

## Serial reversal learning in bumblebees (*Bombus impatiens*)

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**Abstract** Bumblebees are capable of rapidly learning discriminations, but flexibility in bumblebee learning is less well understood. We tested bumblebees (*Bombus impatiens*) on a serial reversal learning task. A serial reversal task requires learning of an initial discrimination between two differentially rewarded stimuli, followed by multiple reversals of the reward contingency between stimuli. A reduction in errors with repeated reversals in a serial reversal task is an indicator of behavioural flexibility. Bees were housed in a large indoor environment and tested during foraging flights. Testing free-flying bees allowed for large numbers of trials and reversals. All bees were trained to perform a simultaneous discrimination between two colours for a nectar reward, followed by nine reversals of this discrimination. Results showed that bumblebees reduced errors and improved their performance across successive reversals. A reduction in perseverative errors was the major cause of the improvement in performance. Bees showed a slight increase in error rate in their final trials, perhaps as a consequence of increasing proactive interference, but proactive interference may also have contributed to the overall improvement in performance across reversals. Bumblebees are thus capable of behavioural flexibility comparable to that of other animals and may use proactive interference as a mechanism of behavioural flexibility in varying environments.

**Keywords** Bumblebees · Reversal learning · Behavioural flexibility · Discrimination learning

### Introduction

Animals are constantly learning about their environment, making discriminations, and forming associations in order to complete foraging, navigation, and other tasks. This constant processing of information raises the question of whether, when faced with the same learning problem repeatedly, performance changes with experience (Mackintosh 1974; Shettleworth 1998, 2010)? The ability to recognize change in the environment and update associations based on new information is adaptive. It is possible to address this issue using serial reversal. Serial reversal learning consists of initial discrimination learning in which one stimulus is rewarded and another not, followed by repeated reversals of the reward contingencies. This requires an animal to reverse its pattern of responding in response to the changing environment in order to obtain reward. With repeated presentation of similar problems, as in a serial reversal, a measure of improvement in learning as a result of experience can be obtained. The requirement that behaviour constantly change to meet the task demands makes serial reversal learning a measure of behavioural flexibility.

The serial reversal task has been used extensively to compare improvement over the course of discrimination reversals in many different species (Davey 1989; Shettleworth 1998, 2010). At one point, serial reversal learning was widely used as a general measure of animal intelligence (Mackintosh 1969; Bitterman 1969; Davey 1989). At the time, explanations of inter-reversal improvements were largely attributed to attentional processes and proactive interference (Mackintosh 1974). In more recent research, the serial reversal task is used to explore the ability of animals to ‘learn to learn’ and to investigate their acquisition of abstract strategies (Shettleworth 1998, 2010).

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Optimal performance on a serial reversal task consists of only a single error following each reversal and results from adopting a ‘win–stay, lose–shift’ strategy (Shettleworth 2010). Comparative cognition research using serial reversal learning has produced some interesting results (Bitterman 1965). It has been shown that species differ dramatically in their abilities and strategies when solving serial reversal problems, from proactive interference in pigeons and fish (Gonzalez et al. 1967; Squier 1969; Behrend et al. 1970), to abstracting rules in primates (Rumbaugh et al. 1996). Even closely related primate species differ in their ability to solve the reversal task and the strategy they use to do so (Rumbaugh et al. 1996). Serial reversal is a useful task for exploring the similarities and differences in learning among both distantly and closely related species. The differences found between species in serial reversal tasks are often qualitative rather than quantitative making them less susceptible to the problem of matching testing conditions across species (Bitterman 1965).

The serial reversal task is also undergoing a resurgence as a measure of learning flexibility. This research often examines the behaviour of animals whose ecology differs in ways that suggest there may be differences in the adaptive benefits of behavioural flexibility (Day et al. 1999; Bond et al. 2007). Bond et al. (2007) compared a number species of corvids on both visual and spatial discrimination reversals. Species with a more complex social structure, hypothesized to increase demand for flexible learning, performed better on reversals. Similarly, Day et al. (1999) found that lizards with an active foraging strategy performed better on reversals than sit and wait foragers. Thus, the serial reversal task can be used as a method for studying behavioural flexibility in addition to its long standing use in studying the ability to ‘learn to learn’.

Foraging bees make numerous discriminations as they acquire flower and place preferences and navigate to and from their colony. How bees accomplish this feat with a small and relatively simple brain is the topic of much research and discussion (Menzel and Giurfa 2001; Chittka and Niven 2009). The impressive repertoire of bees includes at least 59 different behaviours (Chittka and Niven 2009), including the ability to form a simple association faster than a human infant (Pearce 2008). Experiments with honeybees have found abilities previously seen only in vertebrates, such as concept and rule learning (for review see Zhang 2006). Bees’ performance on the serial reversal task could help determine how they compare to other animals in basic mechanisms of learning (Mackintosh 1969; Bitterman 1969; Davey 1989).

Early attempts to explore serial reversal learning in bees provided contradictory results. Menzel (1969) trained honeybees on a two-colour discrimination and then repeatedly reversed the reward contingencies. Rather than

showing improved learning in later reversals, bees began to respond to both stimuli equally, failing to discriminate between the two stimuli. Couvillon and Bitterman (1986) found that four reversals consisting of eight trials each were readily acquired by bees. When bees were required, however, to learn eight reversals consisting of four trials each, their learning pattern resembled that found in Menzel’s study (1969), with accuracy approaching chance. These results suggest that bees are sensitive to changes in reward contingencies and do show flexibility, because they can reverse their learned response pattern. Honeybees, however, failed to show inter-reversal improvement and in fact showed the opposite pattern of a decline in performance.

To clarify the contradictory findings of previous work, Mota and Giurfa (2010) revisited serial reversal learning in honeybees, using the proboscis extension reflex (PER) developed by Bitterman et al. (1983). PER requires that bees are harnessed so that only their proboscis and antennae are free to move. Their reflexive proboscis extension to sucrose is then conditioned to odour stimuli administered to their antennae, the bees’ olfactory sensory organ. Mota and Giurfa (2010) conditioned honeybees to differentially respond to two odour stimuli. Once the bees had completed five trials with each odour, the reward contingencies were reversed. Bees experienced a total of four reversals. Bees significantly changed their response patterns after each reversal, but in the final reversal, bees’ ability to discriminate between the two stimuli declined and their responses approached equal responding to both stimuli. As found in the previous serial reversal work, bees’ began to generalize their responding to both stimuli after repeated reversals. Although this study, like previous ones, showed a generalization of responding in bees following reversal, the PER protocol limits the number of trials that bees can be given in any experiment. While harnessed in the apparatus bees are unable to return to their colony to deposit nectar and become satiated when their honey crop is filled. The number of trials and reversals is, therefore, limited by this testing paradigm.

There has been at least one demonstration of improvement by a bee on a serial reversal task. Chittka (1998) trained a bumblebee (*Bombus impatiens*) to enter a T-maze and choose the right arm, rather than the left, to receive a sucrose reward. The bee was trained on this task for 100 trials and then required to switch repeatedly from the right arm to the left arm after 100 trials on each. The initial reversal resulted in a significant increase in the number of errors. The bee’s high error rate at the start of each session continued until the fifth reversal. Importantly, however, the bee’s performance eventually improved and after eight reversals, the bee performed the reversal with only three errors. This suggests that under certain conditions, such as

a vastly increased trial number, bees are capable of showing improvement as measured by reduction in errors. As with previous serial reversal work in bees, however, there is cause to interpret these results with caution. The study was performed on only a single bumblebee. In addition, the bee was performing spatial reversals, not visual or olfactory reversals. Serial reversal learning can vary depending on the modality tested (Bitterman 1965). Improvement in spatial reversal cannot therefore be taken as evidence that a similar pattern will occur in other modalities. The experiment was also conducted with a bumblebee, but because no comparable spatial task has been used with honeybees, this cannot be taken as evidence of a species difference in reversal learning.

It is notable that the only clear demonstration of inter-reversal improvement in a serial reversal task occurred in a bumblebee (Chittka 1998). This may be due to the larger number of trials used in the bumblebee experiment compared to those used in the honeybee experiments. Von Helversen (1974) tested honeybees on two colour reversals and found that with very few trials between each reversal, the bees' performance declined, whereas in reversals with larger trial numbers bees maintained their initial performance. The honeybees failed to show improvement with repeated reversals even with the larger trial numbers, but this does suggest that there is a relationship between trial number and performance on reversal learning tasks in bees.

We examined serial reversal learning in bumblebees. Bees were trained on a simultaneous colour discrimination followed by repeated reversals. Bees were tested in a simulated natural foraging environment where they collected nectar and carried it back to their colony throughout testing. The pivotal differences between this study and previous investigations were (1) a considerably increased number of trials and reversals and (2) free-flight testing.

## Methods

### Subjects

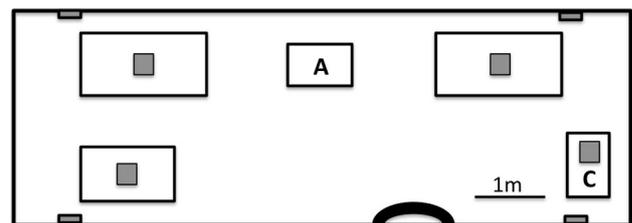
Subjects were 14 bumblebee workers (*Bombus impatiens*) from four colonies obtained from a commercial supplier (Biobest Canada Ltd., Leamington, ON). Colonies were kept on a 12-h light/dark cycle (light onset 0600). All four colonies were housed in a 3.0 × 8.5 m room (Fig. 1). The room contained eight artificial foraging patches. Four patches consisted of ten artificial flowers each, placed on tables, and four patches consisted of five artificial flowers each mounted on the walls. Artificial flowers were 1.7-ml microtubes (Axygen Inc., Union City, CA) with clear plastic corollas. Two of the colonies had additional stimuli in the room (i.e. artificial plants placed on the tables;

artificial vines hung from the ceiling and placed around wall-mounted foraging patches; carpet; brightly coloured felt, and foam panels placed on the tables and walls) as part of a pilot study exploring environment enrichment. Bees foraged ad libitum at artificial flower patches for 15–17 % sucrose solution. Pollen was given directly to the colony in the hive box. Colonies were given at least 5 days after arrival in the laboratory to begin foraging and habituate to their housing conditions before bees were tested.

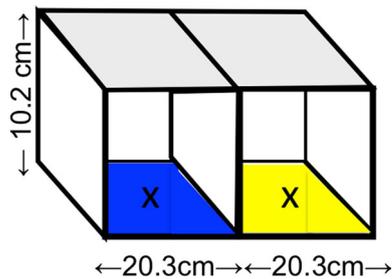
Prior to testing, bees were collected while foraging or taken directly from the colony, restrained in a marking tube, and tagged for individual identification with either plastic number tags (Betterbee Inc., Greenwich, NY) affixed with cyanoacrylate glue or Posca paint markers (Mitsubishi Pencil Co.). During testing, bees were identified by their colour and number.

### Apparatus

The apparatus consisted of two boxes (20.3 × 20.3 × 10.2 cm), each with a clear plastic lid (Fig. 2). One 20.3 × 10.2 cm side was open to provide an entrance. Boxes were placed together side by side during testing, and their relative left–right position could be changed during testing. Each box contained a 19.0 × 19.0 cm piece of polystyrene foam with a single 1.7-ml microtube embedded in the centre to administer sucrose solution reward. Flowers were filled or drained from outside the apparatus using 3-ml syringes (Becton, Dickinson and Company, Franklin Lakes, NJ) connected with 20 cm of PE-60 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, NJ) to the bottom of each flower. Stimuli consisted of 19.0 × 19.0 cm blue and yellow Creatology™ foam sheets (Michaels Stores Inc.) affixed to the polystyrene foam inside each box. The coloured Creatology™ foam covered the entire bottom surface of each box. The apparatus was placed on a table 4.0 m from the colony (Fig. 1). Following each testing session, the apparatus was removed from the room and the Creatology™ foam was wiped with 70 % isopropyl alcohol.



**Fig. 1** The testing room housed the bee colony (C) as well as eight foraging patches (shaded boxes). The apparatus was brought into the room and placed on a table (A) during testing



**Fig. 2** The apparatus consisted of two adjacent boxes, each with a front entrance. Stimuli were presented on the floor of the boxes. Artificial flower locations are designated with 'x'

## Behavioural task

### Pre-training

Prior to testing, bees were shaped to enter the apparatus with no colour stimuli present. Bees were collected either while foraging or taken from the colony and placed on a portable artificial flower filled with 35–40 % sucrose solution. While taking sucrose solution, bees were moved to the apparatus. This procedure was repeated until the bees made foraging trips to the apparatus by themselves, at which point testing began. Pre-training trials only occurred in the first testing session unless absolutely necessary to initiate foraging trips by a reluctant bee.

### Testing procedure

Bees were individually tested in two sessions per day on five consecutive days for a total of ten sessions. Sessions 1 and 2 in a day were separated by 1.5–8 h.

During each testing session, the apparatus contained two colours, blue and yellow, one in each box. Only one colour was rewarded in each session. The rewarded colour in the first session (start colour) was counterbalanced across bees. Each session consisted of 40 trials. Position of colours on the left or right was pseudorandomized for each trial with an equal number of right and left positions for each colour in a block of ten trials and no more than three trials in a row of one position. A single choice was recorded for each trial, and complete entrance into a box was considered a choice. If bees made a correct choice and entered the chamber of the rewarded colour, the artificial flower was immediately filled with 35–40 % sucrose solution and the bee was allowed to fill to repletion. If bees made an incorrect choice, they were allowed to exit the apparatus and make a second choice. A barrier between the two boxes forced bees to exit the apparatus completely before making a second choice. If bees left the area of the apparatus without making a second choice, the trial was considered finished and the

bee's next appearance was the start of a new trial. To ensure bees could not use olfactory cues from the sucrose solution, there was no reward present in the apparatus until after a bee made a choice.

Criterion performance for the initial discrimination in the first session was 8 out of 10 correct choices in a sliding block of 10 trials, and only bees meeting this criterion in the first session were tested on the serial reversal task. In the serial, reversal task reward contingencies were reversed at the start of each session. The first reversal began in session two. Bees completed a total of nine reversals over the course of ten sessions.

### Olfactory control condition

It was not possible to eliminate olfactory cues left by bees during a testing session. To determine whether olfactory cues influenced performance, seven bees were tested on the initial discrimination under identical conditions to bees in the serial reversal task but with the colour stimuli removed.

### Data recording and analysis

Each bee's first choice in each trial was recorded. The number of errors made in each session was used as a measure of learning across sessions (intersession comparisons). Learning within sessions (intrasession comparison) was measured by dividing each 40-trial session into four consecutive blocks of 10 trials each and calculating the number of errors per block for each bee. The effect of start colour on acquisition of the initial discrimination was measured by comparing the first session performance of bees trained with blue rewarded to the performance of bees trained with yellow rewarded using an independent samples *t* test. Data from bees in the olfactory control condition were divided into four blocks of 10 trials and analysed using repeated-measures ANOVA to assess intrasession learning. Learning on the serial reversal task was analysed using the errors in each trial block for each bee and a repeated-measures ANOVA design with trial block and reversal as within subject factors and start colour and foraging environment (the pilot study manipulation) as between subject factors. Trends across reversals were explored using regression analysis. Chi-square tests were used to compare bees' choices on the first trial in each session to chance.

Seven of the fourteen bees tested did not complete all nine reversals. Complete data were collected and analysed from fourteen bees for the first four reversals, and additional separate analyses were done for the seven bees that completed all nine reversals.

## Results

### Initial discrimination in the first session

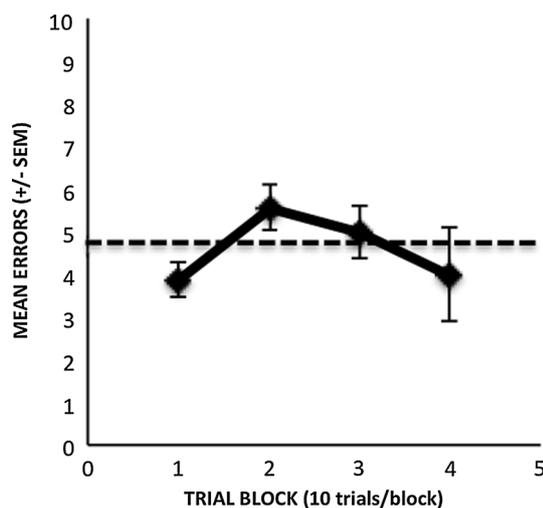
Bees readily acquired the initial simultaneous colour discrimination and met the 8 out of 10 correct criteria within the first 40-trial session. The environment enrichment had no effect on acquisition of the initial colour discrimination ( $t(12) = -.583$ ,  $p = .579$ ). A difference was found in acquisition between those bees for whom blue was the rewarded stimulus in the initial discrimination (start colour) compared to those for whom yellow was rewarded, with a response to blue acquired more rapidly ( $t(12) = 2.389$ ,  $p = .03$ ). Consequently, start colour was included in subsequent analyses as a between subjects factor.

### Uncued discrimination

Bees in the simultaneous discrimination task with no colour cues showed no evidence of learning (Fig. 3). Performance in the session did not differ from chance ( $t(6) = .638$ ,  $p = .55$ ). Performance was further analysed by dividing the 40-trial session into blocks of ten trials and using a repeated-measures ANOVA to determine whether performance improved over the course of the session. Performance did not change across trial blocks ( $F(3,12) = 1.55$ ,  $p = .251$ ).

### Serial reversal learning

The mean number of errors made by bees on each reversal is shown in Fig. 4. Mean errors in the first four reversals,

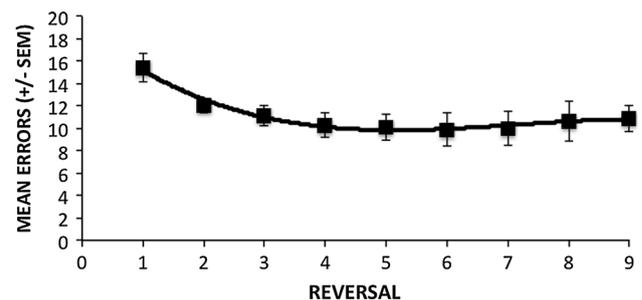


**Fig. 3** Simultaneous discrimination without colour cues. The mean number of errors (out of 10) made by bees in a single session of uncued discrimination does not differ from 5, the number expected by chance

which were completed by all bees, decreased significantly with successive reversals ( $F(3,30) = 3.841$ ,  $p = .019$ ). The environment enrichment had no significant effects ( $F(1,10) = 3.312$ ,  $p = .099$ ) or start colour ( $F(1,10) = 3.113$ ,  $p = .108$ ) and no significant reversal  $\times$  environment enrichment interaction ( $F(3,30) = .553$ ,  $p = .650$ ) or reversal  $\times$  start colour interaction ( $F(3,30) = 1.452$ ,  $p = .247$ ). A separate analysis using the 7 bees who completed all nine reversals also found a main effect of reversal ( $F(8,24) = 3.444$ ,  $p = .009$ ), and no significant effect of environment enrichment ( $F(1,3) = .542$ ,  $p = .515$ ) or start colour ( $F(1,3) = 1.275$ ,  $p = .341$ ). Curve estimation was conducted using mean errors per session and showed significant logarithmic ( $R^2 = .644$ ,  $F(1,7) = 12.67$ ,  $p = .009$ , AICc = 5.56), quadratic ( $R^2 = .920$ ,  $F(2,6) = 34.43$ ,  $p = .001$ , AICc =  $-3.03$ ) and cubic ( $R^2 = .974$ ,  $F(3,5) = 62.73$ ,  $p < .001$ , AICc =  $-5.98$ ) regressions. The corrected Akaike information criterion (AICc) was used to select the cubic regression as the best fit (Fig. 4).

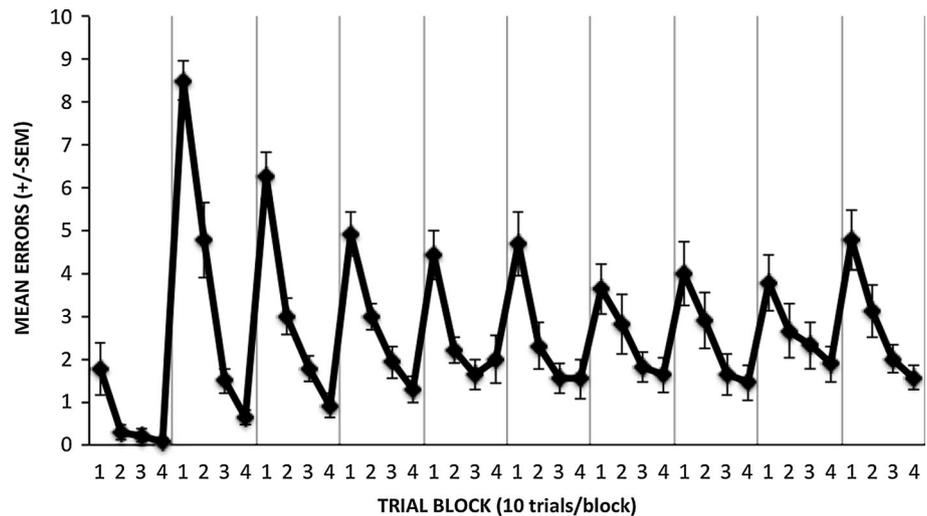
Learning within sessions was analysed by dividing the 40 trials per session into four blocks of ten trials each. The mean number of errors for each trial block is shown in Fig. 5. Analysis of the block data from the 14 bees that completed the first four reversals confirmed the main effect of reversal ( $F(3,30) = 3.818$ ,  $p = .02$ ) along with a significant main effect of block ( $F(3,30) = 105.439$ ,  $p < .001$ ) and a reversal  $\times$  block interaction ( $F(9,90) = 5.885$ ,  $p < .001$ ). No significant effect of foraging environment was found. Separate analyses using only bees that completed all nine reversals gave the same result: a main effect of reversal ( $F(8,24) = 3.444$ ,  $p = .009$ ), a main effect of block ( $F(3,9) = 77.110$ ,  $p < .001$ ), and a reversal  $\times$  block interaction ( $F(24,72) = 4.043$ ,  $p < .001$ ).

To further explore the reversal  $\times$  block interaction, the errors for each trial block were plotted and analysed (Fig. 6). The change in errors across reversals in the first trial block (Fig. 6 Trial Block 1) was significant in analyses of both the first four reversals ( $F(3,39) = 11.016$ ,  $p < .001$ ), and all nine reversals ( $F(8,64) = 4.455$ ,  $p < .001$ ). Planned

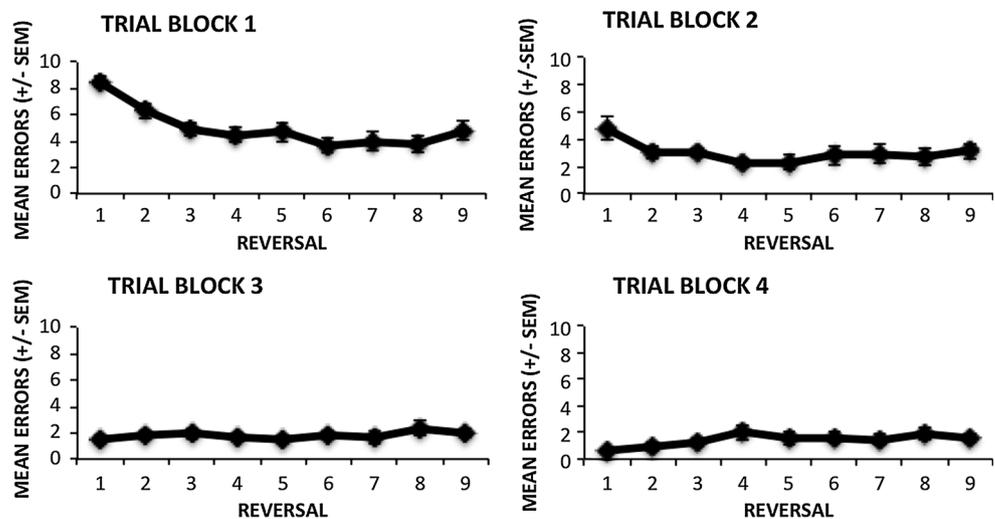


**Fig. 4** Mean number of errors per reversal. The mean number of errors changed significantly across the nine reversals. There is a significant cubic relation between mean errors and reversal

**Fig. 5** Mean number of errors per trial block. Each session was divided into 4 blocks (numbered 1–4) with ten trials per block. The first four-block session is data from the initial discrimination, and reward contingencies were reversed on the first trial of each subsequent four-block session. *Vertical lines* separate the four-block sessions and indicate when reversals occurred



**Fig. 6** Trial block errors as a function of reversal. The mean number of errors in each trial block is shown separately. *Each panel* shows the mean errors for one block across nine reversals



repeated contrasts found a significant change in errors from reversal one to reversal two in both the first four reversals ( $F(1,13) = 7.803$ ,  $p = .015$ ), and for the bees who completed all nine reversals ( $F(1,8) = 11.571$ ,  $p = .009$ ). No other significant contrasts were found. Analysis of the second trial block (Fig. 6 Trial Block 2) for the first four reversals found a significant main effect of reversal ( $F(3,39) = 4.065$ ,  $p = .013$ ), but no effect for the bees who completed nine reversals ( $F(8,64) = 1.571$ ,  $p = .151$ ). The analyses for the third trial block (Fig. 6 Trial Block 3) found no significant effects of reversal in either the first four reversals ( $F(3,39) = .317$ ,  $p = .813$ ), or all nine reversals ( $F(8,56) = .851$ ,  $p = .563$ ). The effects of reversal on the fourth trial block (Fig. 6 Trial Block 4) were also found to be non-significant for the first four reversals ( $F(3,36) = 1.807$ ,  $p = .163$ ) and all nine reversals ( $F(8,48) = 1.067$ ,  $p = .401$ ).

For the first and second trial blocks, quadratic regression of errors across reversals was significant (Trial Block 1  $R^2 = .94$ ,  $F(2,8) = 43.51$ ,  $p = .0003$ ; Trial Block 2  $R^2 = .74$ ,  $F(2,8) = 8.6$ ,  $p = .017$ ).

A comparison of bees' choices on the first trial of each session was performed. Bees' choices were not different from chance in the first trial of the initial discrimination ( $\chi^2(1, N = 14) = 2.571$ ,  $p = .109$ ). Bees' first trial choice did differ from chance in the first reversal ( $\chi^2(1, N = 14) = 14$ ,  $p < .001$ ), showing that they preferentially chose the stimulus rewarded in the initial discrimination. On all subsequent reversals, bees' choice on the first trial did not differ from chance: reversal 2,  $\chi^2(1, N = 14) = 2.571$ ,  $p = .109$ ; reversal 3,  $\chi^2(1, N = 14) = 2.571$ ,  $p = .109$ ; reversal 4,  $\chi^2(1, N = 14) = 1.143$ ,  $p = 2.85$ ; reversal 5,  $\chi^2(1, N = 9) = 1$ ,  $p = .317$ ; reversal 6,  $\chi^2(1, N = 9) = .111$ ,  $p = .739$ ; reversal 7,  $\chi^2(1, N = 9) = .111$ ,  $p = .739$ ; reversal 8,  $\chi^2(1,$

$N = 9) = .111, p = .739$ ; and reversal 9,  $\chi^2(1, N = 9) = 1, p = .317$ .

The interval between sessions 1 and 2 within a testing day averaged 232 min, and the interval between session 2 and session 1 the following day averaged 954 min. A comparison of first trial choices on all first sessions in a testing day to all second sessions revealed no significant differences between the two ( $t(3) = .650, p = .56$ ), demonstrating that the large difference in intersession intervals did not influence bees' first trial choices.

## Discussion

Bumblebees were able to respond to the changing reward contingencies in a serial reversal task and to improve their ability to reverse their responding with experience, as shown by the reduction in errors with repeated reversals. The pattern of errors within trial blocks showed that the improvement in performance was due predominantly to a reduction in perseverative errors immediately following a reversal. Bees improved their performance rapidly after only a couple of reversals and then maintained a consistent performance before a slight increase in errors in the final reversals. Bumblebees are thus capable, by the serial reversal criterion, of behavioural flexibility. The bees' performance on the serial reversal task did not improve, however, to the point of one-trial reversal that has been shown in rats (Duforth et al. 1954) and some primate species (Rumbaugh 1971). These findings are also different from much of the previous serial reversal work with honeybees and bumblebees, which found no consistent improvement in performance with repeated reversal.

### Trial number

There are a number of differences between this study and prior investigations of serial reversal learning in bees that may explain the difference in results, the most obvious of which is the number of trials. Much of the prior work used very small trial numbers, in some instances due to the restrictions imposed by PER conditioning (Mota and Giurfa 2010). The trial number in this study was much greater than in previous studies and may explain the differences. The only previous evidence of improvement in serial reversal learning in bees also used large trial numbers (Chittka 1998), and a relationship between trial number and reversal learning has been previously observed in honeybees (Von Helversen 1974).

There are multiple explanations for why large trial numbers may facilitate serial reversal learning. A phenomenon that has long been observed in reversal learning is the overtraining reversal effect (ORE) (Mackintosh

1974). It was found that, in some situations, animals that received training trials beyond criterion were facilitated when subsequently tested on a reversal compared to controls trained only to criterion (Mackintosh 1974). These results are counterintuitive because increasing pairings with a reward should strengthen an animal's association between a response and a stimulus, increasing the difficulty of reversal. Nevertheless, OREs have been replicated, and the effect appears frequently, depending on testing conditions (Mackintosh 1974). This study cannot be considered a demonstration of the ORE because bees were not specifically overtrained, and there was no comparison group without overtraining. In a number of sessions, however, the majority of bees reached criterion prior to the end of the session and therefore received overtraining trials. Thus, the connection between overtraining and performance on reversal task does provide a possible explanation for the bees' performance in this study.

It has been shown that length of training can affect how bees perceive visual stimuli, consequently affecting their learning (Giurfa et al. 2003; Stach and Giurfa 2005). Stach and Giurfa (2005) showed that increasing the number of acquisition trials on a visual discrimination increased the bees' generalization to novel stimuli following acquisition. If bees in our study were undergoing a similar process of increased generalization, it is likely that their performance would have declined, through generalizing between the two colours. However, the improvement in performance suggests that bees were not generalizing and were maintaining their ability to discriminate between the two colours. It is possible that some of the previous reversal work in bees that demonstrates a decline in performance with repeated reversals (Mota and Giurfa 2010) can be explained by generalized responding from the rewarded to the unrewarded stimulus. If generalization, similar to that shown by Stach and Giurfa (2005), was influencing bees' performance on the serial reversal task, however, it would predict worse performance in our study where bees were extensively trained compared to previous work with few trials, which is not supported by our data.

### Timing

In our study, sessions 1 and 2 each day occurred consistently in the morning and afternoon, respectively, although the actual start time of the sessions was highly variable. As the reward contingencies were reversed at the start of each session, it is possible that bees associated one response with the AM session and the other with the PM session. Such an association would facilitate learning and contribute to their reduction in errors. Bees are capable of integrating circadian information with a visual association to determine which of a number of feeding locations will be

rewarded (Pahl et al. 2007). Additionally, honeybees have been shown to respond preferentially to circadian cues when learning colour associations (Prabhu and Cheng 2008). Prabhu and Cheng (2008) found that bees trained on two conflicting colour associations use circadian information to solve a subsequent test rather than the recency of reward with a particular stimulus. Despite bees' easy acquisition of circadian learning, there are a number of reasons to believe it was not a factor in this study. First, the circadian learning experiments involved testing at precise circadian time points, whereas here the testing times were highly varied, devaluing any circadian information. Additionally, if bees were using the session time of day as a cue, we would expect them to select the correct response on the first trial. Bees chose randomly on the first trial, however, in all sessions except the first reversal in which they performed worse than chance. This pattern of randomly responding on the first trial with repeated reversals has been found in rats and occurred when rats were predictably reversed midday (Mackintosh et al. 1968), where it was also taken as evidence of a lack of circadian learning. Although it cannot be ruled out that bees were aided by circadian cues, it seems unlikely given the pattern of their performance.

Although bees' progression towards chance accuracy on the first trial of the reversals is not consistent with circadian timing, it might be predicted by the temporal weighting rule (TWR) (Devenport and Devenport 1994). The TWR uses a weighted average of previous experience, with most recent experience weighted most heavily, to predict selection on the first trial of a session. The rule predicts a strong preference for the most recently rewarded stimulus on the first trial of the first reversal, followed by progression towards chance accuracy on the first trial of subsequent reversals, because the influence of the most recent experience is reduced with each experience that is added to the weighted average. TWR also predicts that bees' selection at the start of a session should be influenced by time since the most recent experience, the intersession interval, because of the greater weighting of recent experience. TWR, therefore, makes a clear prediction that bees' should select the most recently rewarded stimulus in the first trial of each session to a greater extent in session 2 of each day than in session 1, because of the shorter intersession interval between sessions 1 and 2 within a day, compared to the intersession interval between session 1 and session 2 of the previous day. We calculated first trial preference for the most recently rewarded stimulus according to the TWR (Devenport and Devenport 1994) and found that TWR predicted first trial preferences ranging .55 to .57 for session 1 of each testing day and first trial preferences ranging from .75 to .84 for session 2 of each testing day (excluding the first reversal, for which TWR

predicts a preference of 1.0). This difference in first trial preferences between sessions 1 and 2 is not supported by our data. Bees' selection on the first trial of each session was not significantly influenced by the intersession interval and is not consistent with bees' use of the TWR.

### Modality

Although a number of reversal studies in bees have used colour stimuli (Menzel 1969; Chittka 1998), most studies have used olfaction (Couvillon and Bitterman 1986; Mota and Giurfa 2010). Because this study was done with colour, it is possible that the difference in performance is due to the difference in modality. It has been shown in the past that testing conditions can have a significant impact on serial reversal performance, with some species showing improvement in one modality and not others (Bitterman 1965). Prior to the present study, Chittka (1998) found serial reversal learning in bees using a spatial task, not the more commonly used olfactory discrimination. Direct comparisons of performance on matched olfactory, visual, and spatial tasks would be required to test the hypothesis that modality is an important factor but the available results are consistent with the idea that bees are more constant, that is, less able to reverse, learned olfactory discriminations compared to other modalities.

### Type of discrimination

Traditionally serial reversal experiments are conducted using differential conditioning, where there is a rewarded and an unrewarded stimulus which the subject must discriminate between, as was done here. This is in contrast to absolute conditioning, where one stimulus is paired with reward in the absence of an unrewarded stimulus. It has been shown that bees perform differently on colour discrimination tests depending on whether they are trained using differential or absolute conditioning (Dyer and Chittka 2004; Giurfa 2004). When given, absolute conditioning bees are unable to discriminate between similar colour stimuli, but readily discriminate between perceptually very different stimuli (Dyer and Chittka 2004; Giurfa 2004). However, bees trained using differential conditioning are able to discriminate between both similar and dissimilar stimuli (Dyer and Chittka 2004; Giurfa 2004). The stimuli used in our study are comparable to the perceptually very different stimuli used in these experiments (blue to the human eye vs. yellow to the human eye). It is possible that our results would hold regardless of initial conditioning training with our stimuli, but may differ considerably if more similar stimuli were used.

An additional consideration of the type of discrimination used in the present study is that our reversals involved a

simultaneous discrimination and much of the previous work involved successive discriminations (Chittka 1998; Mota and Giurfa 2010). Simultaneous discriminations were used by Couvillon and Bitterman (1986), who did find that bees showed accurate performance with repeated reversals in one of their conditions. Studies using successive discrimination in which bees' performance declined with repeated reversals attributed the decline to a reduction in bees' ability to discriminate between the stimuli. Using a simultaneous discrimination may improve the bees' ability to discriminate between stimuli and provide a buffer against declining discriminability with repeated reversals. Chittka's (1998) results, however, are an exception to this pattern because the bumblebee tested demonstrated improvement in a successive serial reversal task.

### Species differences

We have thus far considered previous work on serial reversal learning in bees as a whole, but there may be important species differences between honeybees and bumblebees. Differences in ecology and behaviour, and their impact on behavioural flexibility, may make it difficult to generalize across even closely related species. Bond et al. (2007) compared closely related corvids and found significant differences in performance on a serial reversal task that was associated with differences in ecology. Similar differences may exist between bumblebees and honeybees.

Serial reversal learning by bumblebees occurred in this study and in previous work (Chittka 1998). In contrast, attempts to show serial reversal learning in honeybees resulted in a decline in bees' discrimination performance (Menzel 1969; Couvillon and Bitterman 1986; Mota and Giurfa 2010). There are differences in the natural foraging strategies of honeybees and bumblebees that may lead to species differences in behavioural flexibility. One of these differences is that honeybees direct fellow bees to nectar sources using the honeybee dance language (Von Frisch 1967; Riley et al. 2005), whereas bumblebee communication is limited to increasing nestmate foraging activity (Dornhaus and Chittka 2001) and scent marks (Saleh and Chittka 2006). Thus, bumblebees must determine good nectar and pollen sources largely by sampling a number of flower species (Heinrich 1976, 1979). Bumblebees need to determine the best sources of nectar and pollen without the help of their nest mates, and this may have resulted in greater behavioural flexibility than found in honeybees.

### Colour preference

Another result of this study was faster acquisition of the initial discrimination when blue was the rewarded colour

compared to when yellow was rewarded. This preference is consistent with a number of previous studies demonstrating a preference for blue (Heinrich et al. 1977; Keasar et al. 1997). A preference for yellow over blue by bees and wasps has been shown before (Real 1981), but the authors attributed that preference to a difference in contrast to the background colour. Here the preference for blue did not persist throughout the study, because there were no differences in performance on the reversals between those bees who started on blue and those bees who started on yellow. This is notable because differences in reversal of foraging preferences in bumblebees trained to go to blue, and those trained to go to yellow have been shown before (Heinrich et al. 1977). Heinrich et al. (1977) found that bumblebees that were trained to be constant to blue flowers did not switch to foraging on white flowers when reward contingencies changed. In contrast, bees that were trained to be constant to white flowers did learn to switch when reward contingencies changed. There are a number of differences in procedure between that study and this one, the most important of which is that the bees here were foraging on a single flower for each colour, whereas in Heinrich et al. (1977), bees foraged on patches with multiple flowers of each colour. The reward contingencies also differed. Bees in this study were given all or nothing rewards, whereas in Heinrich et al. (1977), flowers varied in their reinforcement with one colour being more rewarding on average than the other. The bees' behaviour in the present study, however, indicates that they can overcome their innate preferences when the task demands require them to do so. This finding is supported by previous work in which bees readily overcame prior colour preferences when the schedule of reinforcement favoured the less preferred colour (Real 1981). It has been shown that innate preferences may persist in bee while unexpressed (Gumbert 2000), so we cannot claim that the preference for blue shown by our bees was eliminated as it could have been retained but successfully inhibited.

### Mechanism of improvement in the reversal task

What mechanism might be responsible for the observed improvement by bumblebees on the serial reversal task? Previous studies of reversal learning have found that similar performance patterns can be attributed to different types of learning including abstract understanding of the task demands, rule learning, and the accumulation of proactive interference (Shettleworth 2010). Although our study was designed to see whether the bees would show improvement rather than to determine the mechanism by which they improved, the pattern of the bees' errors provides information about mechanism. In the first few reversals, the bees perseverated significantly on the previously rewarded

colour in the early trials in each session (Fig. 5). After making one or two correct responses to the newly rewarded stimulus, bees rapidly switched their pattern of responding and reached a near perfect performance in the final trials similar to their level of accuracy in learning the initial discrimination. The improvement in the bees' performance over the course of the experiment was driven largely by a reduction in the perseverative errors in the early trials in each session. Two additional important patterns can be found in the bees' performance. The first is that the bees never reached perfect reversal performance, which is defined as single-trial switching. The second is that in all reversals excluding the first, bees' responses on the first trial of each session were random and did not depend on the previously rewarded colour. Interestingly, a similar pattern of performance—initially large errors followed by improvement—was shown on a task-switching experiment with bumble bees (Dukas 1995). The bees were trained on two different colour discriminations and required to repeat switch between the two tasks. Initially, performance was poor following a switch, but experienced bees were able to switch repeatedly without a significant decrease in performance.

Of the possible mechanisms listed above for improvement in serial reversal tasks, only proactive interference explains the bees' pattern of responding. Abstract knowledge of the task demands or use of a rule-based strategy, such as win–stay, lose–shift, would both result in perfect or near perfect performance. Therefore, we propose that bees' are not capable of extracting general knowledge about the task demands, or 'learning to learn', but instead experience memory error that reduces their perseveration on previously rewarded stimuli and allows them to behave more flexibly. This could explain why successful serial reversal was observed in the present study but not in previous research. The conditions under which bees were tested in previous studies may have produced different levels of proactive interference and thus little improvement on the reversal task.

There is some work that seems to argue against our conclusion that bees do not 'learn to learn'. In Chittka (1998), a single bee improved performance on a reversal task almost to the point of one-trial reversal. Such performance would be suggestive of rule learning. In order to confirm that such learning occurred, a transfer test would be required. If general information about task demands was extracted, that is learning beyond the level of stimulus control, then learning on a similar task involving different stimuli would be faster than learning on the initial task. Komischke et al. (2002) found that bees trained on successive PER olfactory reversals, in which discriminations included an odour from a previous discrimination with a reversed reward value, performed better on a subsequent

PER olfactory reversal than those that experienced discriminations without reversals. The authors describe this as 'learning to learn' though they state that the mechanism behind the bees performance is not clear. The bees were matched on experience with the particular stimuli, so these results appear to be unexplainable in terms of proactive interference. It is possible, however, that the reversals caused proactive interference not for particular stimuli, but for the task demands. This could potentially result in more tenuous discriminations and easier reversal for those bees with reversal experience. Komischke et al.'s (2002) testing paradigm is, however, considerably different from the one used in the present study. There is additional research not involving reversal learning that suggests bees are capable of rule learning (Giurfa et al. 2001; Avarguès-Weber et al. 2011). It is possible that bees are capable of rule learning, but unable to extract the win–stay, lose–shift rule in reversal tasks. This could be because of the large accumulation of proactive interference in reversal tasks, through conflicting reward history with each stimulus, which is absent in rule learning tasks. Regardless of their rule learning capabilities, the bees were not able to acquire a win–stay, lose–shift rule in our task.

## Conclusions

Although proactive interference is usually regarded as a memory error, it may be that proactive interference is beneficial in highly variable environments. Bees are able to solve a remarkable number of learning tasks considering the rudimentary structure and small size of their brain, the serial reversal task included. Determining the mechanisms behind their success is an essential step in unravelling the 'amazing mini-brain' (Giurfa 2003). If bees are behaving flexibly because of proactive interference rather than a more cognitively demanding strategy such as rule learning or abstract thinking, it follows that the mechanisms underlying other remarkable learning abilities of bees may also be relatively simple.

This study shows that bumblebees can improve performance on a serial reversal task with experience. In doing so, bees exhibit the capacity to recognize changes in their environment and flexibly change their behaviour. Bees showed two patterns of performance. One was a reduction in perseverative errors and the other was a slight increase in errors in the final reversals. The latter pattern is much more pronounced in other studies (Menzel 1969; Couvillon and Bitterman 1986; Mota and Giurfa 2010), with bees completely losing the ability to discriminate after a number of trials. Bees in the present study maintained criterion performance and only showed a small increase in errors. Nonetheless, an increase in overall errors with repeated

reversals in serial reversal learning is consistent across almost all studies in bees. We take this pattern of performance to demonstrate that bees are able to behave flexibly because of their susceptibility to proactive interference in a highly variable environment.

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