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Learning Behaviour in Bumblebees: An Analysis with a Control Group

Introduction

This dataset is part of a study on bumblebee learning led by Loïc Goulefert's team. Understanding the impact of environmental stressors on the cognitive and navigational abilities of pollinators like bumblebees *Bombus terrestris* is increasingly crucial in ecological and behavioural research. The authors' aim is to investigate how exposure to specific stressors, such as pesticides and radiation, can affect the cognitive functions of bumblebees. To achieve this, they assessed several behavioural parameters, including the bees' performance in the Y-maze, their learning rate, and their movement patterns through the maze. This paper analyses the data from the control group to establish baseline learning patterns in an environment free from external stressors.

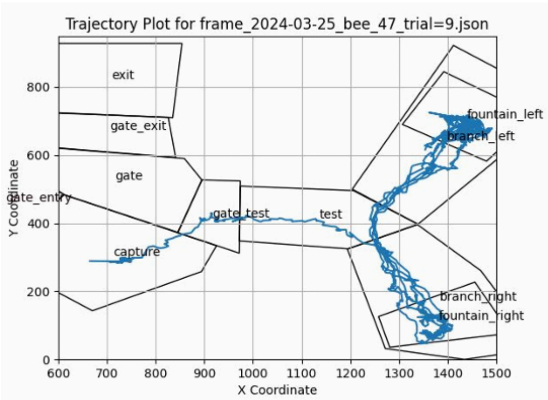


Figure 1. The trajectory of normal bumblebee

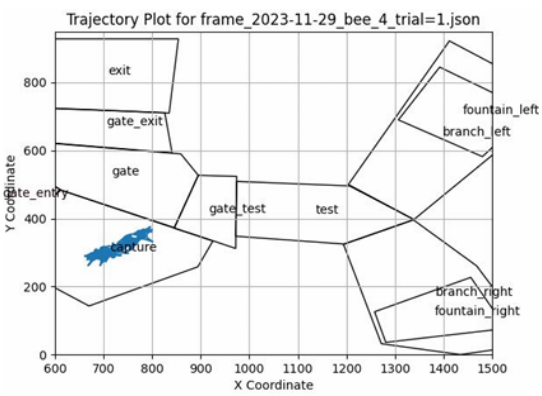


Figure 2. Invalid trajectory

Materials

The dataset consists of 187 trajectory recordings of healthy *Bombus terrestris* bumblebees from an automated Y-maze learning experiment. Each recording is saved as a CSV file with recording parameters: time frames, x-y coordinates of the bee's position in pixels, the compartment it was in, reinforced colours and sides compared to the side of the food zone, ID of each bee and the number of trials up to 31 trials for some individuals. Each centimetre of movement is represented by 75 pixels, with a resolution of 1640 x 928 pixels. Each frame records a single data point for movement, allowing for detailed tracking (*Figure 1*). As we are interested in the learning behaviour with the food area, the anomalous data showing bees not reaching the food area have been eliminated in the statistical analysis. (*Figure 2*).

Method and Results

Task 01: What is the proportion of bees choosing the fountain on the same side as their reinforced gate in the Y-maze?

In this task, we tested whether bees chose the fountain on the same side as their reinforced side in a Y maze at the early stage of learning with the trials from 1 to 15. We also tested for the first visit at the fountain. The number of times the bees matched the side of their reinforced door (left to left or right to right) was counted and divided by the total number of trials. This fraction indicates whether the bees learned or preferred the reinforced side. In the first 15 trials, 52.2 ± 4% of the bumblebees chose the fountain on the same side as their reinforced side door, while 47.8 ± 4% chose the opposite side (*Figure 3*). This result may reflect an early stage of learning, where bees are starting to form an association but have not yet developed a strong preference.

Figure 3. Learning Effects with Side Reinforcement

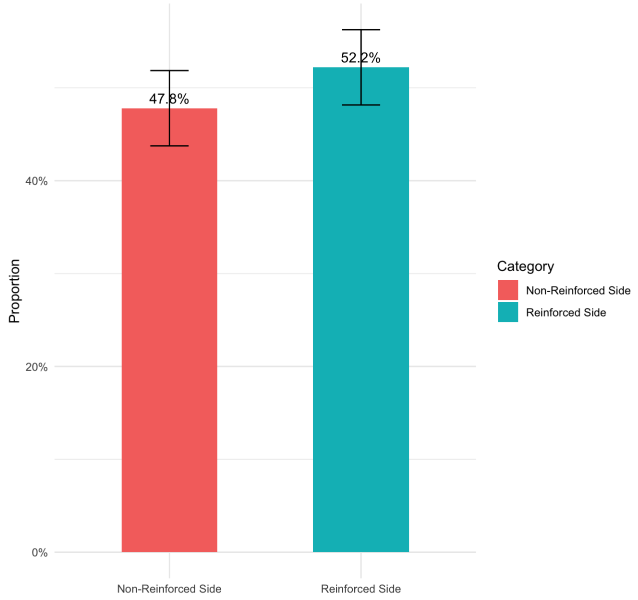
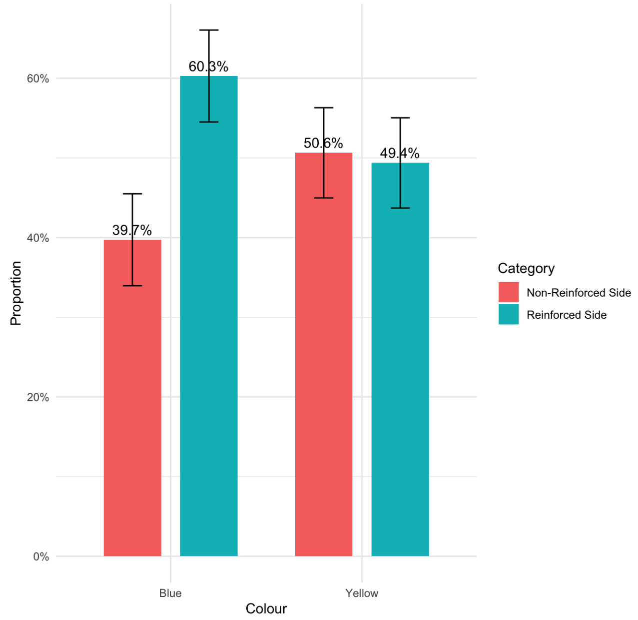


Figure 4. Learning Effects with Side and Colour Reinforcement



Task 02: Which colour of light (blue or yellow) affects the bees' learning to follow the reinforced side to the fountain?

For each trial, we checked whether the bee went to the fountain on the same side as the reinforced side and noted whether the reinforcement was done under blue or yellow light. We counted the number of times the bees chose the same side and the opposite side for each colour. Then we calculated the fraction by dividing these numbers by the total number of trials for each colour. This allowed us to see whether the bees were more likely to learn to follow the reinforced side under blue or yellow light.

In trials with blue light, 60.3 ± 5.7% of bumblebees chose the reinforced side, while 39.7 ± 5.7% chose the non-reinforced side. In contrast, under yellow light, the bees showed almost no preference, with 50.6 ± 5.6% choosing the non-reinforced side and 49.4 ± 5.6% the reinforced side (*Figure 4*). These results suggest that bumblebees exhibit a stronger preference for the reinforced side when exposed to blue light. This difference indicates that light colour may play a role in influencing the bees' learning or preference behaviour.

Task 03: Does the time required for the bees to reach the fountain change over repeated trials?

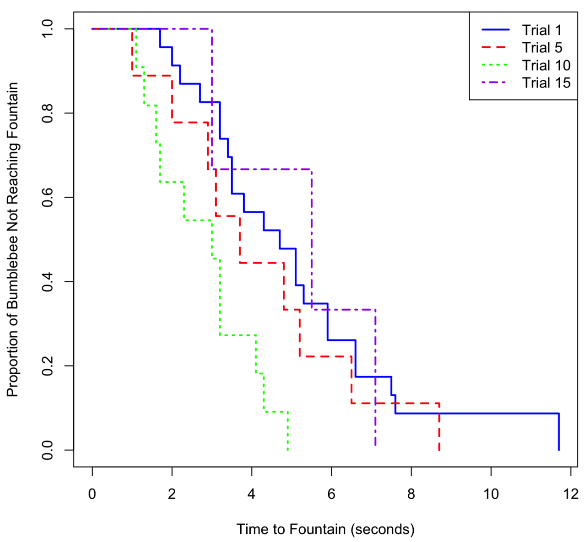
The results of the first two tasks show a slight tendency for the bumblebees to choose the reinforced side. Even though the first 15 trials show very little learning, this may be due to a progressive learning process, with low learning rates in the beginning and higher learning rates later, which then cancelled each other out. To address this, we calculated the time bumblebees took to reach the fountain in separate trials 1, 5, 10, and 15.

For each trial, we identified the last time the bee was recorded at the gate_test before its move to the fountain, and the time of its first arrival at the left or right fountain. We then calculated the latency and converted this latency from frames per second (fps) to seconds (s) by subtracting the time of the last visit to the gate_test from the time of the first visit to the fountain. Finally, we calculated the average latency for each trial to see if bees reached the fountain faster over successive trials.

The average latency decreased progressively from **Trial 1** (5.10s) to **Trial 10** (2.79s) which shows that bumblebees reached the fountain more quickly as trials progressed. However, in **Trial 15**, the average latency increased slightly to 5.2s, likely due to the small sample size for this trial (only three bumblebees for trial 15).

To visualise the data, we plotted a survival curve for each trial (1, 5, 10 and 15) over time to show the proportion of bumblebees that had not yet reached the fountain (*Figure 5*). In Trial 1 (blue line), it took the longest for all the bees to reach the fountain at 12s, with a relatively high proportion of bees still on the way as time passed. In trial 5 (red line), the time to reach the fountain decreased significantly to 8.5s. This trend continued in trial 10 (green line), with all the bees reaching the fountain faster at 5s and fewer bees arriving later in the day. In trial 15 (purple line), the curve drops rapidly, but bees take slightly longer than the others in trial 10 (7s). The overall pattern is that the bees become more efficient in their search for the fountain over the course of the trials.

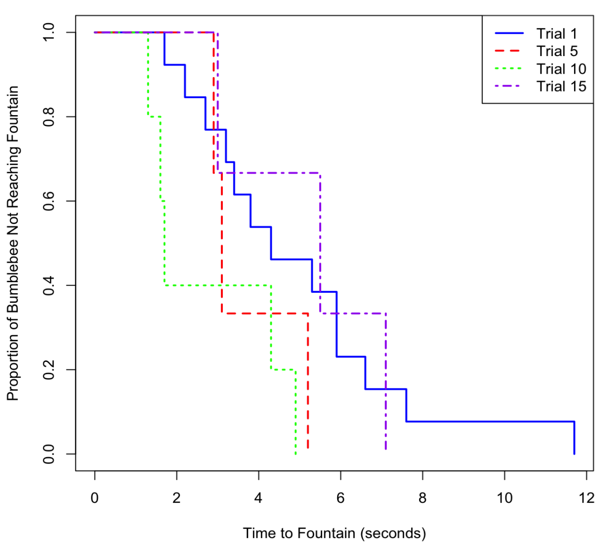
Figure 5. Time to Reach Fountain Across Trials



Task 04: Do the bumblebee reach the fountain more quickly after trials of learning to follow the reinforced side?

Task 03 confirmed our prediction that the effect of learning was cancelled out in Tasks 01 and 02. The bumblebees took less and less time to reach the fountain as the trials progressed. To better assess learning over time, Task 04 focused on trials 1, 5, 10 and 15 to track the improvement in reinforced learning.

Figure 6. Time to Reach Fountain for Congruent Reinforcement



Task 04 follows a similar approach to task 03. We measured latency by identifying the last recorded time the bumblebee was at gate_test and the first time it reached the fountain. The data is then divided into two categories: congruent (where the fountain side of the bee matches the reinforced side) and incongruent (where it does not). Next, the data in each category is stored separately across different trials. Finally, average trial latencies are calculated for both groups.

The results show that bumblebees reached the fountain faster over time, especially when using the reinforced side, which dropped from 4.95s in trial 1 to 2.76s in trial 10. In trial 15, the latency increased to 5.2s, possibly due to limited data (*Figure 6*). In the non-reinforced group, latency also decreased from 5.29s in trial 1 to 2.82s in trial 10. By trial 15, the bees appeared to be learning the reinforced side, although the small sample size suggests caution in interpreting these results.

Overall, the results suggest that the bees navigate the maze more quickly over time, particularly when they follow the reinforced path. In addition, factors such as becoming familiar with the Y maze configuration over time may have influenced their performance.

Task 05: What is the average speed of the bumblebees as they learn to navigate the maze over the number of trials?

As latency decreased across trials, we expected the speed of the bumblebees to increase as they became more familiar with navigating the maze during trials 1, 5, 10 and 15. As bees could travel back and forth between their initial departure point and their first arrival at the fountain, averaging their speed between departure and arrival appeared to be inaccurate. Instead, we measured speed based on the distance between the last time recorded at the gate_test and the first arrival at the fountain, using x and y coordinates (converted from pixels to centimetres) and time (converted from fps to seconds). Finally, the average speed and standard error were calculated for each trial.

Overall, in line with our expectation, the average speed of the bumblebees increased from trial 1 to trial 15. These findings show that repeating the trials leads to an improvement in the speed of the bees. In trial 1, the average speed was only 0.022 ± 0.048 cm/s. In trial 5, the average speed increased to 0.2 ± 0.16 cm/s. In trial 10, the average speed increased continuously to 1.09 ± 0.46 cm/s. This shows that the bumblebees become faster as they gain experience. By trial 15, the average speed reached 1.326 ± 0.37 cm/s. The average speed of the bumblebees appears to be lower than the typical speed observed in the natural environment. However, this measurement is based exclusively on the time between leaving the gate and reaching the fountain for the first time. As such, these speeds should be interpreted as relative comparisons between trials rather than as representative of the average speed of bumblebees in the natural environment.

Conclusion

This paper examines the learning effects of the bumblebee *Bombus terrestris* as it navigates a Y-maze to reach a food source. The experiment was carried out by training the bumblebees with reinforced gates and colours to see if they would learn to approach the corresponding side of the fountain. For the first 15 trials, we saw that the reinforced colours and reinforced side had an effect on bumblebee’s learning. As the trials progressed, the bumblebees showed a decrease in the time taken to travel from the gate_test to the fountain, which revealed improved learning and efficiency in reaching the food zone. Notably, the bees showed considerably faster speeds travelling from the gate_test to the fountain over the trials, although their average speeds were lower than typical speeds observed in natural environments. This analysis is part of a larger study investigating the effects of environmental stressors and helps to establish a baseline for further research.