ring after one visit to a filled ring. It would not be necessary for them to have learned an actual number; they might simply have recognized that they had returned to their starting point by using visual or olfactory cues. However, this idea is not consistent with all the data. If it is considered that the results from all bees that visited two of the full–full pairs within a foraging bout in Experiment 1, there is little difference: the respective means for first and second rings of first pairs were 10.9 and 8.4, and on second pairs the same means were 10.3 and 8.7 ($N = 18$). If the bees learned that there were eight wells per ring, therefore, they must have forgotten it very quickly, on the order of less than 30 seconds. The same pattern was seen in Experiment 2B; there, individuals for whom multiple visits had been recorded consistently repeated the 10–then–8 pattern, and in Experiments 2C and 2D the bees again visited fewer wells in 2 μL rings if they had first visited an empty ring. In addition, bees from three separate colonies consistently probed about ten wells on the first encountered 2 μL ring (respective means from Experiments 1, 2C and 2D = 10.1, 10.4 and 10.3).

If some form of cue or number learning does not explain the above results, then the cause may involve the form of patch assessment the bees used to make their departure decisions.

In Experiment 1, there were two distances between umbel rings, the long distance between pairs (25 cm) and the short distance between rings within a pair (4.5 cm). The bees departed more quickly after the shorter travel distance, as predicted by foraging theory when rewards within a patch are obtained at a diminishing rate [Charnov 1976, Parker & Stuart 1976]. They did this even though the energy gain function was linear: bees foraged systematically around the rings of wells, and handling times per well did not generally increase within rings (**Fig 6**). This means that the bees should theoretically have stayed the same time in the rings, regardless of the interpatch travel distance. In this respect, the bees resembled the starlings studied by Kacelnik & Cuthill [1986]. Birds foraging from a central place in that study departed from patches more quickly given shorter travel times when energy gain was a decelerating function of patch time, as theory predicts; however, the starlings also departed sooner after shorter travel times when the gain function was linear. In that case, certain factors might have made the gain function that the birds actually experienced decelerating; for example, the energy needed to carry loads home. While this may have been true for bees on the second of a full–full pair, it does not explain why they also visited only about 8 wells on full rings after first visiting an empty ring.

The bumble bees might have used a simple departure rule that related a give-up number or time to interpatch travel distance. In that case the bees would have visited fewer wells on second rings of pairs simply because of the shorter distance between rings within a pair. In the second set of experiments, using triplets, it was possible to test this hypothesis. On the triplets, the bees visited their first ring after a long, intertriangle distance and then two subsequent rings after identical shorter, intervening distances. If the bees based their departure decisions on the interpatch travel distance, then the numbers of wells visited on the second and third rings of the triplets should have been equal in Experiments 2A and 2B. However, the bees consistently visited fewer wells on the third rings.

The changes in the number of wells visited from first to third rings in Experiments 2A and 2B suggest that departure decisions were influenced by a declining factor operating between successive rings. Since the bees increased the number of wells probed on the first ring of a second triplet relative to that of the third ring of the first triplet, the decline was not due to storage constraints, ie the bees having filled their honey stomachs. Note that in many cases the bees departed from the rings before visiting all available wells, in rings that contained both 2 and 4 microliters sucrose solution. This may in part be due to a decline in the within-ring rate of nectar uptake that is difficult to observe; within rings, each individual may have reached a point at which rate of delivery of nectar from tongue/esophagus to the honey stomach slowed temporarily, making the bee depart immediately without probing further wells. In that case, the handling time for the last well probed may have varied little from those for previous wells. To test this it will be necessary to use a more sophisticated monitoring system, one in which rates of nectar uptake can be measured on a very small time scale. Since there may also have been carryover effects between rings, as in Experiment 1, it is difficult to separate these effects. What the behavior on the filled rings does show is that whatever caused the sequential decline in the number of wells probed, it was fairly constant in effect, since the proportional decline in the number of wells probed remained constant within each triplet.

The behavior on empty rings (Experiments 2C and 2D) is easier to interpret, since any differences in the numbers of wells probed between treatments must be due to carryover effects from other rings. Both experiments strongly suggest that experience in previous patches influenced the bees' departure decisions. This is consistent with thought about how animals' cognitive architectures can be incorporated into patch models [Cuthill et al 1990, Kacelnik & TODD 1992, Todd & Kacelnik 1993, Cuthill, Haccou, & Kacelnik 1994].

The results reported here suggest that both nectar volume and the order of encounter were important.

The way in which nectar volume affected the numbers of wells probed on subsequent empty rings was apparently not a simple summation of the individual effects of volume. Each of the amounts of sucrose that were used increased the number of wells probed on a following empty ring; apparently the effect saturated near 2 μL, since there was no difference between the numbers of wells probed on empty rings after either a 2 or 4 μL ring. However, the bees actually probed fewer wells on empty rings if they had previously visited two such rings. As noted above, the gross rate of energy expenditure may have been greater on empty rings when the bees had just finished gathering such a relatively large amount of nectar (48 μL total after visiting a 2 and a 4 μL ring, which is roughly half the capacity of most workers). However, this is not consistent with the results from Experiment 2D, in which bees probed more wells after first visiting a 2 and then a 1 μL ring than in any other situation. The fact that the bees probed fewer wells on empty rings after visiting two filled rings in a row suggests that the same decline in response seen in experiments 2A and 2B was operating.

The asymmetry between the final two experiments suggests a pattern in the temporal weighting of past experience. In Experiment 2D the bees probed more wells on empty rings after visiting a 2 μL and then a 1 μL ring than vice versa. There was no such asymmetry in 2C with 2 and 4 μL. This implies that the carryover effect from previous rings on departure number in empty rings saturated at around 2 μL. If so, assuming that the carryover effect was greater for 2 μL than for 1 μL, then the earlier experience was apparently weighted more in the effect on the subsequent empty ring. Such a temporal weighting would have had no effect on the number of wells probed on empty rings in experiment 2C, assuming that the separate effects from 2 and 4 μL were approximately equal.

Taken as a whole, the results did not support the threshold departure rules [HODGES 1985, PLEASANTS 1989] that have been proposed as the mechanism underlying patch departure in bumble bees. The bees probed up to four wells before departing empty patches, with the number depending on the type of patches encountered beforehand. Thus, the data suggest that the mechanism is more akin to what has been seen in studies with birds or parasitic wasps: departure decisions apparently involve a form of patch assessment that depends on past events, and is more complex than a simple summation of experience in previous patches [WAAGE 1979, TODD & KACELNIK 1993, CUTHILL et al 1994].

The most important aspect of the results, with regard to future research, is the constancy and repeatability of the departure decisions. Individual worker bees from three separate colonies visited about ten wells on their first 2 μL ring, for example, and this did not change with experience. If the eventual goal of research into mechanisms of patch departure is to produce a predictive model, the numerical constancy of bees' responses in the system used here is encouraging. Since bees in this laboratory situation behave as bees do in the field, the system holds promise for understanding how bees in nature make their departure decisions.

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