

Learning in two contexts: the effects of interference and body size in bumblebees

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Summary

We examined the effect of learning a new task on the performance of a previously learned task with the same set of visual cues in bumblebees, *Bombus impatiens*. Previous studies have shown that given a binary choice at each task, bumblebees do not show retroactive interference, or mistakes in the first task, if the two tasks are in different contexts, feeding and nest location. Here we tested whether adding a third, unrewarded choice to each task affects the performance of bees learning in two contexts. In addition, we examined whether workers differ in their expression of interference and learning ability based on size. Performance of workers at a feeder task was degraded by the introduction of training to a second task at the nest entrance. Mistakes at the feeder were biased toward the color cue that was not rewarding in both tasks; suggesting that irrelevant or background stimuli are more prone to decay or forgetting during

interference. With respect to interference, we did not find an effect of body size on the amount of interference; however, size was related to how quickly interference occurred. Among individuals showing retroactive interference, larger bees showed interference earlier in phase 2 than did smaller bees. Overall, larger workers learned each task more rapidly than smaller workers. We conclude that the timing of interference is a tradeoff between acquisition of the new task and performance at a previously learned task. Given that foragers in nature tend to be larger than nest workers, we suggest that size-related learning differences be considered as a factor in division of labor between large and small bumblebees.

Key words: interference, learning, bumblebee, *Bombus impatiens*, body size, contexts, foraging.

Introduction

Individual insects can learn and remember an impressive number of cues or motor patterns more or less at the same time (Gould, 1987; Srinivasan et al., 1998). For instance, honeybees can learn to approach a petal in one orientation to get a reward at one time of day and a petal in a different orientation at a different time of day (Gould, 1987). However, insects also behave in ways suggestive of constraints on learning or memory. For example, pollen or nectar foraging insects often show fidelity to flowers of one or a few plant species even though flowers of other species are equally or even more rewarding, a phenomenon known as flower constancy (Waser, 1986). Because constancy increases travel time between flowers, it is potentially costly, and is often explained as a product of learning and memory constraints that limit the number of flower types an individual can efficiently learn and handle (Proctor and Yeo, 1973; Waser, 1983, 1986; Lewis, 1986, 1989; Woodward and Laverty, 1992). Some limited evidence supports this explanation; in bees and butterflies, learning to forage on a novel floral type degrades performance on a previously learned type (Lewis, 1986; Chittka and Thomson, 1997). Errors in a previously learned task caused by learning of new associations with similar stimuli, termed retroactive interference (RI), is thought to be a major cause of

forgetting in humans and other animals (Keppel, 1968; Bouton, 1993; Anderson, 2003; Wixted, 2004). For this paper we consider forgetting as either a problem of permanent decay of information or a more temporal problem of failure to retrieve the appropriate information at a given time. Chittka and Thomson (1997) found RI, or mistakes in the first learned task, in bumblebees trained separately on two tasks in successive blocks.

If constancy to a floral type can be interpreted in terms of learning and memory constraints, perhaps so too can the tendency that insects have for partitioning their activities in time or among individuals. Many solitary insects, for example, segregate activities, such as feeding and egg-laying, into extended bouts so that different activities are not mingled in time. Social insects are further characterized by a division of labor, in which a given individual within the colony engages in a limited set of activities, such as foraging or nest maintenance, at any one time in its life. In honeybees, foragers additionally specialize in either pollen or nectar collection, although some mixing of these tasks occurs (Page and Fondrk, 1995). In general, division of labor between individuals in social insect colonies can be considered to limit the number of tasks an individual must learn, possibly in part as a response

to problems in learning and memory. Division of labor notwithstanding, even within an activity, such as nectar foraging, individuals must necessarily learn multiple tasks intermingled closely in time. Nectar-foraging bees learn the location of rewarding patches and species of flowers, the location of the nest or hive, the color, odor, shape and texture of rewarding flowers, and the means by which to extract nectar successfully from flowers of diverse morphology (Gould, 1990; Giurfa and Nunez, 1992; Gerber et al., 1996; Scheiner et al., 1999).

In the present study, we were interested in determining whether learning of multiple visual cues associated with nectar source and nest entrance location posed a challenge to bees in terms of learning and memory. In a study bearing on this issue, Colborn et al. (1999) found no RI when bumblebees learned similar cues (patterns of lines of differing orientations) first at a feeder and subsequently at the nest box entrance. The contrast between their results and those of Chittka and Thomson (1997), where both tasks involved nectar sources, led Colborn et al. (1999) to conclude that differences in the context in which the two visuomotor associations were learned prevented interference. For instance, the distinctive locations of feeder and nest may have provided contextual cues that facilitated retrieval from memory of the pattern pertinent to a particular task. Even in Colborn et al.'s experiments, however, there were indications of possible interference between tasks: while error rates at the feeder did not increase after the competing association began at the nest, hover time in front of the feeder rose, possibly indicating some confusion about learned cues.

The Colborn et al. (1999) study presented bees with a binary choice for each task, one cue rewarded in one task, the other rewarded in the alternative task. A binary choice design of this type has the potential to detect interference if an animal tends to confuse which training cues were associated with which task. However, interference may take another form that is not readily detected by a test that includes only the cues trained in the two tasks. Animals trained to a given task may be able to withhold responses to cues learned in an alternative task but less able to withhold responses to novel, non-rewarded cues. In the present study, we asked whether interference occurred in a design similar to that of Colborn et al. (1999) but that involved a trinary choice, the two visual cues used in the two tasks and a novel non-rewarded visual cue. Because multiple types of mistakes were possible in either context, we were able to examine whether interference affected rewarded and non-rewarded cues equally.

To the extent that learning of multiple tasks during foraging posed a challenge in terms of learning and memory, we were secondarily interested in whether bumblebees organized their division of labor to minimize any such challenge. In bumblebees, division of labor is correlated with body size, with foragers being larger, on average, than nest workers (Goulson et al., 2002). Among foragers, size is positively correlated with foraging efficiency (Goulson et al., 2002) and foraging rate (Spaethe and Weidenmüller, 2002). Among the various activities in which worker bees engage, foraging is a

particularly complex task. We wondered if division of labor in relation to size is a strategy to cope with problems of learning and memory within the complex task of foraging. If so, larger bees should be better at learning nectar source cues and should experience fewer problems of interference between nectar source and nest location tasks. We therefore examined visual cue learning and interference with respect to size. In doing so, we provide one of the only assessments, to our knowledge, of size-related differences in learning and interference in animals.

Materials and methods

Bees

Bumblebee (*Bombus impatiens* Cresson) colonies were obtained from Koppert Biological Systems (Michigan, USA). Individual foragers were measured for two morphological traits, head width and radial cell length, and marked with a unique number. The colony was provided sucrose solution and free pollen when not being used in experiments. The pollen was placed directly into the nest, and bees were allowed to forage freely at sucrose-saturated wicks at a feeder station.

Apparatus and pre-training procedure

Our foraging assays took place in an experimental foraging arena (120 cm × 75 cm floor area and 36 cm high) constructed of plywood sides and bottom and a top of clear plexiglas (Fig. 1A). The plywood interior of the arena was painted gray. Foragers had access to the arena *via* a holding box with a swinging door. By manipulating the door, bees could be released singly into the foraging arena during experiments. The arena was illuminated overhead by two 500 W halogen lamps suspended 40 cm above the arena and by a dual-element 40 W fluorescent lighting fixture (Sylvania) mounted 110 cm above on the ceiling of the observation room.

Before training to colors began, foragers fed freely in the arena at three feeder holes arranged in a triangle on the arena wall (Fig. 1). Feeder holes consisted of sucrose-saturated wicks inserted into wells of 1.4 cm i.d. vinyl tubing. Braided cotton dental wicks (Richmond Dental) were 2.3 cm diameter and extended out from the holes approximately 5 cm into the arena. Bees were permitted to return to the nest through a single hole (2.3 cm diameter) in the wall at the other end of the arena (Fig. 1). This pre-training stage familiarized bees with the arena and the two contexts.

Training colors

Training colors and the colored background against which they were presented were cut from standardized color papers of the HKS-N-series (Hostmann-Steinberg K+E Druckfarben, H. Schmincke & Co., Erkrath, Germany). We chose colors that had been studied by Gumbert (2000) and mapped in the color-opponent-coding diagram model (Backhaus, 1991) for bee color vision. We used the following three colors for training at either the feeder or the nest entrance: HKS 46n – human light blue, HKS65n – human light green, and HKS6n – human orange. We used HKS58n – human green, for the background

surrounding the training colors. Gumbert (2000) showed that bumblebees can easily distinguish the training colors, and further that the three training colors were relatively equal in color distance from the background green under laboratory conditions.

Phase 1: training to feeder color only

Training consisted of two phases. Phase 1 involved training to a color at the feeder only. In phase one, the arena had one hole through which a bee could exit or enter the nest located on one wall at one end of the arena and the three holes that held feeder tubes at the opposite end (Fig. 1B). A 5 cm diameter circle of colored paper surrounded each feeder hole, and the three circles were arranged against a colored background consisting of a 26 cm × 20 cm rectangle.

Bees were randomly assigned to a rewarding color (light blue, light green, or orange); the remaining two colors were non-rewarding. Bees could feed *ad libitum* at any wick of their choosing. The wick for the rewarding color was saturated with 20% sucrose solution; the other two wicks dispensed distilled water. To prevent bees from developing a side preference, the orientation of colors at the feeder was changed every trial in a random fashion. The colored circles and background paper were covered with an acetate sheet to prevent transfer of odor cues to the paper. We cleaned the sheet with water between trials.

A bee was determined to have made a 'correct' choice at the feeder when its first landing upon leaving the nest in a given trial was at the rewarded wick and it began to feed. A trial ended when the bee returned to the nest. For a bee to move on to phase 2, it had to have completed a minimum of eight trials, and to have been correct on at least four out of its last five ($\geq 80\%$) trials. Bees were removed from the experiment if they did not meet the 80% criterion by the 20th trial.

Phase 2: training to feeder and nest entrance

Phase 2 involved continued training to a color at the feeder combined with initiation of training to a different color at the nest entrance. Phase 2 began immediately after phase 1. In phase 2, the nest hole used in phase 1 was concealed and three surrounding nest holes arranged in a triangle were opened (Fig. 1B). Colored circles identical to those used at the feeder holes were placed around each of the three nest holes. Bees that had been assigned to one rewarding color (light blue, light green, or orange) at the feeder were now randomly assigned one of the two remaining colors as rewarding at the nest entrance. The third color was not rewarded in either task. The two non-rewarding holes at the nest entrance contained tubes that were blocked by a transparent acetate covering that prevented bees from entering the nest. The orientation of colors at the nest was changed every trial. A total of five bees were assigned to each of the six possible combinations of rewarding

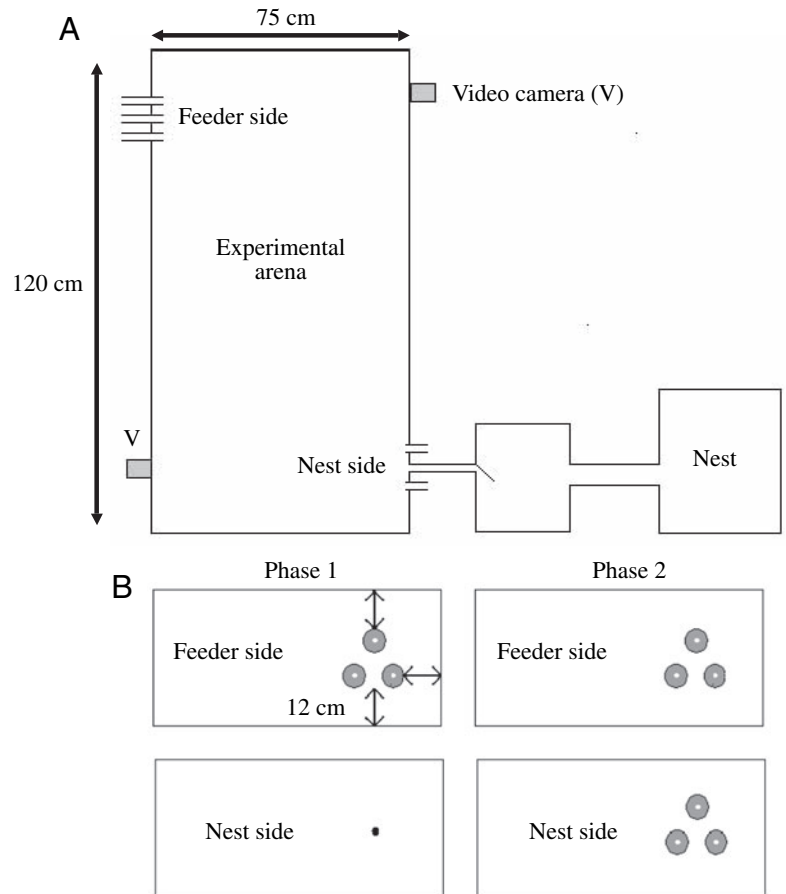


Fig. 1. Apparatus. (A) Plan view of the apparatus. Bees entered the experimental arena *via* a transparent tunnel that could be closed. Video cameras (V) recorded activity at both the feeder and nest. (B) Plan view of the feeding and nest areas during phase 1 (single context) and phase 2 (dual context). During phase 1, three choices with three colors (green, blue, orange) were present at the feeder area and only one, background colored hole was present at the nest area. In phase 2, both feeder and nest had three choices cued by three different colors (green, blue, orange).

colors. Test bees were allowed to forage for eight feeding/nest entrance trials in phase 2, a trial defined by a bee entering the foraging arena, feeding at the feeder holes, and returning to the nest.

Control experiment

In the previous experiment, a test bee was deemed to have learned the rewarded color in phase 1 when four out of five consecutive landings were on the correct color. It is possible that having this criterion at the end of phase 1 but, obviously, no selection criteria during phase 2, increased the likelihood of observing more mistakes during phase 2 even if interference was not a problem. To determine if this was the case, we ran a second experiment in which we directly compared performance between bees that were continuously trained in one context (feeder) only with bees that trained in the second context (nest entrance) during phase 2. Bees were trained at the feeder until they met a minimum of five trials and had been

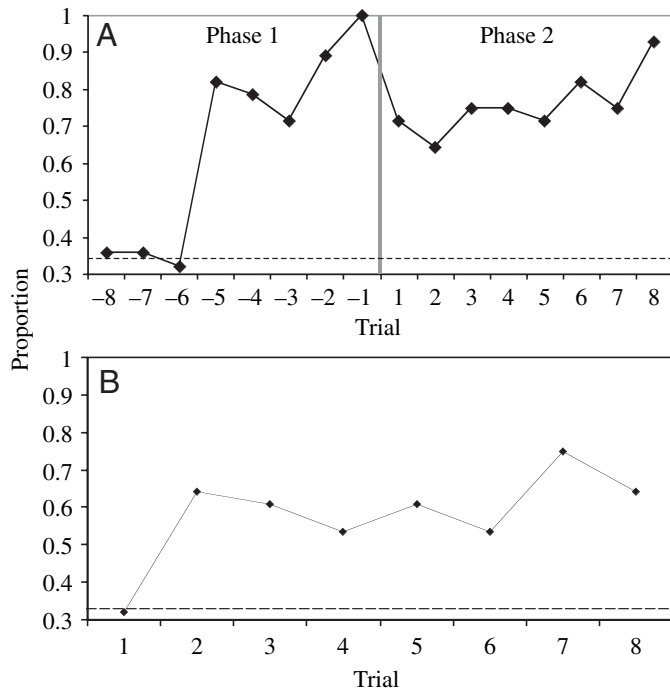


Fig. 2. First choice of color (A) at the feeder, and (B) at the nest. Graphs plot the proportion of bees (out of 30) that made the correct choice in each trial. The dashed line represents no choice or chance expectation (33%). Solid gray line divides phase 1 and 2. Phase 1 is represented by trials -8 to -1, and phase 2 by trials 1 to 8. The last eight trials are graphed from phase 1.

correct on their last four of five trials. Bees were then randomly assigned to single-context training or two-context training during phase 2. Single context training consisted of eight trials performed in the same manner as phase 1 (training only at the feeder). Two-context training involved the addition of color cues and training at the nest entrance. Because the previous experiment indicated that results did not depend on color combination, we only tested a single color combination (feeder S+, orange; nest S+, blue).

Odors

Because bumblebees use scent marks left on flowers as landing cues (e.g. Goulson et al., 1998), visual learning studies in bees must address the concern that innate responses to odors rather than learned responses to colors account for results obtained. For several reasons we feel that this is unlikely to be the case. First, feeder wicks were changed between trials so odors deposited on them should not affect subsequent choices. Second, the positions of the colors in the array were changed across trials. Hence, any odors left on the acetate sheet would not be a reliable cue for reward, and bees using odor as a landing cue could not have improved their performance during phase 1 as a consequence. Third, non-visual cues at a particular location could not account for the interference pattern we found. In particular, there is no reason to expect 'single context' bees in the control experiment to perform better in phase 2 than 'two

context' bees because of odor cues, as there was no difference in odors at the feeder between the two treatments.

Data collection and analysis

Landings measurement and analysis

The feeder array was videotaped during each trial in both phases by a video camera located directly opposite the feeder hole array (Fig. 1). A second video camera was placed opposite the nest hole array for phase 2 and all trials recorded. From the videotapes, we scored the bee's first landing at either the nest holes or feeder wicks. All landings that a bee made during a trial were scored for the last two trials of phase 1 and the first two trials of phase 2.

In all analyses, we pooled data across color combinations, having discerned no obvious effect of color combination on either acquisition or interference. As a statistical check that bees meeting our 80% criterion had actually learnt the task at the feeder during phase 1, we counted the number of correct choices all bees made during the last five trials of phase 2. We applied a binomial test to determine whether the choice frequency pooled across bees was significantly different from 33% (random choice).

To determine whether the new learning of nest entrance color in phase 2 interfered with performance at the feeder, we asked whether or not an individual bee's performance at the feeder at the beginning of phase 2 was worse on average than its performance at the end of phase 1. We first generated bins of trials consisting of one, two, three, four and five trials on either side of the transition from phase 1–2, and computed performance (= total number of correct choices) within each bin for all test bees. To each pair of bins of matched size, we applied a matched-pairs *t*-test to compare performance before *versus* after the nest entrance task began. We set five trials as the upper limit because our criterion for learning at phase 1 was set at 80% correct during the last five trials at the feeder. In longer bins, bees may not yet have learned the feeder context during phase 1, according to our 80% criterion.

To examine differences among categories of colors in landing frequency at the feeder during the transition from phase 1 to phase 2, we used matched pair *t*-tests. In analyzing all landings a bee made, we examined the trials where mistakes were the greatest, the first two trials of phase 2 (see Fig. 2). Fig. 2 shows that the drop in performance during phase 2 bottoms out at the second trial. Therefore, we made the decision to analyze landings and hover times only for the four trials that immediately bracketed the switch from phase 1 to phase 2 (that is, two trials before the switch and two trials afterwards). We calculated the proportion of all landings that were made at three categories of color: the feeder-rewarded color, the nest-rewarded color, or non-rewarded colors. An arcsine transformation was applied to the proportions data before analysis.

Hovering time measurement and analysis

We also recorded the amount of time that a bee spent hovering before a colored circle at either the feeders or nest

Table 1. Pairwise comparisons of performance at the feeder during x number of trials before and after the introduction of the nest entrance task (phase 2)

Number of trials (x) on either side of phase transition	Mean difference*	S.E.M.	t -statistic	P
1	-0.30	0.08	-3.5	0.001
2	-0.57	0.16	-3.6	0.001
3	-0.57	0.23	-2.5	0.009
4	-0.63	0.26	-2.4	0.02
5	-0.73	0.30	-2.5	0.01

*Mean difference is average number of correct choices in phase 2 minus the average number of correct choices in phase 1. $N=30$.

entrance. We defined hovering as the time a bee spent facing the array while in flight when it was within approximately 15 cm directly in front of a colored disk. As with landing frequencies, hovering time at feeder holes was analyzed for the last two trials of phase 1 and the first two trials of phase 2. Hovering time at the nest entrance was analyzed for the first two trials of phase 2. For each of these trials at each location, we calculated the relative hovering time for each color, defined as the amount of time spent hovering in front of a colored circle divided by the sum of hovering times for all circles within a given context. Data were then grouped as relative hovering time in front of the rewarded color and relative hovering time in front of the two possible non-rewarded colors. Time at the non-rewarded colors could be further subdivided as to whether the colors were rewarding or non-rewarding in the other context.

To examine differences in hovering times at the feeder during the transition from phase 1 to phase 2, we used a t -test. Hovering times were converted to proportion of time hovering in front of a particular color out of all time hovering in front of holes at a given task. We applied an arcsine transformation to the proportions data before analysis.

Body size measurement and analysis

We examined the effect of body size on learning and interference, using head width as a proxy for body size. Upon completion of phase 2, bees were frozen. Their head width was subsequently measured using digital calipers. We examined pooled performance of all bees at the feeder during phase 1. We used the point at which the pooled performance of all bees was significantly greater than chance (33%) as the trial for measuring overall performance of each bee. As a measure of an individual's rate of acquisition, we used the number of correct choices at the feeder during these trials. We then used correlation analysis to test for an association between head width and number of correct choices.

As a measure of an individual's expression of interference, we scored all test bees' performance at the feeder for bins of trials consisting on one, two, three, four and five trials on either side of the transition from phase 1–2 as discussed above. Bees

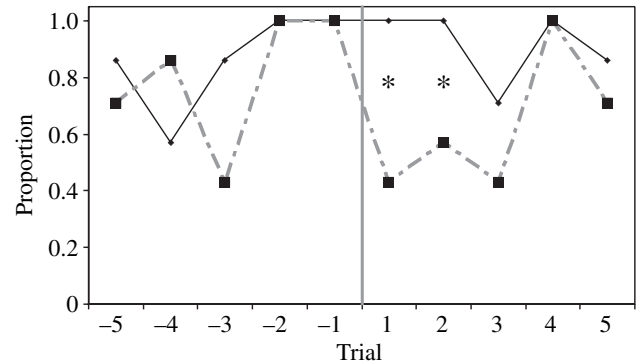


Fig. 3. First choice of a color at the feeder for control experiment. Graph plots the proportion of bees (out of seven) that made the correct choice in each trial. The solid line represents bees that were trained to only a single context (feeder) and the dashed line represents bees trained to two contexts (feeder + hive) during phase 2. The last five trials of phase 1 (-5 to -1) and the first five trials of phase 2 (1–5) are shown. Solid gray line divides phase 1 and 2. *Indicates significant difference (see text for statistics).

were categorized by their performance as either having performed worse (showing interference) or the same/better (not showing interference) in phase 2 compared with phase 1. Head widths were compared between these two categories of bees using a t -test.

Finally, we assessed the latency to expression of interference, in relation to head width. Latency was measured as the trial in phase 2 in which a bee first made a mistake at the feeder. We tested for a correlation between head width of bees and the latency to expression of interference.

Results

Learning in two contexts

In this experiment, 30 bees (five for each possible feeder/nest color combination) were trained through phases 1 and 2. Bees learned to associate the correct color at the feeder with the sucrose solution, as indicated by the progressive improvement in correct choices over trial number [Fig. 2A; binomial test on last five trials of phase 1, 126/150 trials (84%) correct, $P<0.0001$]. Bees took varying numbers of trials to reach the 80% criterion; for convenience, only pooled data for the last eight trials in phase 1 are shown in Fig. 2A.

For bees that met criteria, introduction of color cues at the nest entrance diminished performance at the feeder, indicative of interference (Fig. 2A). The number of correct choices was consistently smaller at the beginning of phase 2 than at the end of phase 1, independent of how many trials were included in the analysis (Table 1). The control experiment verified the finding of interference, as bees learning a color in a second context made more mistakes than bees that were trained only to the feeder context at the beginning of phase 2 (Fig. 3). Performance between bees trained to one ($N=7$) and two ($N=7$) contexts differed only for trials 1 and 2 of phase 2 (t -tests: $t=2.8$, $P=0.02$; $t=2.1$, $P=0.05$, respectively). If the criterion for

success in phase 1 (4/5 correct) inflated performance estimates, we would expect the performance of bees trained in only a single context (control) to decline at the beginning of phase 2. Our results do not support this as single-context learners did better on average (but not significantly, $P>0.30$) in the first five trials of phase 2 than they did in the final five trials of phase 1.

We analyzed the landing mistakes made at the feeder during the first two trials of phase 2 to determine whether bees were biasing their mistakes toward a particular color category (Fig. 4A). During the last two trials of phase 1, bees landed relatively equally on the two possible incorrect colors ($4.5\pm 8.9\%$ at the color that would be rewarding at the nest in phase 2 vs $6.1\pm 13.3\%$ at other color; matched-pairs t -test; $t=-0.58$, $N=30$, NS). However, landing mistakes (Fig. 4B) were significantly biased toward the color not rewarded in either context once the nest context was introduced in phase 2 ($10.2\pm 13.4\%$ at nest color vs $17.4\pm 16.5\%$ at the other color; matched-pairs t -test; $t=-2.5$, $N=30$, $P<0.05$).

Although not significant, bees tended to hover before the feeder holes longer overall at the beginning of phase 2 compared with the end of phase 1 (t -test on difference $\neq 0$,

$t_{29}=1.8$, $P=0.08$). The difference in hovering time was related to a bee's propensity to make a mistake at the feeder in phase 2. The 14 bees that made mistakes during the first two trials of phase 2 hovered 3.75 ± 5.76 s longer in phase 2, compared with the slight decrease in mean hovering time (0.25 ± 2.26 s) shown by the 16 bees that did not make a mistake in the beginning of phase 2 (t -test on difference, $t_{28}=-2.486$, $P<0.05$).

At the nest, bees learned to make the association between the color and nest hole within the eight trials [Fig. 2B; Binomial test on last five trials, 99/150 (66%) correct, $P<0.0001$]. As with mistakes at the feeder (Fig. 4), mistakes at the nest could be divided into two groups: landings on the color rewarded in the other context (feeder) and landings on the color not rewarded in either context (other). In contrast to results at the feeder, landing mistakes at the nest entrance were biased not toward the non-rewarded color but toward the color rewarded at the other location (Fig. 5; feeder color, $24.9\pm 17.0\%$; other color, $14.9\pm 14.4\%$; $t_{29}=2.16$, $P<0.05$). Similarly, mean relative hovering time near incorrect colors was biased toward the feeder color (feeder color, $36.0\pm 12.6\%$; other color, $23.3\pm 12.9\%$; $t_{29}=3.1$, $P=0.005$).

Effect of bee size

Not all bees that began the first phase made it to phase 2, and the rate of acquisition showed substantial variation between bees. Analysis of the pooled data indicated that bees made the correct choice significantly greater than chance (33%) by the seventh trial in phase 1 [binomial test; 21/39 (54%) correct, $P=0.02$]. To determine the relationship between size and learning, we examined the performance of all bees through the seventh trial of phase 1. Head width was positively correlated with the number of correct choices during this stage of phase 1 (Fig. 6; $r_p^2=0.11$, $N=39$, $P<0.05$).

Head widths were compared between bees that performed worse or same/better during phase 2 for comparisons of 1–5 trials on either side of the transition from phase 1–2 (Table 2). In general, performance differed by size for comparisons

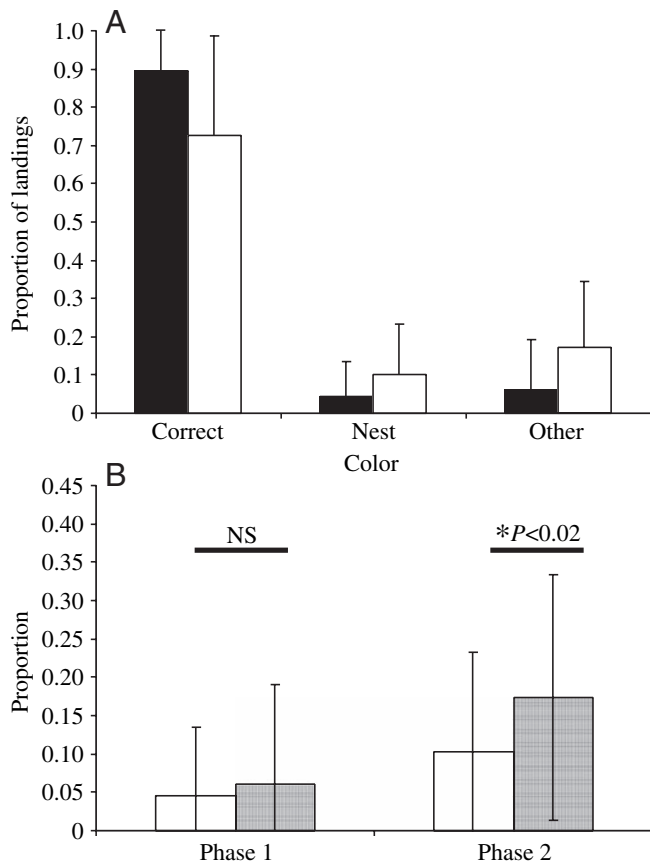


Fig. 4. The proportion of landings at the feeder during the last two trials of phase 1 and the first two trials of phase 2. (A) Landings at either the color rewarding at the feeder (correct), color rewarding at the nest (nest), or color not rewarding in either context (other) for phase 1 (solid bars) and phase 2 (open bars). (B) Landing mistakes at the nest color (open bar) and other non-rewarding color (hashed bar).

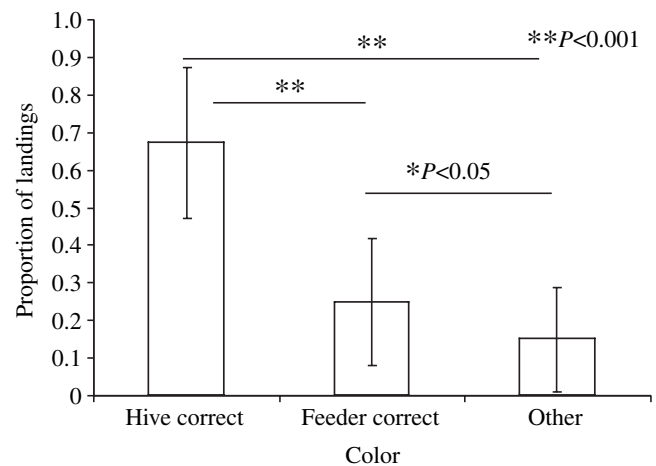


Fig. 5. Proportion of landings at the nest during phase 2. Colors choices were either rewarding at the nest (correct), rewarding at the feeder (feeder), or not rewarding in either context (other).

immediately before and after the introduction of the second context (small bin sizes), but the difference disappeared as the bin size increased. The strongest size difference occurred for comparisons of two trials on either side of the transition, indicating that larger bees showed more interference during this timeframe.

The previous analysis suggests that the timing of interference is affected by a bee's size. To further explore this effect, we examined the latency to the first mistake at the feeder during phase 2. Among bees that made at least one mistake at the feeder, head width was correlated with the trial in which a bee made its first mistake during phase 2 (Fig. 7; $r_p^2=0.25$, $N=21$, $P<0.05$). Larger bees made mistakes earlier in phase 2 than did smaller bees. Bees that made mistakes at the feeder during the first two trials of phase 2 were larger than bees that did not make a mistake (head widths: 3.95 ± 0.14 mm and 3.69 ± 0.28 mm, respectively; $t_{28}=-3.01$, $P<0.01$).

Given an indication that feeder performance in phase 2 varied with bee size, we were interested in whether bees showing relatively rapid acquisition of the nest entrance task tended to be relatively larger. By chance, bees had a 0.33 probability of making the correct choice at the nest. Because the effect of size on interference (Table 2) was most pronounced during the first 2–3 trials, we examined performance of bees at the nest during the first three trials of phase 2. We grouped bees into 'good performers' (≥ 2 of the 3, or at least 66% trials correct) or 'poor performers' (≤ 1 of the 3, or 33% trials correct or less). The head widths of good performers (mean 3.90 ± 0.21 mm; $N=15$) were significantly larger than those of poor performers (mean 3.72 ± 0.27 mm; $N=15$; $t_{28}=-2.28$, $P<0.05$).

Discussion

The nature of interference across tasks

This study demonstrated that *Bombus impatiens* bumblebees can learn two distinct color cues for two tasks, feeding (F) and accessing their nest (N), but that learning the second task, N,

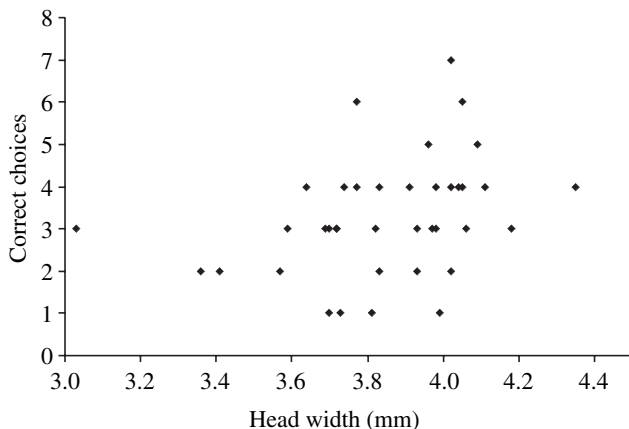


Fig. 6. The relationship between head width (mm) and the number of correct first landings at the feeder during the initial eight trials. A total of 39 bees were tested.

interfered retroactively with performance on the first task, F. As bees learned the nest entrance task, they showed an increase in errors at F, although performance overall never declined to levels at the beginning of phase 1 (33%).

Our findings contrast with those of Colborn et al. (1999), where no evidence of interference was found. A principal difference between our study and that of Colborn et al. (1999) is that we provided three choices for bees instead of two. This design feature allowed us to examine the relative choice of two incorrect colors, one that was rewarding for an alternative task and one that was novel and non-rewarding. At the feeder, foragers made the majority of mistakes at the color that was not rewarding at either task. This result means that retroactive interference between tasks was reflected not by a confusion of one task's stimuli with another's, but rather by an elevated response to irrelevant stimuli. Thus, the addition of the third, non-rewarded choice in our experiment may explain why we detected RI in dual-task learning but Colborn et al. (1999) did not.

In general, studies of interference in animals have typically used assays involving a binary choice to examine the effect of learning a new task on the performance at a previously learned task (see Bouton, 1993). This design limits the amount of information that an individual needs to process. In nature, bees are exposed to a multitude of cues, only some of which are relevant to locating a nectar source or nest entrance. Bees must not only learn to orient to rewarding stimuli, but must also learn to ignore non-rewarding stimuli; a process termed conditioned inhibition. In his review of context effects on interference and memory, Bouton (1993) pointed out that conditioned inhibition (no reward) and conditioned excitation (with reward) differ in their robustness to change in context or passage of time. In general, conditioned inhibition is more sensitive than conditioned excitation to changes in context. In phase 1, bees were conditioned to ignore two of the colors at the feeder (conditioned inhibition), and respond to one color *via* conditioned excitation. In phase 2, there were still two colors that were non-rewarding at the feeder, but one of these colors was being trained by conditioned excitation at the new task. Learning or responses to the two cues that were trained

Table 2. Comparisons of head size (mm) of bees that performed worse or not during x number of trials of phase 2 compared with the previous x number of trials in phase 1 at the feeder

Number of trials (x) on either side of phase transition	Head size		t -statistic	P
	Worse (N)	Not worse (N)		
1	3.93 ± 0.05 (9)	3.77 ± 0.06 (21)	-1.63	0.11
2	3.96 ± 0.04 (15)	3.68 ± 0.07 (15)	-3.42	0.002
3	3.90 ± 0.05 (15)	3.74 ± 0.07 (15)	-1.89	0.07
4	3.90 ± 0.06 (17)	3.76 ± 0.07 (13)	-1.53	0.14
5	3.83 ± 0.07 (18)	3.80 ± 0.06 (12)	0.35	0.73

Means \pm S.E.M.

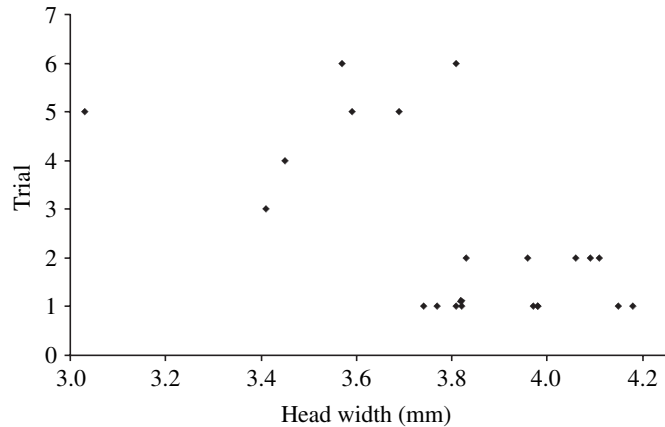


Fig. 7. Latency to expression of interference as measured by the trial in which a bee made a mistake at the feeder during phase 2 *versus* head width.

by conditioned inhibition at the feeder decayed unevenly. It appears that conditioned excitation for the color rewarded at the nest reduced the decay of the learned inhibition for that color at the feeder once the second context was introduced. As an alternative to this interpretation, bees may have been learning specificity of context (that is, learning that something that is available in one task is not rewarding in the other task). Our experiments were not set up to distinguish these two possibilities.

Another difference between this study and Colborn et al. (1999) is that bees in our study had 80% accuracy before adding the second context, compared to 100% accuracy of bees in the Colborn et al. study. Bees may be more vulnerable to interference prior to reaching perfect or peak performance levels. Indeed, Fauria et al. (2002) found that bumblebees made cross-contextual mistakes when learning both contexts simultaneously but not when the second context was added after the first had already been learnt. Nonetheless, the bias in mistakes toward the non-rewarding cue cannot be explained simply by a failure to learn the first task to 100% accuracy.

Interestingly, mistakes at the second task, N, were biased toward the color rewarded at F. Why this difference? One possibility is that when first confronted with a new task, bees may transfer outcomes of previous experiences to new ones. For example, honeybees (*Apis mellifera*) trained to differentiate symmetrical patterns from non-symmetrical patterns transferred this discrimination to novel patterns within the same task (Giurfa et al., 1996). Likewise, *Battus philenor* butterflies made more errors on a color rewarding in another learned task, relative to novel colors (Weiss and Papaj, 2003). Instead of inhibiting responses to the previously conditioned stimulus in the feeder context, bees may have transferred what they learned from the feeder to the new task at the nest.

Our experimental design may have inadvertently facilitated this kind of transfer. Phase 2 involved not only reinforcement of a new color at N, but also a display of three colors at N that resembled quite closely the display at F. A bee approaching the new display at N may have responded as though it was

approaching F and thereby responded more frequently to the feeder-rewarded color than expected. Such linking of contextual cues with reinforced cues is common in animals (Bouton, 1993). To examine proactive interference more rigorously, one would need to measure learning the novel task (nest entrance) with and without prior learning of similar cues at the feeder. At this point, our explanations for behavior at the nest entrance must remain speculative, as our experiment was not designed to assess proactive interference, or interference on a novel task.

Coping with interference in nature: individual-level considerations

Contextual cues are believed to alleviate interference in memory retrieval (reviewed by Bouton, 1993). For example, rats showed significant RI after learning a second task, but RI was significantly reduced when the context (lighting, size of arena, and texture of arena) of each task was made more different from each other (Rodríguez et al., 1993).

Our experiment was designed in a number of ways to make interference more likely to be detected. First, the local context for the feeder and nest tasks was quite similar, owing to the close proximity between nest entrance and feeder. In nature, flowers will tend to occur further from the nest than in our experimental arena. The visual scene around nest and feeder will likely be more different than it was in our experiment and for that reason, interference issues might pose less of a problem.

Additionally, in nature, there will probably be more distinctive differences in more cue types between flowers and nest than appeared in our experiment. During nectar-foraging, for instance, bees learn not only the color but also the distinctive shape, patterns, odor and microtexture of flowers (Chittka and Thomson, 2001). At the nest entrance, they could be learning another suite of similarly distinctive cues. The distinctiveness of the entire ensemble of cues in each task will probably diminish interference with respect to any given cue in the ensemble. Even in this study, interference effects were not severe, and it is possible that contextual cues may have reduced conflict between the memories at the hive and at the nest.

Coping with interference: colony-level considerations

In nature, bumblebees and other social insects may be able to avoid much interference by task specialization and division of labor. Bumblebee workers do not appear to have the strong age-based polyethism that is found in honeybees (although this remains open to debate; see Goulson, 2003). Instead, division of labor within a bumblebee colony is size-related, with foraging bumblebees being larger, on average, than bees that remain in the nest (Cumber, 1949; Goulson et al., 2002). A variety of hypotheses have been put forth to explain this difference in specialization and efficiency relative to size (reviewed in Goulson, 2003). One hypothesis not addressed to date is that larger bees are better learners and, therefore, better at the most learning-intensive task, foraging (for discussion on

learning ability and division of labor, see Withers et al., 1993). Our study provides support for this hypothesis.

With respect to interference, we did not find an effect of body size on the overall amount of interference; however, size was related to how quickly interference occurred. Among individuals showing retroactive interference, larger bees showed interference earlier in phase 2 than did smaller bees.

Why do larger bees, which learn faster, show interference more rapidly? Possibly, the size effect reflects an acquisition/interference tradeoff. Larger bees made the nest/color association more rapidly, but at the expense of a more rapid expression of interference at the feeder. Such a tradeoff is consistent with human studies wherein the amount of training in the second task (or set of associations) increases performance at the second task but decreases performance (interference) at the first (previously learned) task (Barnes and Underwood, 1959; Tell and Schultz, 1972). Large bees learned the nest task faster and therefore showed interference at the feeder sooner because, interference, by definition, occurs when a new association is formed with a familiar cue, competing with previous learned associations with that cue (Bouton, 1993).

In conclusion, the results of this study suggest that there is a decline in performance associated with switching tasks when cues are similar between tasks. Bees, like many other animals, forget by retroactive interference even when there are contextual cues (internal state, location, direction) present. As yet unclear is whether such interference poses a problem for bees in the wild and, if so, whether they possess strategies for coping with it. In some cases, interference between tasks may be alleviated by specialization of behavior or by reducing the frequency of switching between tasks (e.g. by engaging in bouts of one task or another). In nature, more cues might be available for any given task, and bees might use multiple cues to reduce the effects of interference. The use of multiple cues might provide mutually-reinforcing context for any one cue (Dale et al., 2005). On the other hand, bees must learn many more than two tasks in nature and so opportunities for interference may be greater than in the relatively simple laboratory environment. How these factors shape behavior will require studies on interference in multiple contexts in more realistic settings, using more tasks and more cues.

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