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Short communication

Susceptibility of four different honey bee species to *Nosema ceranae*

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ABSTRACT

In this study, we investigated the infectivity of *Nosema ceranae* and the immune response of the European honey bee, *Apis mellifera* and the Asian honey bee species, *Apis cerana*, *Apis dorsata* and *Apis florea* when inoculated with two isolates of *N. ceranae* isolated from different climates (Canada and Thailand), using cage experiments. The results indicated that the local isolate of *N. ceranae* (Thailand) had high infectivity in *A. mellifera*, *A. cerana* and *A. dorsata* but only a few spores were observed in *A. florea*. However, we found that only two honey bee species, *A. mellifera* and *A. dorsata* became infected when inoculated with *N. ceranae* isolated from Canada. Finally, our results showed that transcript levels of antimicrobial peptides (AMPs) in Asian honey bees were significantly higher than that of *A. mellifera* in both the control and *N. ceranae* inoculated bee groups. Comparing the expression of AMPs between the control and inoculated bees in each species, it was evident that *N. ceranae* inoculations did not affect the expression level of abaecin in all four honey bees species investigated in this experiment. Nevertheless, we found a significant up-regulation of apidaecin in *A. cerana* and *A. florea* when inoculated with *N. ceranae* (Canadian isolate). Also, the mRNA levels of hymenoptaecin were significantly increased in *A. cerana* after inoculation by *N. ceranae* isolated from Canada as compared with the Thai isolate.

1. Introduction

Nosema ceranae, a microsporidian parasite first known to infect the cavity nesting Asian honey bee, *Apis cerana* (Fries et al., 1996), has become a cosmopolitan species in the European honey bee, *Apis mellifera* (Fries et al., 2006; Cox-Foster et al., 2007; Klee et al., 2007). Moreover, *N. ceranae* has been found to infect two additional Asian honey bee species, the giant Asian honey bee (*Apis dorsata*) and the dwarf Asian honey bee (*Apis florea*; Chaimanee et al., 2010). This previous study also showed that the *N. ceranae* infection rate in *A. mellifera* is higher than that in Asian honey bee species in the natural environment, possibly due to the differences in the host response to infection. There is a significant applied and fundamental interest in clarifying *N. ceranae* infection dynamics and host immune responses to infection.

After honey bees are infected by pathogens, they enlist mechanisms to protect themselves from these infections including mechanical, physiological and immune responses (Evans and Spivak, 2010). Mechanical defense involves the epithelial layers which prevent pathogens from entering or invading the body. Moreover, changes in gut chemicals and pH can inhibit pathogen invasion (Crailsheim and Riessberger-Galle, 2001). Finally, the cellular and humoral immune responses are another strategy used by honey bees to eliminate pathogens. Cellular immunity includes phagocytosis, encapsulation and

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melanization (Osta et al., 2004) while humoral immunity relates to the secretion of antimicrobial peptides and other effectors as a defense against pathogens. There are many studies on immune responses of honey bees to infections by bacteria, viruses, fungi and parasites. When honey bees are infected by pathogens, different antimicrobial peptides including, apidaecin, abaecin, defensin and hymenoptaecin are synthesized (Casteels et al., 1989, 1990, 1993; Casteels-Jonsson et al., 1994). Expression of the antimicrobial peptide-encoding genes abaecin and defensin was investigated by Evans (2004) in honey bee larvae infected by the bacteria *Paenibacillus larvae*. Yang and Cox-Foster (2005) reported that the infestation of parasitic mite, *Varroa destructor* suppressed antimicrobial peptide expressions in *A. mellifera*. Moreover, the effect of *Nosema* infection on honey bee immunity was also investigated. Antúnez et al. (2009) demonstrated that strong *N. ceranae* infections suppress the immune response of *A. mellifera* when compared to the *Nosema apis* infection and control honey bees. In a recent study, Chaimanee et al. (2012) reaffirmed that infection of *N. ceranae* isolated from United States suppressed antimicrobial peptides in *A. mellifera*. However, little is known about the immune response of Asian honey bees when infected with the pathogens. Suwannapong et al. (2011) examined cross infection of *N. ceranae* isolated from *A. florea* in *A. cerana* with different doses (10,000, 20,000 and 40,000 spores/bee) of *N. ceranae* spores. Their results showed that *N. ceranae* isolated from *A. florea* cross-infected *A. cerana* and affected the hypopharyngeal protein production and increased the mortality of *A. cerana*. However, the infectivity between *A. florea* and *A. cerana* was not determined in the study.

The objective of this study was to compare the infectivity of *N. ceranae* from different geographic origins in four different honey bee species. Also, the differential expressions of antimicrobial peptide genes of each honey bee species when exposed with *N. ceranae* were investigated.

2. Materials and methods

2.1. Honey bee species

Four honey bee species (*A. mellifera*, *A. cerana*, *A. dorsata* and *A. florea*) were used in this study. The experiments were conducted in March 2011 at the Bee Protection Center (BEEP), Department of Biology, Faculty of Science, Chiang Mai University, Thailand. Frames of sealed brood were obtained from three colonies of each honey bee species from Chiang Mai, Thailand. The colonies had not presented any visible clinical symptoms of disease. Before the experiments, fifty bees from each colony were checked for *Nosema* spores under light microscopy and were further confirmed to be free from *Nosema* by PCR method as described by Chen et al. (2008).

2.2. Inoculum preparation

In this study, two isolates of *N. ceranae* were used. *N. ceranae* spores were isolated from *A. mellifera* colonies infected with *N. ceranae* obtained from apiaries in Canada and in Thailand. The Canadian isolate was provided by Dr. Steve Pernal from Agriculture and Agri Food in Beaverlodge, Alberta, Canada and transported to Thailand as pelleted spores. The Thai isolate was obtained from heavily infected *A. mellifera* workers collected from the Bee Protection Center in Chiang Mai province. The midguts of individual infected bees were removed and crushed in distilled water. Spore numbers were estimated by counting spores with light microscopy after the method of Cantwell (1970). *N. ceranae* was confirmed as the infecting species using PCR amplification after the methods described by Martín-Hernández et al. (2007) and Chen et al. (2008). Additionally, the inocula were prepared by mixing spores with 50% (w/v) sterile sucrose solution to obtain a final concentration of 10^6 spores/ml.

2.3. N. ceranae infection experiment

Brood combs were put in an incubator at $34 \pm 2\,^{\circ}C$ and following eclosion the newly emerged worker bees from each of three colonies per species were divided into three groups of twenty bees. Two groups of bees were inoculated with either strains of *N. ceranae* (Canadian and Thai isolates) and another group was fed with only 50% (w/v) of sterile sucrose solution as a control group. The worker bees in each group were placed in new screen cages. The top and the bottom of the cages were made from plastic plates. The top of each cage had a single hole that allowed for a 15 ml centrifuge tube containing sucrose solution (3.5 ml) to serve as a food source. The sucrose solution (3.5 ml) with 10^6 spores/ml (the infectious dose) of *N. ceranae* was used to feed bees for the first 2 days (bees consumed about 100,000 spores each). All cages were kept in an incubator at $32 \pm 2\,^{\circ}C$. After exposure to *N. ceranae*, worker bees were fed ad libitum with a 50% (w/v) sucrose solution throughout the remainder of the experiment. Our exposure method for honey bees was conducted to mimic natural infection in which each bee may get exposed to spores in different amounts over time and through trophallaxis. Control groups were fed with a 50% (w/v) of sucrose solution with no *Nosema* spores. Mortality was recorded daily during experiments. Six bees from each cage were collected at day 6 post-inoculation and kept in a $-20\,^{\circ}C$ freezer until RNA extraction.

Development of *N. ceranae* in different honey bee species was examined via spore counting. The whole abdomen of an individual worker was homogenized in 1 ml of distilled water and *N. ceranae* spores were estimated using a haemocytometer (Cantwell, 1970).

2.4. RNA isolation and cDNA synthesis

After the gut was removed, total RNA was isolated from the rest of the abdomens of individual bees using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. DNA was removed using DNase I (Invitrogen, Carlsbad, CA) incubation at room temperature for 15 min. The DNase I was then inactivated by the addition of 1 μl of 25 mM EDTA solution and incubated at $65\,^{\circ}C$ for 10 min. First-strand cDNA was generated from approximately 5 μg total RNA using SuperScript™ III First-Strand