DEFENCE STRATEGIES AND SELF MEDICATION IN HONEY BEE (*Apis mellifera*)

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Abstract

The defence strategies of the honey bee is strongly related to their behaviour. As a social insect, the risk of infection is very high, therefore, unless humans interfere, they have to deal themselves with the parasites, which sometimes could be very successful. A huge advantage is on the honey bee side, because there are thousands of individuals inside a colony, so they can unify their effort to eliminate the intruders on one hand. On the other hand, based on the honey bee – human relationship too, the honey bees have developed an evolutionary capacity of diagnose the disease and take some action. We here show that despite the virulence of some pathogens, the honey bees are capable of defend themselves and, sometimes, they can even resort to self medication.

Key words: Honey bee, Behaviour, Social insect, Medication

INTRODUCTION

The honeybee – a skilful machine that is perfectly adapted to different tasks, depending on what is needed in the nest, is a social insect exposed to a lot of pathogens. Living in large groups (up to 60.000 individuals in honey bee case) has many benefits. For example, some social insects such as ants or termites, due to this large ”families” have become dominant species in certain habitats [9]. On the other hand, such large groups of individuals are a perfect ”host” to many pathogens, because they can spread very easily [4], [5], [6], such as *Paenibacillus larvae*, the causing agent of lethal American foulbrood. The microsporidians, such as *Nosema ceranae* are also particularly severe when they are present in the nest, causing a energetic stress [1] and as a result, the bee colony might be in danger, especially in early spring, when the honey bees need all their capacity of flying and foraging to ensure the colony enough energy to face the climacteric conditions and to be able to feed the larvae in the hive. To understand better how the defence strategies work in a social group, we have to show how the parasite works. First, the parasite has to enter the nest. This might be done in two different ways: by approaching and actively entering the nest or just wait passively to be transported [7]. Once in the nest, the parasite has to multiply and to spread. Spreading the parasite and implicitly the disease might be done in two different ways: horizontal and vertical. The horizontal transmission way means that the colony members will receive the microbes via nestmates, the hive environment, or hive parasites. The best example is the microsporidian *N. ceranae*, which is spread among the colony members by exchanging the food between the individuals. [3], [7], [8]. The vertical transmission means that, for example, the honey bee queen transmit the viruses to the offspring [2], [10]. This way of transmission is very important and, unfortunately, nowadays, very less understood by the beekeepers that don't follow their queen genetic heredity and spread the viruses and the genetic malfunctions among other beekeepers. We here use the micorsporidian parasite *Nosema ceranae* as an experimental model system to infect nurse bees. *Nosema spp.* is a gut parasite of *Apis mellifera*, that enters, multiplies in and destroys the gut epithelium causing diarrhea and shortening

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the life span of workers. We compare the degree of infection with *N. ceranae* among two types of honey (linden tree and sunflower) two different cages. We also tested the antibiotic potential of these honeys to screen for any adaptive choice behavior.

MATERIAL AND METHOD

Combs with sealed worker brood were taken from a colony and workers were allowed to emerge in an incubator at 37°C, These freshly emerged bees were free of *Nosema* infections and were kept for 5 days in small cages and fed with 50% sugar solution ad libidum. After 5 days we performed a microscopically examination to confirm that there were no spores in bees' midguts. We slightly separated the head together with the midgut from the rest of the body and examined the midgut with the microscope at 40x. As a replicate, we also smashed 10-12 abdomens and mixed it with water. After that we filtered the mixture through a 10µm pore size filter paper, and examined 2µl of the resulting solution, using the same microscope at the same 40x resolution. No spores were detected in both cases. We want to use the freshly emerged bees as a control.

Infection with Nosema ceranae.

We isolated *N. ceranae* spores from a highly infected colony, using the following method: 30 to 40 abdomens were homogenized with a pestle for about 5 minutes and diluted in 20ml of water. We filtered the abdomen-water mixture through 10µm pore size filter paper. The filtrate was centrifuged in Eppendorf tubes for 10 min at 5000 rpm. We discarded the supernatant, and the remaining spore pellet was mixed again with 2ml of water and counted, using a Neubauer counting chamber. We used different dilutions to obtain a quantifiable spore number, which we have mixed with 50% sugar solution to feed the bees.

The bees were starved for 3 hours and subsequently individually fed workers were either fed with a sugar solution containing $8x10^4$ spores. After infection the bees were kept in cages for another five days, to ensure that the spores will be present in the midgut. After five days we ensured that the bees were infected by squeezing some abdomens and the content was under the 40x microscope. The spores were present, so we could move on with the experiment. We put the bees in three cages, in the first one the *N. ceranae* free bees as control, in the second one the infected bees free to feed themselves with linden tree honey, in the third cage infected bees free to feed themselves with sunflower honey. We checked the degree of infection in days 1, 4, 8 and 10. The result is shown in the figure 1:

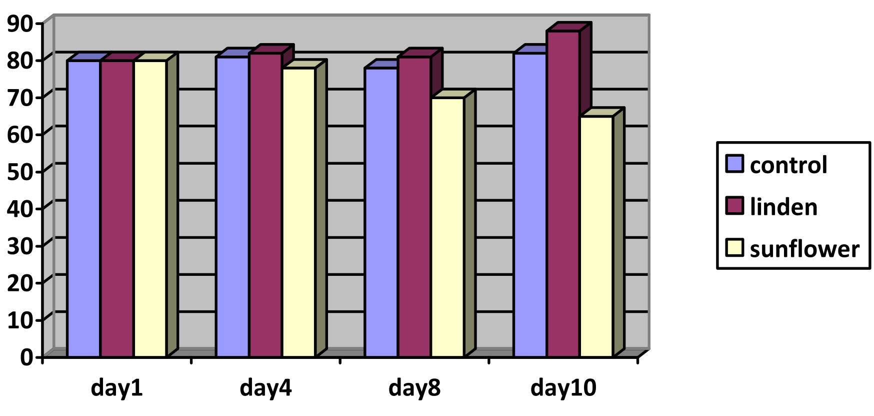

Fig. 1 Degree of infection among 10 days

RESULTS AND DISCUSSIONS

As we can easily see, the bees that fed themselves with sunflower honey recorded a slightly decrease of infection with the microsporidian *N ceranae*. In day 4, the infection was down to 78k spores, in day 8 to 70k spores and in day 10 to 65k spores. This result might lead us to the hypothesis that honey bees have the capacity to treat themselves, due to the evolution of the species. Also, we don't know yet what is the mechanism that makes the *N. ceranae* infection to decrease when honey bees are fed with sunflower honey. Anyway, this is a very good starting point to dig in for the solution to have healthier honey bees. In the same time, we can observe that the infection slightly increases when the honey bees were fed with linden tree honey or in case of the control group, which is quite normal, because, in natural condition, when honey bees feed themselves with nectar, the infection with the microsporidian *N. ceranae* increases, if the beekeepers don't interfere. Of course, it is of good interest to test more types of honey, in order to find out if there are benefits in using that certain type as an alternative to classic medication, which in some cases is not well received. It is obvious that this behaviour, combined with the study of immune system, can contribute to understand how the regulatory system is organized in general [7].

CONCLUSIONS

As we know, there is a huge difference between what is happening in honey bee natural environment and what is happening inside the hive. The honey bee behaviour has a lot of "question marks", starting from the communication between individuals to foraging behaviour, hygienic behaviour and pathogen defence behaviour. With this study we have proved that even if the environment is, sometimes, a starting point to infections and a lot of other undesired threats. We want this study to be very good understood by the scientific community as well as by the beekeepers who might think better for a practical solution to let the honey bee work for itself, not only for them.

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