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Effects of *Nosema* on Honey Bee Behavior and Physiology

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Currently there are two species of *Nosema* infecting the Western honey bees, *Apis mellfiera*. *Nosema apis* is the first species described by Zander in 1909. In 1995, Ingmar Fries described a new species of *Nosema* in the Asian honey bee, *Apis cerana*, thus it was named *Nosema ceranae*. It was thought that *N. ceranae* mostly infected *A. cerana*, although there was a mention that *A. mellifera* could become infected with *N. ceranae* under laboratory conditions. In 2005, natural infection of *N. ceranae* was reported in *A. mellifera* colonies from Taiwan (Huang et al., 2005). Shortly thereafter, the infection of *N. ceranae* to *A. mellifera* was reported in Europe, United States, China, and worldwide. In this article I will review old studies done on *N. apis* and recent studies on *N. ceranae*, from a behavioral and physiological perspective. For basic biology, diagnosis and control of *N. ceranae*, please refer to Tom Webster's article at **http://www.extension.org/pages/27064/*Nosema*-ceranae-the-inside-story**.

1. Differences among the two species

The differences between the two *Nosema* species are listed in Table 1, but I will only discuss differences in morphology and symptoms here, other differences are dealt with in more detail in the following sections. *N. ceranae* spores are slightly smaller, less symmetrical and the two ends are sharper, compared to *N. apis* spores (Fig. 1). Cross sections of *N. ceranae* spores show a fewer number of coils of polar filament compared to *N. apis* spores. *N. ceranae* is more resistant to high temperature, Fenoy et al (2009) claims that at 60°C for 1 month, over 90% of *N. ceranae* spores were still viable. *N. ceranae* spores lost ~90% infectivity at freezing temperature for one week, while *N. apis* spores retained 100% of its activity. The typical symptoms for *N. apis* infection are also lacking in *N. ceranae* infected bees: such as defecation near or inside the hive entrance during winter (Fig. 2), and milky color of midgut in heavily infected bees (Fig. 3). Spanish studies claim that *N. ceranae* infection rates do not show the typical changes with season, while prior studies of *N. apis* indicate that infection rate drops down in summer but stays high in spring.

2. Is *N. ceranae* more virulent than *N. apis*?

In a widely cited study conducted in Spain (Higes et al. 2007), bees infected with *N. ceranae* in the laboratory cages showed 94.1% and 100% mortality seven and eight days after inoculation, respectively. Although *N. apis* was not compared in the same experiment, by comparing cage studies using *N. apis* conducted earlier, it was suggested that *N. ceranae* is much more virulent than *N. apis*. More recent studies from other laboratories failed to see this difference. Forsgen and Fries (2010) compared the mortality of bees infected with either species and did not see a difference between

Table 1. Differences between *N. apis* and *N. ceranae*.

	N. apis	*N. ceranae*	Section
Spore size	6 x 3 μm	4.4 x 2.2 μm	1
Spore morphology	Ends rounded, symmetrical	Ends sharper, less symmetrical	
Polar filament coils	>30	18-21	1
Resistance to high temperature	Low	High	1
Resistance to freezing	High	Low	1
Seasonal fluctuations	May have infections in summer	Lack of seasonality	1
Defecation near or inside the hive	Yes	No	1
Milky color of midgut	Yes	No	1
Virulence	Low	Higher	2
Earlier foraging	Yes	unknown	3
Enhanced JH production	Yes	unknown	3
Homing ability	Likely reduced	Reduced	4
Energy cost	Low	High	4
Immuno-supression	No	Yes	4

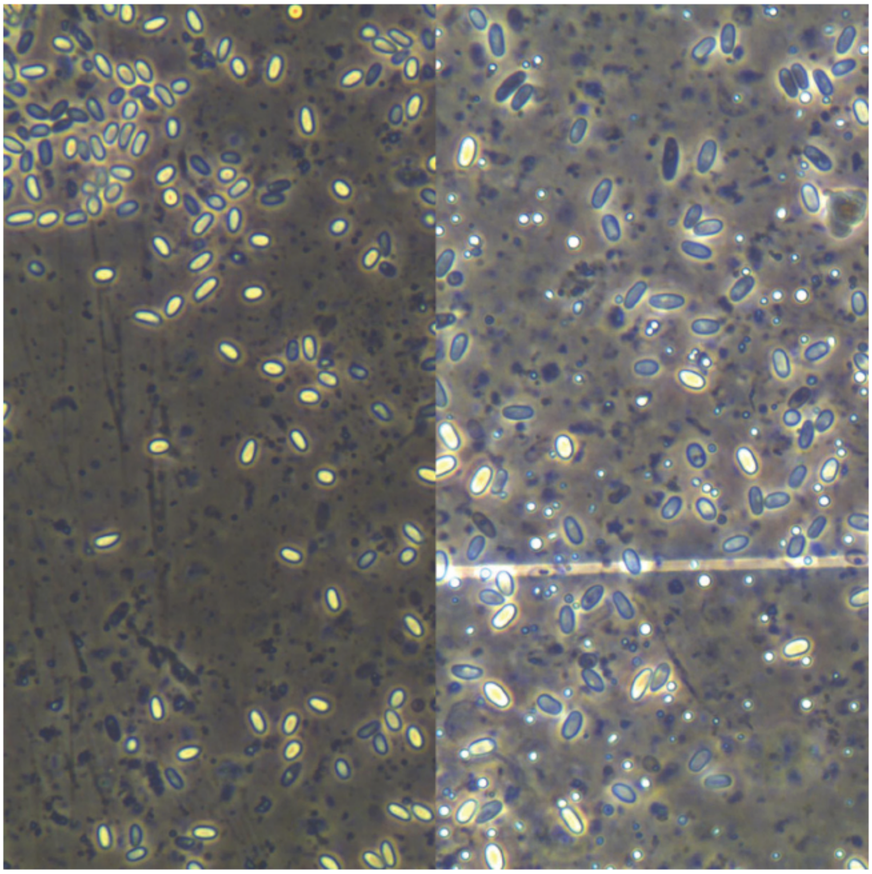

Fig. 1. Spores of *Nosema ceranae* (left) and *Nosema apis* (right) and under the same magnification (400x) using a compound microscope. *N. ceranae* is about 20% smaller and more almond shaped.

Fig. 2. Defecation on hive bodies by bees with dysentery. This occurs in the Midwestern U.S. in March to May. Dysentery can be caused by many factors, one of which is a *N. apis* infection. *N. ceranae* seems to cause this symptom occasionally, but it is not clear if it is due to mixed infection with *N. apis.*

the two. The MSU honey bee laboratory also failed to see a difference in mortality between bees infected with either species of *Nosema* (Z.Y. Huang, unpublished data). It is not clear whether the Spanish *Nosema* strain is more virulent, or whether the Spanish honey bees (*Apis mellifera iberica*) are more susceptible to *N. ceranae*.

3.Effects by *Nosema apis*

3.1. Effects on workers and queens

In the 1990s, T.P. Liu in Canada conducted many studies, most of them at the ultra-structural level on the effects of *N. apis* on honey bees. His studies indicated that workers infected with *N. apis* show ultrastructural changes in the cells from midgut epithelium, hypopharyngeal glands, and corpora allata (sources of juvenile hormone). Oöcytes in queens infected with *N. apis* for only 7 days were already degenerated. The ovariole sheath became wrinkled. In the oöplasm, yolk granules broke down into small spheres and granular substances and the oöcytes became extensively autolysed. It is not clear whether the oocyte degeneration in infected queens is due to a pathological process, a lack of protein nutrition, or to increased juvenile hormone production as a result of *Nosema* infection (see below).

3.2. *N. apis* causes earlier foraging, and higher JH production

Worker bees infected by *N. apis* have smaller hypopharyngeal glands and show an earlier regression in gland size than un-infected bees. In addition, *Nosema* infected bees show a more rapid behavioral maturation than un-infected bees (Wang and Moeller, 1970). Infected bees also guarded more frequently, and performed the following behaviors earlier than un-infected bees: orientation flight, dance following, and foraging. Infected bees also show a decreased tendency to feed the queen. As predicted by a theoretical model, which states that workers should take more risks when they are parasitized or were "not as worthy to the society" as others, Woyciechowski and Kozlowski (1998) demonstrated that *N. apis* infected workers showed more foraging activity than healthy foragers during adverse weather conditions.

The changes in *N. apis* infected bees are very similar to those induced by artificially applying juvenile hormone (JH), which is low in nurses but high in foragers. Huang et al. (2001) studied whether the earlier foraging in infected bees was due to an early rise of JH levels. They found that infected workers foraged at an earlier age and showed higher haemolymph juvenile hormone (JH) titers than control bees in preforaging bees. This suggests that *N. apis* infection induces workers to forage earlier via higher JH titers. The higher JH titers could be achieved by several alternative mechanisms: enhanced JH production by host corpora allata (CA), reduced JH degradation, or JH production by *Nosema* directly. The same study found that rates of *in vivo* JH biosynthesis as well as JH degradaton were higher in *Nosema*-infected bees than in control bees. Workers with their source of JH (CA) removed, but fed *Nosema* had no detectable levels of juvenile hormone in hemolymph and these bees did not forage early. These results suggest that *Nosema*-infected workers forage at an earlier age than control bees due to higher JH titers, which arise through increased JH production, and despite the increased JH degradation in infected bees. The data also suggested that *Nosema apis* does not produce JH directly.

Most likely the smaller hypopharyneal glands and earlier foraging in *N. apis* infected workers are due to the fact that *Nosema* infects the epithelial cells of the midgut, therefore greatly reducing the host's ability to digest pollen, which is needed for the development of glands. Impaired protein metabolism is deduced by lower proteolytic activity of the mid-gut, lower amount of amino acids in hemolymph, lower levels of proteins in the fat bodies (reviewed by Kralj and Fuchs, 2010) and lower protein levels in hemolymph in infected bees (Z.Y. Huang and T. Zhou, unpublished data). Inadequate nutrition could fail to increase vitellogenin, which normally inhibits JH production. Therefore, JH increases prematurely in these bees with poor protein nutrition.

It is not clear whether *N. ceranae* causes the same changes in workers as *N. apis* or not: e.g. earlier foraging and higher JH production. However, based on that fact that both species affect the midgut epithelial cells, *N. ceranae* will most likely cause the same early foraging and enhanced JH production in workers. This is now being studied at Marla Spivak's laboratory.

4. Effects by *N. ceranae*

4.1 Learning and homing behavior affected by *N. ceranae*

When Kralj and Fuchs (2010) studied the homing behavior of bees mainly infected with *N. ceranae*, some bees were coinfected with *N. apis*. They found that infected bees released 6 and 10 m away from the colony took longer times to return. The percentage of bees that did not return home was higher in the infected bees compared to the healthy bees when released 30 m away from the colony. They also found a lower rate of infected bees among the returning foragers compared to departing foragers, suggesting some infected bees did not return home successfully. It is not clear why infected bees did not return home as well. The study used bees of known ages, so this is not because infected bees were developing precociously. The alternative is that infected bees did not have proper protein nutrition which affected their brain development and capacity of learning. It is not clear whether *N. apis* causes the same effect in honey bee learning and homing behavior. We have tried to determine if *N. apis* infected bees drifted more to surrounding colonies, but failed to find if this is the case (Z.Y. Huang and H. Lin, unpublished data).

4.2. *N. ceranae* causes higher energy costs

Mayack and Naug (2010) compared the effect of energy stress on healthy and *N. ceranae*-infected bees and found that *N. ceranae* infection caused an energy stress in bees from several lines of evidence. First, bees infected with *N. ceranae* were more responsive to sucrose solution (not as picky as healthy bees) and would extend their proboscis at a lower sugar concentration. Second, infected bees consumed about 87µl of 30% sucrose solution in 24 hours, while healthy bees consumed only 60µl. Therefore, it appears that *N. ceranae* made the bees more "hungry" and caused them to drink more syrup. Third, control bees fed with 5, 10, 20 or 30 µl of syrup survived better compared to infected bees fed the same amounts during a 24-hour period. However, if bees were fed *ad libitum*, or not fed at all, the two groups did not show any difference in their survival. The authors therefore concluded that *N. ceranae* caused energy stress in infected bees, and speculated that this might be the main reason for the shortened survival of infected bees inside colonies.

Martín-Hernández et al. (2011) compared the energy cost of both *Nosema* species. Using caged bees, they showed that both mortality and sugar syrup consumption are the highest in *N. ceranae* infected bees, intermediate in *N. apis* infected bees and lowest in uninfected control bees. This study further demonstrates that *N. ceranae* has subtly different effects on honey bees compared to *N. apis*.

4.3. *N. ceranae* causes immune suppression

Antúnez et al. (2009) studied the immune response of honey bees after infection with either *N. apis* or *N. ceranae*. They measured gene expressions of several antibiotic peptides, abaecin, defensin and hymentoptaecin, produced inside honey bees after bacterial infection. In all three genes, *N. apis* infection caused an elevation of gene expression in either 4 or 7 days post infection, but *N. ceranae* did not show any difference in gene expression compared to the control (uninfected bees), or even significantly reduced it (abaecin at 7 days). These data suggest that *N. ceranae* actively suppresses the immune response in infected honey bees, while *N. apis* does not.

Alaux et al. (2010) studied whether a neonicotinoid (imidacloprid) and *Nosema* (a mixture of both species) would show a synergistic interaction in affecting honey bees. They found that the combination of both agents caused the highest mortality and food consumption. They also found that the activity of glucose oxidase, an enzyme bees use to sterilize the colony and brood food, was significantly decreased only by the combination of both factors compared with control, *Nosema* or imidacloprid-only groups, suggesting a synergistic interaction between the two agents. Because the combined group showed similar *Nosema* spore counts to that of *Nosema*-infected bees alone, it seems that the synergistic effect is due to the immune suppression of *N. ceranae*, causing bees to be more sensitive to

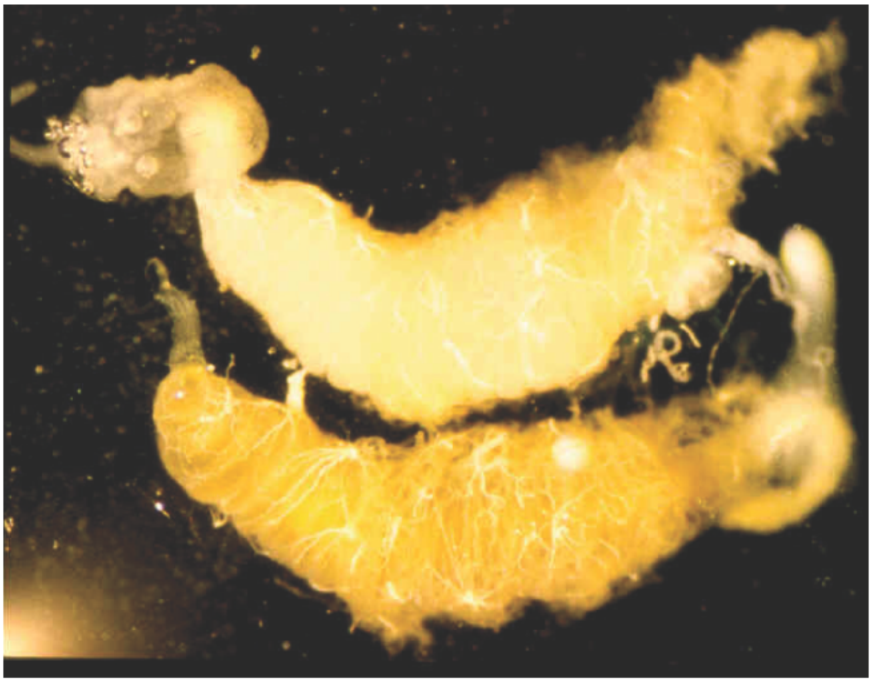

Fig. 3. Midgut (ventriculus) tissue of a bee infected by *N. apis* (top) and a healthy bee (bottom). Healthy bee midguts are straw colored, translucent and ring-like structure can be seen, while infected midguts are milky and the structures are not as clear. It has been said that *N. ceranae* infection does not show this symptom, which is typical of *N. apis*.

the pesticide, rather than the pesticide reducing bee resistance to allow more severe damage by *Nosema*.

In a more recent study, Vidau et al. (2011) found a similar synergistic effect between pesticides and *N. ceranae*. After being exposed to sublethal doses of fipronil or thiacloprid, *N. ceranae*-infected bees showed a higher mortality than in uninfected ones. The synergistic effect of *N. ceranae* and insecticide on honey-bee mortality was not linked strongly to a decrease of the insect detoxification enzymes. This is because *N. ceranae* infection induced an increase in glutathione-S-transferase activity in the midgut and fat body, but not in the 7-ethoxycoumarin-O-deethylase activity. It is not clear how tightly the insect detoxification system and the immune system are linked – they might well not be tightly linked since one is induced by pesticides and another by parasites.

4.4. *N. ceranae* affects queen health

Alaux et al. (2011) studied the effect of *N. ceranae* infection on 8-day-old honey bee queens. They found that *N. ceranae* did not affect the fat body content, which is an indicator of energy stores, but changed the vitellogenin titer, which is an indicator of fertility and longevity, the total antioxidant capacity and the queen mandibular pheromones. The strange thing is that these changes were contrary to the predicted direction in that they were all increased in *Nosema*-infected queens. It is possible that these are only seen in 8-day-old queens, perhaps due to accelerated development as seen in *N. apis*-infected worker bees. It is not clear whether in older queens these changes will remain or reverse themselves.

5. Conclusions

Studies on *N. apis* were mostly done during the 1970-1990s, but there has been a huge interest in *N. ceranae* since 2007, especially after Colony Collapse Disorder (CCD) appeared in this country. *N. ceranae* infection by itself does not seem to explain colony loss in the US, but in Spain it was speculated to be the main cause for CCD. There are clear differences in how the two species of *Nosema* affect our bees (Table 1). However, there are still many unanswered questions in the biology, epidemiology, and pathology of *N. ceranae* despite these many studies. We are not even certain of how many of the old studies attributed to *N. apis* might be actually from *N. ceranae*, and how long *N. ceranae* has been in the U.S. Until now we did not have a single sample showing that prior to a certain time, we only had *N. apis* and *N. ceranae* came after that point. The notion that *N. ceranae* is replacing *N. apis* is also based on circumstantial evidence, however with ongoing monitoring of whether and how *N. apis* is slowly disappearing, this might help clarify the answer.

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