SWIMMING ASSAY TO TEST LEARNING BEHAVIOR IN PR HONEYBEES, *Apis mellifera*

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Introduction

Honeybees demonstrate in their behavior an ability to learn and memorize, using spatial cognition. Mainly the foraging worker bee, needs a great ability to spatially memorize the location of both the food and its hive. We want to develop a procedure to test spatial learning and memory capacity in honeybees. This procedure will be used later in studies with honeybees infested with the *Varroa destructor* mite, which is a vector of the Deformed Wing Virus (DWV).

Richard Morris (1984) developed a procedure to study spatial learning in rats in a water maze. This was based on the idea that the rat could remember through images, where there is a dry base that serves as an escape. Because honeybees demonstrate both individually and as a colony the ability to memorize, we wish to adapt Morris's (1984) water maze model for our swimming-based studies of memory development and learning in honeybees, *Apis mellifera*.

Figure 1. Rat's water maze pool. (Morris, 1984)

Figure 2. Bee's water maze pool.

Honeybees use their wings to move across the surface of the water. They can use their wet wings as hydrofoils for propulsion on the surface of the water (Roh & Gharib, 2019).

Several tests were performed to check the variability in swimming ability of bees. We made a comparison between two beehives, one completely healthy and the other where the presence of the *Varroa* mite had been observed.

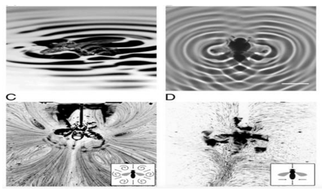

Figure 3. Surface wave and flow visualization. (Roh & Gharib, 2019)

Objectives

1. Validate the water maze protocol used by Morris to study spatial memory in honeybees in our laboratory.
2. Measure the survival of swimming honeybees.
3. Evaluate the swimming directionality of the honeybee and its ability to find a dry surviving surface.
4. Check the ability to remember where the survival surface is and find it in less time.
5. Measure the endurance of swimming honeybees.

Results for Endurance Experiments

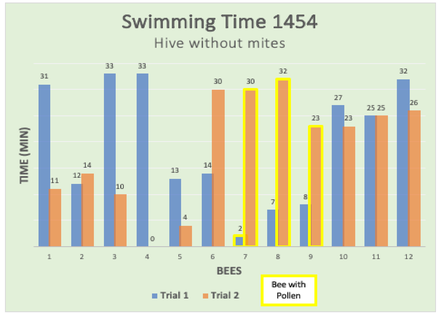

Figure 4 & 5. Comparison of resistance of bees during swimming between hive with mites and hive without mites.

Methods

Endurance Experiments

Figure 6. Twelve swimming pools.

Figure 7. Swimming bee seen from below.

Figure 8. Swimming bee with pollen.

Twelve bees were put to swim at the same time. → Each minute it was noted whether the bee was swimming or not. → After the test at 33 minutes, the bees were released.

Figure 9. Endurance Experiments Protocol.

Methods

Water Maze protocol

Figure 10. Symbols to help with spatial memory.

Figure 11. Security camera on the surface.

Figure 12. Bee swimming on our fist water maze model.

Figure 13. Rest cage and Bee Feeders.

Figure 14. Bee drinking 50/50 water sugar solution.

Figure 15. Second water maze model.

Findings

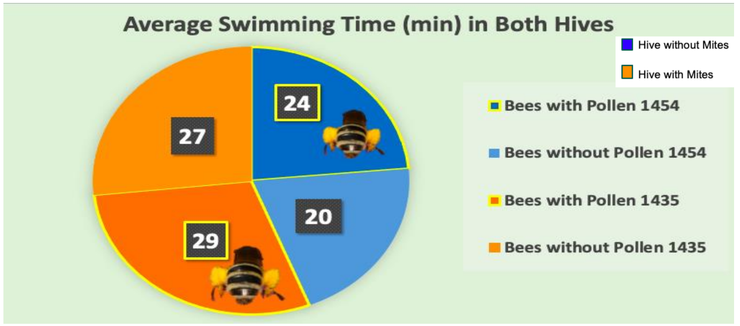

Figure 16. Average Swimming Time comparison.

1. *Aphis mellifera* bees have a resistance to keep swimming for more than 20 continuous minutes.
2. We had 33% bees that kept swimming for more than the thirty-three minutes of the study.
3. Our spatial memory tests could be up to 20 minutes long.
4. There is no significant difference between bees with pollen on their legs or not, nor between hives with mites and those without.
5. Further evidence is needed to define the presence of any directionality in bee swimming.
6. The learning protocol with the Morris water maze is viable for studies with bees, but there are still many details to perfect, such as:
   a. Improve controlled release of bees into water.
   b. Explore ways to hide the platform in the water without use of paint.
   c. Improve type of platform to provide better grip for the bees.

Acknowledgements

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References

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