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Observations of ethanol exposure on the queen honey bee *Apis mellifera anatoliaca* (Preliminary note)

Ibrahim CAKMAK[1], Charles I. ABRAMSON[2], Selvinar SEVEN-CAKMAK[3], Peter NENTCHEV[4], Harrington WELLS[5]
[1]*Beekeeping Development and Research Center, Uludag University, M. Kemalpasa MYO, Bursa, Turkey*
[2]*Laboratory of Comparative Psychology and Behavioral Biology, Departments of Psychology and Zoology, Oklahoma State University, Stillwater, OK, USA*
[3]*Uludag University Loj., Bursa, Turkey*
[4]*Department of Apiculture, Thracian University, Stara-Zagora, Bulgaria*
[5]*Department of Biology, Tulsa University, Tulsa, OK, USA*

Abstract

Previous data from this laboratory has shown that consumption of ethanol by worker honey bees disrupts learning, communication and social behaviours. We extend our honey bee model of ethanol induced behaviour by reporting preliminary observations on a queen honey bee (*Apis mellifera anatoliaca* Maa) which had consumed 20µl of a 10%, 2.0 M ethanol solution. Our observations reveal that the inebriated queen is accepted back into the colony but is replaced several days later by a new queen possibly as a result of decreased egg laying behaviour. The new queen – which was an offspring of the previous queen – had less hair on the thorax and an unnatural darker colour. Egg laying was also reduced as suggested by the smaller brood area compared to a control queen. Recommendations are provided when to conduct ethanol experiments with queens.

Key words: social insect model, *Apis mellifera anatoliaca*, honey bee, queen, ethanol.

Introduction

For a number of years we have been developing a social insect model of ethanol induced behaviour using honey bees as our model organism. Previous work has concentrated on the behavioural effects of ethanol on worker honey bees (*Apis mellifera* L.) and several effects common among humans and worker honey bees have been demonstrated. These effects include the ability to self administer ethanol and disruptions in locomotion, social behaviour, communication, complex decision making, and learning (Abramson *et al.*, 2000; 2005; 2007). Moreover, honey bees appear intoxicated at blood alcohol concentrations similar to humans (Bozic *et al*., 2007).

Ethanol consumption during pregnancy has profound effects on both the mother and her offspring in various mammalian species. These effects include learning deficits, weight changes, brain damage, hormonal changes, developmental and behavioural problems, and in the case of rodents reduced litter size (e.g., Sigh and Snyder, 1982; Hayward *et al*., 2004; Julien *et al*., 2008). The question naturally arises whether drinking ethanol also affects the honey bee queen and her offspring.

Materials and methods

In this preliminary report we restricted our observation to two colonies. This was done, in part, because we were confronted with the issue that when a queen dies there is a danger of the colony collapsing in late summer or fall (Laidlaw, 1992; Caron, 1999). Accordingly, one queen received 20 µl of 10% ethanol mixed with a 2.0 M sucrose solution and a second queen 20 µl of a 2.0 M sucrose solution. The second queen served as a control. An observational study with a small sample size can not provide the same type of data as an experiment. Nonetheless, it can answer some basic questions fundamental to subsequent experimental research such as whether a honey bee queen will actually consume ethanol and whether egg laying behaviour is affected.

Two sister queens of the same age (1 year old) (*Apis mellifera anatoliaca* Maa) were obtained from the apiaries of the Beekeeping Development and Research Center, Uludag University in Bursa, Turkey on July 15 and marked for identification. These colonies were maintained in an isolated apiary within the university and continuously examined for parasites and diseases such as mites (*Varroa destructor* Anderson et Trueman), chalkbrood, nosema, wax moth, foulbrood, and tracheal mites and no threats were found. These colonies had bottom board varroa screen and monitored regularly. They were treated in spring with flumethrin and had very low levels of varroa mites.

Observations were made from July 15 through September 30, 2008. Two colonies were used based on similarity in size of the honey bee population (9 frames of bees containing about 36,000 bees). Queens were first captured and kept in queen cages for one hour to increase their motivation to feed and we had interest in knowing whether an inebriated queen would be accepted back into the colony.

After one hour, one queen received 20µl of 10% ethanol mixed with a 2.0 M sucrose solution and the other served as a control receiving 20µl of a 2.0 M sucrose solution without ethanol. The rationale behind the use of a 10% solution was that behavioural effects of ethanol are easily observed (Abramson *et al*., 2000). After 30 minutes post ethanol or post sucrose ingestion, both

queens were returned to their colonies. Subsequently, the two colonies were checked every other day for two weeks thereby having 7 observation sessions. We determined: 1) if the queens were accepted by their respective colonies, 2) whether the queens laid eggs and 3) the health of larvae and pupae.

Results and discussion

We observed several effects. First, we were surprised that the queen consumed 20 µl of a 10% ethanol solution. It took approximately 10 seconds to consume the ethanol solution and is consistent with our data with worker bees (Abramson *et al*., 2000). This observation suggests that it is now possible to feed queens ethanol. This will lead to additional behavioural experiments on, for example, the effect of ethanol on the interaction of the queen and her attendants, the viability of her eggs, and the perception of worker bees on the quality of the eggs. Whether all queens readily feed on ethanol solutions is also an interesting question and it is not known whether there are subspecies differences in the ability of queens to consume ethanol. Our studies with worker honey bees from the United States, Brazil, Slovenia, and Turkey show that workers readily consume ethanol solutions up to 20% (Abramson *et al*., 2007).

Secondly, the queen was accepted into its colony after drinking the ethanol solution. There are several conditions in which a queen is not accepted back in the colony such as injury, age, and disease (Laidlaw, 1992). That the queen is accepted is also consistent with our previous results on the effect of ethanol on the social behaviour of worker honey bees (Bozic *et al*., 2006).

Thirdly, the queen receiving ethanol delayed the onset of egg laying by at least one day compared to the control queen. However, the area of larva and pupae produced by these two queens was not measured and remains to be investigated. Two weeks after ethanol ingestion, the queen was superseded by a new queen. We believe she was killed by worker bees and suspect that this was because she was laying fewer eggs. In effect, the worker bees detected the abnormal behaviour of the queen. There were only a few queen cups on the edges of the frames which is indicative that the queen will be replaced (Caron, 1999).

When the ethanol treated queen was found missing, we decided to remove the control queen as well. The purpose of this manipulation was to make an observation on the egg laying behaviour of a new queen which was the offspring of the ethanol treated queen. By removing the queen that previously received sucrose only both colonies were put under the same queen rearing and egg laying condition.

The colonies were subsequently checked from the beginning of August until the end of September, 61 days after the two original queens were replaced. Surprisingly, the queen that supplanted the ethanol drinking queen looked old, that is, containing less hair and darker colour even though she was only about one month old at the beginning of September (Laidlaw, 1992; Winston, 1992). We believe that this queen was attacked by worker bees as her mother may have been. She too, like her mother, laid fewer eggs. The status of larva and pupae were visually inspected and no abnormalities detected for both colonies. However, it was noted that the brood area was much smaller for the new queen whose mother received the ethanol compared to the new queen whose mother received sucrose only. We found a significant difference between the number of frames containing bees; only four frames of bees (about 16,000 bees) were found compared to eight frames of bees (about 32,000 bees) at the end of September.

Conclusion

That a honey bee queen can consume a large amount of high concentration ethanol, lay fewer eggs, and appear in poor health opens the door to new experiments on the effect of ethanol on development and reproduction. Our future work will be directed toward confirming these results experimentally and studying in detail the effect of ethanol on the developing larvae and pupae. We also have interest in looking at the effect of ethanol on drone semen since it is known that ethanol effects semen quality (Emanuele and Emanuele, 2001; Wallock-Montelius *et al*., 2007).

We would like to note that using queens may be problematic because, if consuming ethanol has a deleterious effect on egg laying, not only in the queen that consumes the ethanol but also her offspring, the number of worker bees may decrease. We suggest that ethanol experiments involving queens be conducted in the spring because new queens can be reared and drones are plentiful. In addition, potentially decreasing the number of bees in the summer and the fall will negatively affect over-winter survival of honey bee colonies (Caron, 1999). Because only the queen in a honey bee colony can reproduce and produce future generations, the effect of ethanol on queen reproduction and behaviour will have serious consequences for colony survival.

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Authors' addresses: Ibrahim CAKMAK, Uludag University, Beekeeping Development and Research Center, MKP MYO, 16500, M. Kemalpasa, Bursa, Turkey; Charles I. ABRAMSON (corresponding author: charles.abramson@okstate.edu), Oklahoma State University, Department of Psychology, 116 N. Murray, Stillwater, OK 74078, USA; Selvinar SEVEN-CAKMAK, Uludag University Loj., 16059, Gorukle, Bursa, Turkey; Peter NENTCHEV, Thracian University, Department of Apiculture, 6000, Stara-Zagora, Bulgaria; Harrington WELLS, Tulsa University, Department of Biology, Tulsa, OK 74104, USA.

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