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Original article

Effects of host age on susceptibility to infection and immune gene expression in honey bee queens (*Apis mellifera*) inoculated with *Nosema ceranae*

Veeranan CHAIMANEE[1], Panuwan CHANTAWANNAKUL[2,3], Yanping CHEN[4], Jay D. EVANS[4], Jeffery S. PETTIS[4]

[1]Section of Biotechnology, Maejo University Phrae Campus, Rong Kwang, Phrae 54140, Thailand
[2]Bee Protection Center, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[3]Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[4]USDA-ARS Bee Research Laboratory, Bldg. 306 BARC-E, Beltsville, MD 20705, USA

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Abstract – *Nosema ceranae* is a microsporidium parasite infecting honey bees worldwide. All colony members including workers, drones, and queens can become infected. In this study, we inoculated queens of age 1, 6, and 12 days post-adult emergence, with *N. ceranae* spores of different doses and allowed them to age an additional 12 days. The results indicated that younger queens were indeed more susceptible to *N. ceranae* inoculation than older queens. Moreover, this is the first report of the effects of *N. ceranae* inoculation on immune gene expression in queens of different ages. Our results demonstrated that the expression of genes related to the bee immunity *apidaecin*, *eater*, and *vitellogenin* in the gut and the remaining abdomen was different among queens of different ages when inoculated with *N. ceranae*. All three ages of queens inoculated by *N. ceranae* showed upregulation of *apidaecin* in gut tissue 6 days after inoculation, but only in queens aged 1 day post-emergence were the differences significant. However, transcript levels of *eater* were increased in all three ages of queens when sampled on day 12, and significant differences were obtained in queens inoculated at 6 and 12 days post-emergence. We clearly show that immune responses to *N. ceranae* changes as queen age and this knowledge may provide clues for understanding the ability of queens to resist infection by this gut parasite.

Nosema ceranae **/ honey bee queen /** ***Apis mellifera*** **/ supersedure / immunity**

1. INTRODUCTION

Two microsporidian parasites, *Nosema apis* and *Nosema ceranae*, have been reported to cause *Nosema* disease of honey bees. *N. apis* was first described in the European honey bee, *Apis mellifera* (Zander 1909) and *N. ceranae* was first known to infect the Asian cavity nesting honey bee, *Apis cerana* (Fries et al. 1996). However, recent studies have shown that *N. ceranae* is becoming the dominant species infecting *A. mellifera* worldwide and may be more virulent than *N. apis* (Fries et al. 2006; Higes et al. 2006; Chauzat et al. 2007; Cox-Foster et al. 2007; Huang et al. 2007; Klee et al. 2007; Paxton et al. 2007; Chen et al. 2008; Williams et al. 2008; Chaimanee et al. 2010). *Nosema* infection occurs when spores are ingested through contaminated food and water

Corresponding author: J. Pettis,
jeff.pettis@ars.usda.gov
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(Webster 1993). The spores can germinate and develop in the epithelial cells of the midgut of all colony members including workers, drones, and queens. Following *N. ceranae* infection, the epithelial ventricular cells of infected honeybees were degenerated (Higes et al. 2007). *N. ceranae* has been detected not only in the midgut tissue but also other tissues including Malpighian tubules, hypopharyngeal glands, salivary glands, and fat bodies using a PCR method (Chen et al. 2009). *N. ceranae* can change the physiology and behavior of honey bees (Dussaubat et al. 2013; Goblirsch et al. 2013). *N. ceranae* infection has been reported to suppress the honey bee immune response (Antúnez et al. 2009; Chaimanee et al. 2012) and to affect hormone production by increasing ethyl oleate content (Dussaubat et al. 2010). Moreover, *N. ceranae* induces an energetic stress (Mayack and Naug 2009; Alaux et al. 2010; Martín-Hernández et al. 2011) and oxidative stress in honey bees (Dussaubat et al. 2012) and decreases the carbohydrate level in honeybee forager hemolymph (Mayack and Naug 2010). Similar to *N. apis* infection, honey bees, when infected with *N. ceranae*, have a shorter life span (Malone et al. 1995; Goblirsch et al. 2013).

Since a honey bee queen is the primary reproductive of the colony and her pheromones and presence produce cohesion between colony members, *Nosema* infection in queens could cause serious problems for the colony. A honey bee queen is susceptible to *N. apis* infection. In commercial queen production, a queen may become infected in the mating nuc or colony via spore ingestion from infected attendants bees in shipment or upon placement in a new colony. *Nosema*-infected queens have smaller ovaries when compared to the non-infected queens (Farrar 1947). Eggs of infected queens show decreased hatching rates and queens are often rapidly superseded once they become infected (Farrar 1947). Similarly, *N. ceranae* can be transmitted horizontally from infected worker bees to queens and can affect the epithelial ventricular cells of queens (Higes et al. 2009). Low levels of *N. ceranae* were detected in tissues of the head, thorax, abdomen, and ovaries from naturally infected queens (Traver and Fell 2012). Recently, Alaux et al. (2011) explored the effects of *N. ceranae* on honey bee queen physiology. Their results demonstrated that *N. ceranae* infection affected the physiological functions and modified pheromone production of queens. This may explain the rapid replacement of infected queens.

In this study, to demonstrate the susceptibility of queens to *N. ceranae* infection, we determined the rate of *N. ceranae* infection in queens of different ages (queens aged for 1, 6 and 12 days post-adult emergence). We hypothesized that younger queens would be more susceptible to *N. ceranae* infection than older queens. The 12-day period was selected as it corresponds with the time that queens often spend in small nucleus colonies for mating before being shipped to the beekeeper. If queens are more susceptible during this initial period of adult life then infection-mitigation measures could improve queen health and queen longevity in colonies. Moreover, even though there are some published studies about the effects of *N. ceranae* on immune response of honey bees, little is known about molecular defense mechanisms of honey bee queens to defend themselves from pathogen infections. Hence, the immune responses of honey bee queens to *N. ceranae* infection were also investigated.

2. MATERIALS AND METHODS

2.1. Honey bee queens

The experiments were conducted in August 2010 in Beltsville, MD. Queen cells containing queens of mixed *A. mellifera mellifera*/*A. mellifera ligustica* stock were obtained from a single queen breeder in Georgia, USA. Queen cells were placed individually in new wooden Benton cages and held at 34±2 °C. The queens emerged within 12 h and were held in individual new cages containing three newly emerged worker bees from a colony with no detectable *Nosema*. Queens aged 1, 6, and 12 days post-adult

emergence were used in this study. Upon emergence, ten queens were randomly sampled and checked for the presence of microsporidian spores using light microscopy and diagnostic PCR amplification (Chen et al. 2008).

2.2. Inoculum preparation

On each day of inoculation, *N. ceranae* spores were isolated from a colony infected with *N. ceranae* obtained from an apiary at the Bee Research Laboratory, USDA-ARS in Maryland, USA. The midguts were removed and crushed in 1 ml of distilled water and *N. ceranae* was confirmed using PCR amplification following methods described by Chen et al. (2008). Additionally, the numbers of spores were estimated by counting spores with light microscopy after the method of Cantwell (1970). The inoculum was freshly prepared in various concentrations by mixing with 50 % sucrose solution to obtain a final concentration of 10^3, 10^4, 10^5, and 10^6 spores/ml and maintained at room temperature until used for inoculation on that day.

2.3. *N. ceranae* inoculation experiment

Queens of three ages: 1, 6, and 12 days post-adult emergence, were used in this study. Each age group of queens (n=150 total) were held individually in new standard wooden queen cages with three newly emerged uninfected attendants. The top of each cage had a single hole that allowed for a 0.5-ml tube containing sucrose solution (500 μl) to serve as a food source. For each age group, 30 queens were fed with 50 % sucrose solution containing 10^3, 10^4, 10^5, and 10^6 spores/ml of *N. ceranae* for the first 2 days and the control group was fed with only 50 % (*w*/*v*) of sucrose solution. After exposure to *N. ceranae*, queens were fed ad libitum with a clean 50 % (*w*/*v*) of sucrose solution throughout the remainder of the experiment. Six queens in each group were collected at day 6 and 12 post-inoculation to be examined for *N. ceranae* infection. The individual queen guts were homogenized in 1 ml of distilled water and *N. ceranae* spores were estimated using a hemocytometer (Cantwell 1970).

2.4. Effects of *N. ceranae* inoculation on expression of genes related to the bee immunity

2.4.1. RNA isolation and cDNA synthesis

Total RNA was isolated from the rest of gut and remaining abdomen tissues of the individual queens (control queens and queens when the cage in which the queen was received a spore suspension with 10^5 and 10^6 spores/ml of *N. ceranae*) using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. DNA was removed using DNAse I incubation at 37 °C for 1 h followed by 10 min at 75 °C. First-strand cDNA was generated from approximately 2 μg total RNA using a master mix containing 50 U Superscript II (Invitrogen, Carlsbad, CA), 2 nmol dNTP mix, 2 nmol poly(dT)$_{18}$, and 0.1 nmol poly(dT)$_{(12–18)}$. Synthesis was carried out at 42 °C for 50 min followed by 15 min at 70 °C as described in Evans (2006).

2.4.2. Quantitative PCR amplification

Real-time quantitative PCR amplification was performed in a 20-μl reaction mixture using the EXPRESS SYBR® GreenER™ qPCR SuperMix Universal (Invitrogen) and 0.2 μM of each specific primer. The oligonucleotide amplification primers are shown in Table I. The PCR reactions were carried out in 96-well microtiter plates using Bio-Rad Icycler (Bio-Rad Crop., Hercules, CA). The amplification was programmed as follows: 95 °C for 2 min followed by 40 cycles of 95 °C

Table I. Oligonucleotide primers used in this study for real-time quantitative PCR (Simone et al. 2009).

Primer name	Sequence 5′ to 3′
Actin-F	TTGTATGCCAACACTGTCCTTT
Actin-R	TGGCGCGATGATCTTAATTT
AmEater-F	CATTTGCCAACCTGTTTGT
AmEater-R	ATCCATTGGTGCAATTTGG
ApidNT-F	TTTTGCCTTAGCAATTCTTGTTG
ApidNT-R	GTAGGTCGAGTAGGCGGATCT
VgMC-F	AGTTCCGACCGACGACGA
VgMC-R	TTCCCTCCCACGGAGTCC

for 20 s, 60 °C for 30 s and 72 °C for 80 s. Fluorescence was measured repeatedly each cycle during the annealing step. This procedure was followed by a melt-curve dissociation analysis to confirm product size.

The amplification results were expressed as the threshold cycle (C_t) value, which represented the number of cycles needed to generate a fluorescent signal greater than a predefined threshold. Threshold cycle numbers for the target gene were subtracted from the reference gene (β-actin) for each sample. The amplification efficiency for target and control products was established via serial dilutions of known templates (e.g., Chen et al. 2008; Simone et al. 2009; Cornman et al. 2012).

2.5. Expression levels of genes related to the bee immunity in gut and remaining abdomen of queens at different ages

Control queens from ages 6 (n=6), 12 (n=12), 18 (n=12), and 24 (n=6) days following adult emergence were analyzed. Total RNA was isolated from gut and remaining abdomen tissues of the individual queens using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. cDNA synthesis and quantitative PCR amplification were described above (Sections 2.4.1 and 2.4.2).

2.6. Statistical analysis

Normality of data was checked using SPSS version 17.0 for Windows (SPSS, Inc.). Statistical significance was analyzed using ANOVA if they fitted a normal distribution. However, where the data were not normally distributed, the nonparametric Kruskal–Wallis H and Mann–Whitney U test were applied.

3. RESULTS

3.1. *N. ceranae* infection of queens aged 1, 6, and 12 days after emergence

When fed with *N. ceranae* spores at different doses, *N. ceranae* spores were quantified 6 and 12 days post-inoculation. No spores were detected in any of the control samples.

When comparing infection rates among queens of different ages, queens aged 1 day after emergence were more susceptible to *N. ceranae* than older queens (6 and 12 days after emergence) (Figure 1a, b). The average number of spores in queens aged at 1 days post-emergence that received a spore concentration of 10^6 spores/ml was significantly higher than those in queens aged 6 and 12 days after emergence at 6 days of inoculation (Kruskal–Wallis H, P=0.03) (Figure 1a). Moreover, there were significant differences in the number of spores between queens aged at 1, 6, and 12 days post-emergence when the cage in which the queen was received a spore suspension of 10^5 spores/ml (Kruskal–Wallis H, P=0.0001) and 10^6 spores/ml (Kruskal–Wallis H, P=0.004) after 12 days inoculation (Figure 1b). The largest difference was exhibited in the 10^5 spores/ml spore dose inoculation. The numbers of *N. ceranae* spores present in queens experimentally inoculated immediately after emergence were about 6 and 73 times higher than that in queens experimentally inoculated at ages 6 and 12 days after emergence, respectively.

The queens aged 1 day post-emergence that received a spore suspension of 10^6 spores/ml all became infected and some spores (average of *N. ceranae* spores=9.92×10^5 spores/queen, SE=5.00×10^5 spores/queen) could be observed after only 6 days post-inoculation (Figure 1a). In the lower spore inoculations (10^3, 10^4 spores/ml), *N. ceranae* spores could be observed after 12 days post-inoculation, with a 16.7 % infection rate (Table II). The number of spores were 4.0×10^7 spores/queen (SE=8.66×10^6 spores/queen) and 3.1×10^7 spores/queen (SE=1.84×10^6 spores/queen), respectively, when inoculated with 10^5 and 10^6 spores/ml, with a 100 % infection rate (Figure 1b and Table II).

Of the queens aged 6 days post-emergence and then fed *N. ceranae*, they became infected when receiving a spore suspension of 10^5 and 10^6 spores/ml, with an infection rate of 16.7 and 50 % at 6 days post-inoculation, respectively (Figure 1a), and a 100 % infection rate after 12 days post-inoculation (Table II). The average spore numbers were 6.64×10^6 spores/queen

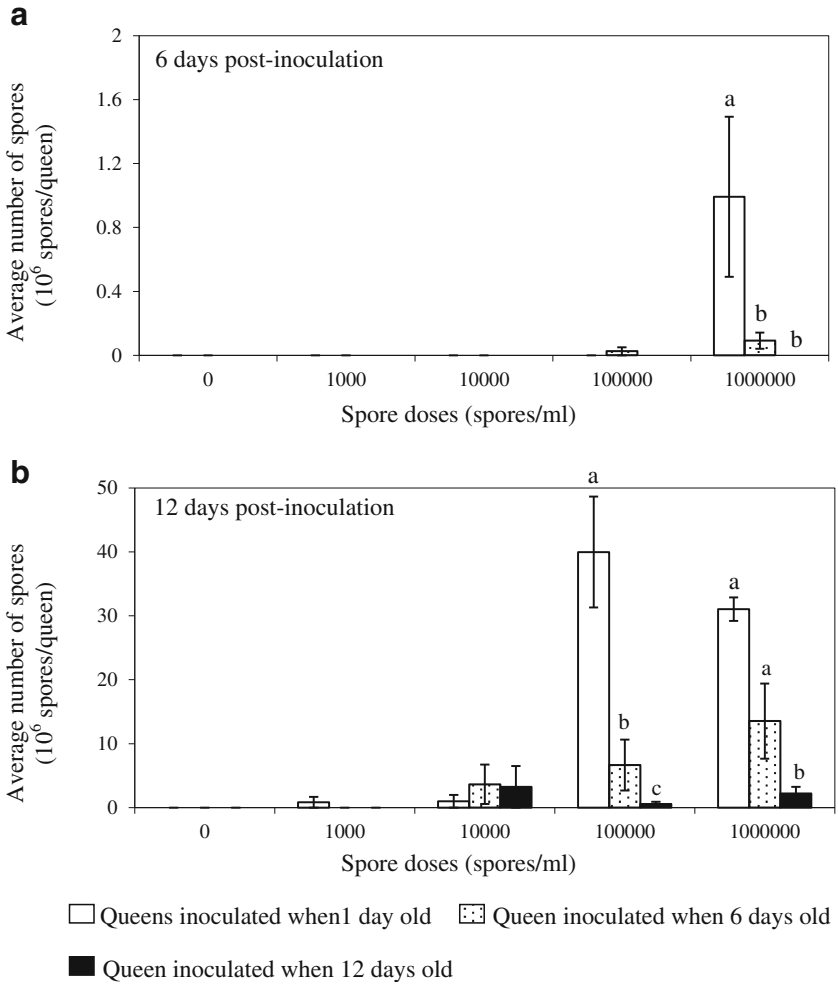

Figure 1. *N. ceranae* infection of different age honey bee queens at day 6 (**a**) and 12 (**b**) post-inoculation (mean of spore number±SE). The queens aged 1, 6, and 12 days old were inoculated with 10^3, 10^4, 10^5, and 10^6 spores/ml. *Vertical bars with different letters* indicate significantly different means (Kruskal–Wallis *H* and Mann–Whitney *U*).

(SE=3.99×10^6 spores/queen) and 1.352×10^7 spores/queen (SE=5.87×10^6 spores/queen) at 12 days after inoculation when the cage was inoculated with 10^5 and 10^6 spores/ml, respectively (Figure 1b).

When queens were aged for 12 days post-emergence and then fed *N. ceranae*, no spores were observed in queens, 6 days post-inoculation (Figure 1a). By 12 days post-inoculation, the infection rates had increased to 16.7, 33.3, and 100 % when inoculated with 10^4, 10^5, and 10^6 spores/ml respectively, indicating decreased susceptibility with increasing queen age (Figure 1 and Table II).

3.2. Effects of *N. ceranae* infection on immune related gene expression in queen gut tissue

Expression of immune-related genes, *apidaecin*, *eater*, and *vitellogenin* of queens of different ages inoculated with *N. ceranae* at the

Table II. Percentage of *N. ceranae* infection in honey bee queen at different ages after inoculated with *N. ceranae* spore doses of 10^3–10^6 spores/ml.

Age of queen (days after emergence	Day of inoculation (days)	Percent of infection				
		Control	10^3 spores/ml	10^4 spores/ml	10^5 spores/ml	10^6 spores/ml
1	6	0	0	0	0	100
	12	0	16.7	16.7	100	100
6	6	0	0	0	16.7	50
	12	0	0	50	100	100
12	6	0	0	0	0	0
	12	0	0	16.7	33.3	100

spore dose of 10^5 and 10^6 spores/ml was analyzed at 6 and 12 days post-inoculation. The tissues of gut and remaining abdomen were investigated for transcript levels of these genes. We found that gene expression varies with queen age.

In queens aged for 1 day post-emergence, there were no significant differences in gene expression of *eater* in gut tissues between inoculated and control queens after 6 days (ANOVA, $P>0.05$) (Figure 2a). Transcript levels of *eater* in queens inoculated with 10^6 spores/ml of *N. ceranae* were three times higher than those in control queens after 12 days of inoculation (Kruskal–Wallis H, $P>0.05$) (Figure 2d). The mRNA levels of *apidaecin* were significantly upregulated in *N. ceranae*-inoculated queens at day 6 of inoculations (ANOVA, $P<0.001$) (Figure 2b), with mean relative expression values of −0.197±0.561 s.e. and −1.717±0.555 s.e. for queens inoculated with 10^5 and 10^6 spores/ml of *N. ceranae*, respectively. Whereas *apidaecin* expression was not significantly different between control and inoculated queens 12 days after inoculation (ANOVA, $P>0.05$) (Figure 2e).

The expression of *vitellogenin* was varied in queens when inoculated with *N. ceranae*. In inoculated queens with 10^5 spores/ml, transcript levels of *vitellogenin* were significantly increased after 6 days of inoculation (ANOVA, $P=0.004$; mean relative expression of −3.483± 1.086 s.e.); however, there was significantly elevated in inoculated queens with 10^6 spores/ml of *N. ceranae* at day 12 inoculation (mean relative expression of 1.912±0.308 s.e.).

In queens inoculated at 6 days post-emergence, the gene expression of *eater* and *vitellogenin* in gut tissue was significantly lower in inoculated queens compared to control queens at 6 days post-inoculation (Kruskal–Wallis H, $P=0.05$ and $P=0.03$, respectively). Transcript levels of *eater* were about four times lower than control queens, with average relative expression of −14.968±0.500 s.e. and −14.007 ±1.631 s.e. for 10^5 and 10^6 spores/ml of *N. ceranae*-infected queens, respectively (Figure 2a). Also, *vitellogenin* expression in inoculated queens was about seven times lower than control queens (mean of −5.757±0.265 s.e. and −4.217±1.000 for 10^5 and 10^6 spores/ml of *N. ceranae*-inoculated queens, respectively; Figure 2c). At day 12 post-inoculation, queens inoculated with *N. ceranae* concentration of 10^6 spores/ml showed higher expression of *eater* (ANOVA, $P=0.036$), with mean of −13.903± 0.306 s.e. (Figure 2d). Comparing inoculated and control queens, there were no significant differences in transcript levels of *vitellogenin* 12 days after inoculation (ANOVA, $P>0.05$) (Figure 2f). For *apidaecin*, the mRNA levels were not significantly different between inoculated and control queens at 6 and 12 days after inoculation (Kruskal–Wallis H, $P>0.05$ and ANOVA, $P>0.05$, respectively; Figure 2b, e).

Gene expression of *apidaecin* and *vitellogenin* did not change in gut tissue of inoculated

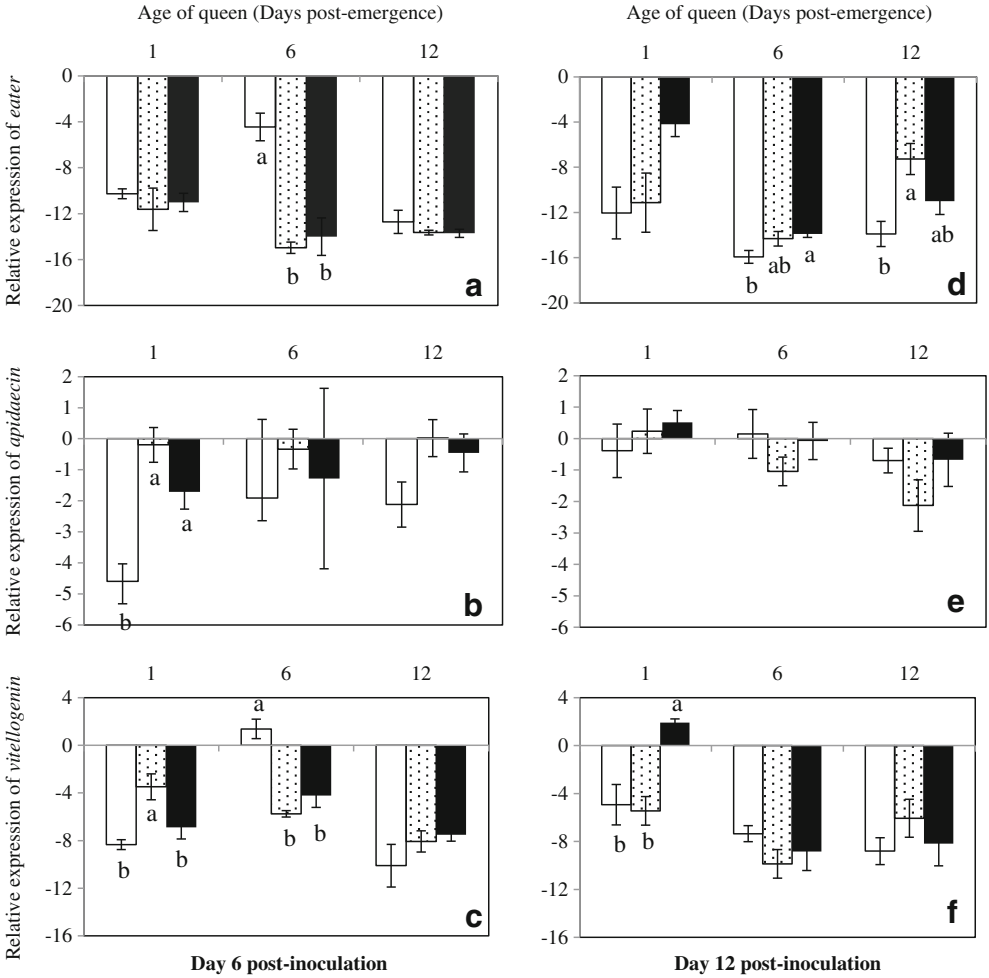

Figure 2. Effects of *N. ceranae* on the expression of *eater*, *apidaecin*, and *vitellogenin* in gut tissue of queens aged 1, 6, and 12 days post-emergence. *Vertical bars with different letters* show statistically significant differences (ANOVA, Kruskal–Wallis *H*, and Mann–Whitney *U*).

queens aged 12 days post-adult emergence after 6 and 12 days (ANOVA, $P>0.05$) (Figure 2b, c, e, f). However, *eater* mRNA was significantly upregulated in inoculated queens with 10^5 spores/ml (ANOVA, $P=0.006$; average of -7.271 ± 1.381 s.e.; Figure 2d).

3.3. Effects of *N. ceranae* infection on immune related gene expression in the remaining abdomen of queens

The mRNA levels of *eater*, *apidaecin*, and *vitellogenin* in the abdomens of inoculated and

control queens aged 1, 6, and 12 days post-emergence were also analyzed. Transcript levels of *eater* and *vitellogenin* in queens aged 1 day post emergence when inoculated with highest spore concentration of *N. ceranae* were significantly increased as compared with control queens (ANOVA, $P<0.001$ and Kruskal–Wallis H, $P=0.001$, respectively; Figure 3a, c). The average relative expression for *eater* and *vitellogenin* was 0.253±0.229 s.e. and 0.898±0.140 s.e., respectively. In contrast, *apidaecin* transcripts were suppressed in inoculated queens and significant expression was obtained in inoculated queens with 10^6 spores/ml at 6 days post-inoculation (ANOVA, $P=0.041$), with mean of −0.280±0.731 s.e. (Figure 3b). Of queens aged 6 days post-adult emergence, the expression of *eater* and *vitellogenin* in inocu-

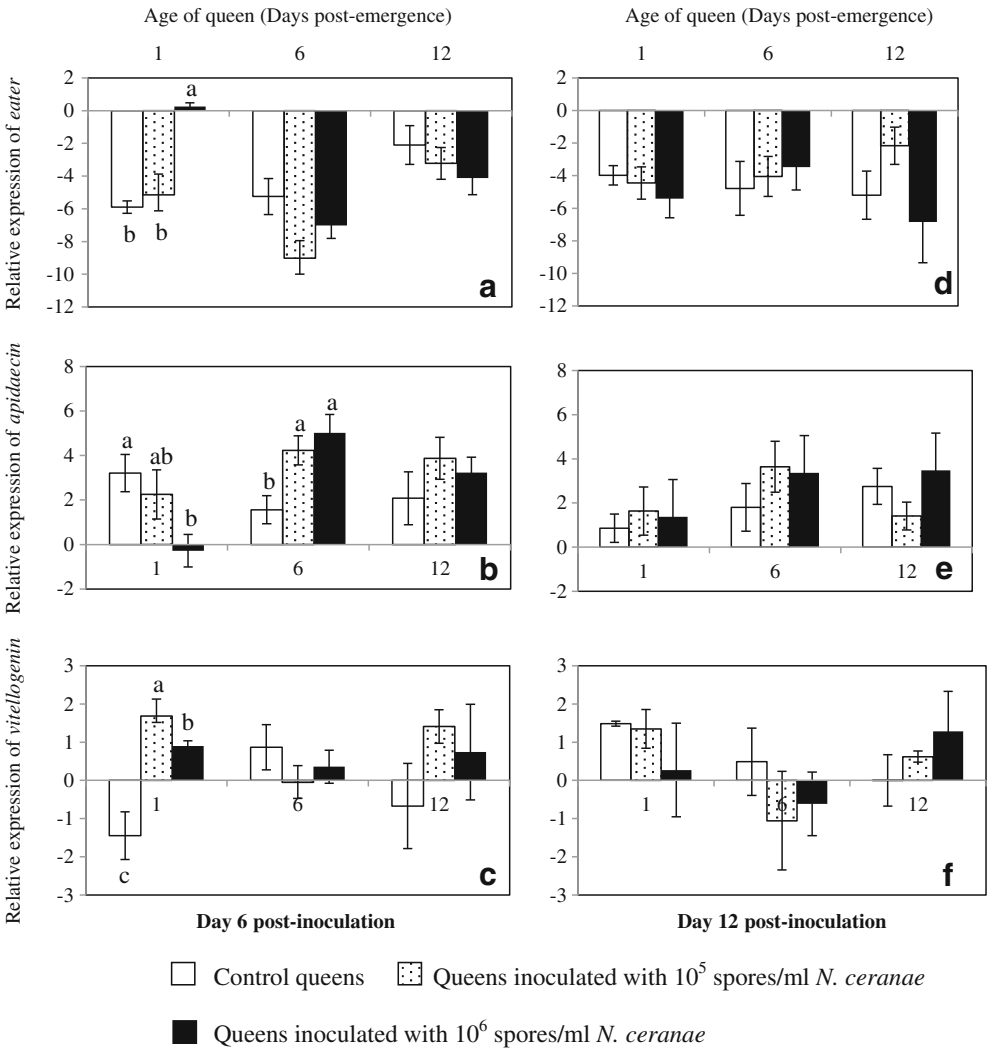

Figure 3. Effects of *N. ceranae* on the expression of *eater*, *apidaecin*, and *vitellogenin* in the remaining abdomen tissue of queens aged 1, 6, and 12 days post-emergence. *Vertical bars with different letters* show statistically significant differences (ANOVA, Kruskal–Wallis H and Mann–Whitney U).

lated queens was lower than those in control queens after 6 days, but not significantly different (ANOVA, $P>0.05$). The mRNA levels of *apidaecin* were about three times higher in inoculated queens than in control queens (ANOVA, $P=0.009$). The average relative expression was 4.230±0.654 s.e. and 5.022± 0.820 s.e. for 10^5 and 10^6 spores/ml of *N. ceranae*-inoculated queens, respectively. Comparing inoculated and control queens aged 12 days post-adult emergence, transcript levels of *apidaecin* and *vitellogenin* in inoculated queens increased as compared to the control after 6 days inoculation (ANOVA and Kruskal–Wallis *H*, $P>0.05$; Figure 3b). By contrast, *eater* expression in inoculated queens was lower than that in control queens (ANOVA, $P>0.05$; Figure 3a). Finally, there were no significant differences in mRNA levels of *eater*, *apidaecin*, and *vitellogenin* in abdomens of all queens after 12 days post-inoculation (Kruskal–Wallis *H*, $P>0.05$; Figure 3d–f).

3.4. Expression levels in the gut and remaining abdomen of queens at different ages

The mRNA levels for the antimicrobial peptide *apidaecin*, and for *eater* and *vitellogenin*, were measured for different-aged queens (queens aged 6, 12, 18, and 24 days old post-adult emergence; Figure 4). For *eater*, the mRNA levels in gut tissue of queens aged 18 and 24 days post-adult emergence were significantly lower than those of younger queens (queens aged 6 and 12 days old post-adult emergence; Kruskal–Wallis *H*, $P=0.014$; Figure 4). Older queens had about 1.6 times lower transcript levels than younger queens with average of −14.329±0.734 s.e. and −13.903± 1.115 s.e. for queens aged 18 and 24 days old, respectively. In contrast, transcript levels of *eater* in the remaining abdomens of the younger queens were not significantly different from those of the older queens (ANOVA, $P>0.05$; Figure 4). *Apidaecin* transcript levels in the gut were sequentially increased in the older queens (Kruskal–Wallis *H*, $P=0.007$). However, mRNA levels of *apidaecin* in remaining abdomen decreased in the older queens but no significant differences could be obtained (Kruskal–Wallis *H*, $P>0.05$). For *vitellogenin*, the mRNA levels in the gut and remaining abdomen were significantly higher in queens aged 12 days old post-adult emergence than in queens aged 6, 18, and 24 days old post-adult emergence (ANOVA, $P<0.001$ and Kruskal–Wallis *H*, $P=0.010$ in the tissues of gut and remaining abdomen, respectively), with means of −1.777±1.303 s.e. and 1.177± 0.700 s.e. for gut and remaining abdomen, respectively.

4. DISCUSSION

In this study, we examined the effect of both queen age and spore dose on the susceptibility of queen honey bees to *N. ceranae* infection. The 12-day period after emergence was selected as it corresponds with the time that queens often kept in small nucleus colonies for mating before being shipped to the beekeeper. We clearly show that queens become less susceptible as they age making their time spent in the mating nuclei critical for *N. ceranae* infection. Our results suggest that any measures that reduce the contamination of *N. ceranae* spores in food sources fed to the newly emerged queens would help to prevent queens from becoming infected with *N. ceranae*.

The results show that queens become infected when inoculated with a high dose of either 10^5–10^6 spores/ml of *N. ceranae* spores, 12 days post-inoculation, except for those queens aged for 12 days, when only those queens dosed with 10^6 spores/ml became 100 % infected. *N. ceranae* fed spores at lower dosages of 10^3 and 10^4 spores/ml did not infect all queens. Younger queens are more susceptible to *N. ceranae* infection than older queens. This age response could be interpreted as the young queen gut cells promoting the growth, development, and germination of *N. ceranae* (Keeling and Fast 2002). Nevertheless, Rinderer and Elliot (1977) indicated that the protein conditions of the host are critical for the development of *N. apis*. It is conceivable that younger queens are in better condition than older queens that

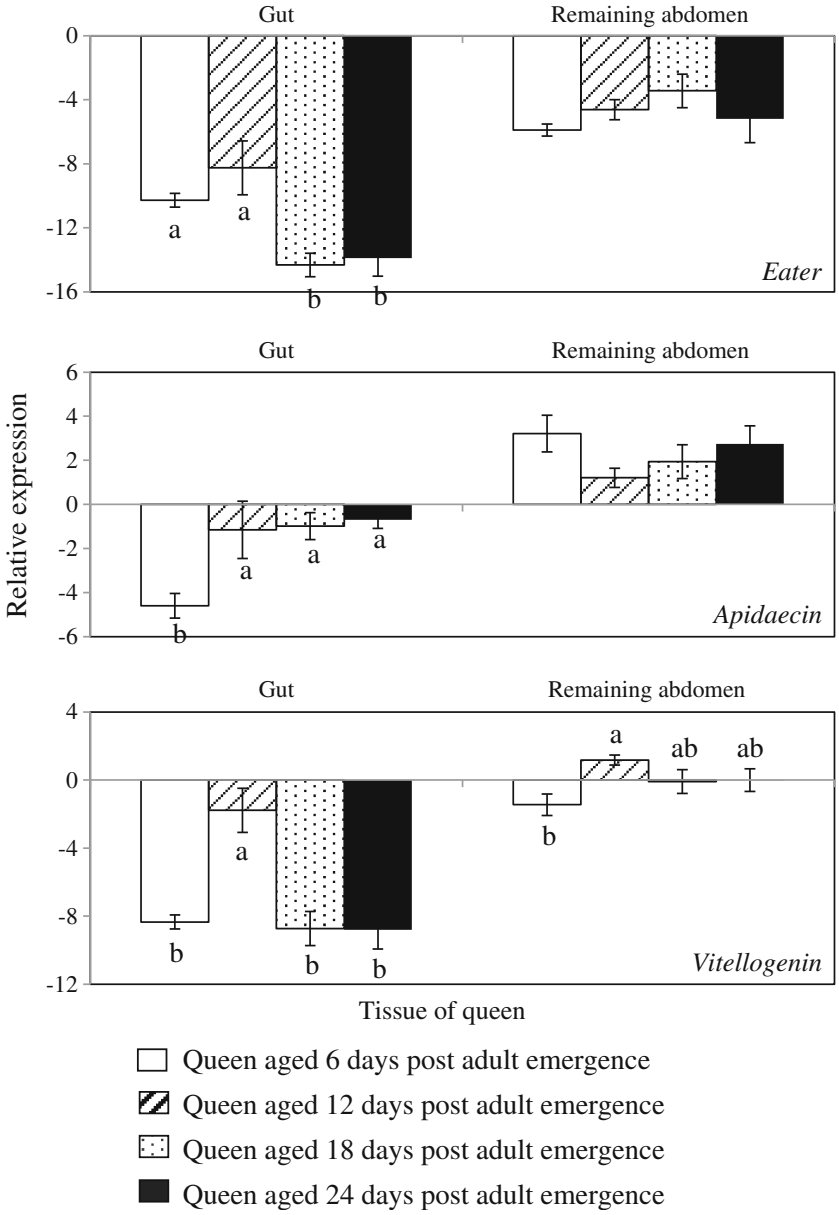

Figure 4. Expression levels of *eater*, *apidaecin*, and *vitellogenin* in the tissues of gut and remaining abdomen of queens of different ages. *Vertical bars with different letters* indicate significantly different means (ANOVA, Kruskal–Wallis *H* and Mann–Whitney *U*).

have been exposed to periods of protein depletion. If queens are more susceptible during this initial period of adult life then mitigation measures could reduce *N. ceranae* infection and

improve queen health and longevity in colonies. When considering the queens aged for 1 day post-emergence, surprisingly the average number of spores in inoculated queens with 10^5 spores/ml of *N. ceranae* was higher than those in queens when inoculated with 10^6 spores/ml. As all honey bee queens originated from the same queen breeder, we suggest that the susceptibility differences likely reflect the genetic variations of host at the individual queen level. The individual immune defense can be considered one of the benefits of genetic diversity in honey bees.

Honey bee queens of all three ages (1, 6, and 12 days) inoculated by *N. ceranae* showed downregulation of *eater* in gut tissue after 6 days inoculation. A significant downregulation of *eater* was especially evident in queens aged 6 days post-adult emergence. *Eater* transcripts after 12 days post-inoculation were upregulated in all queen ages when inoculated with *N. ceranae*. The results suggest that queens may have to produce more eater in this period to defend against *N. ceranae* infection. Since *N. ceranae* spores can rapidly develop and multiply during day 4 to 7 of infection. Thus, eater may play important role to eliminate this pathogen. Also eater has been shown to play a role in the phagocytosis of bacterial pathogens (Ertürk-Hasdemir and Silverman 2005; Kocks et al. 2005).

For *apidaecin*, the upregulation of this gene was observed in the gut of inoculated queens of all ages at 6 days post-inoculation. Our results revealed that *N. ceranae* induces *apidaecin* expression in queens in early periods of inoculation. However, *apidaecin* expression shows an opposing response between queens and workers after inoculated with *N. ceranae. N. ceranae* infection suppresses immune gene response in worker bees (Autúnez et al. 2009; Chaimanee et al. 2012). Queens might be better able to defend themselves from pathogen invasion since they produce more antimicrobial peptides. Nevertheless, different immune signaling between queens and workers following *N. ceranae* infection cannot be excluded.

Our study demonstrates great variation in *vitellogenin* expression between queens of different ages. *Vitellogenin* transcripts were increased in inoculated queens aged 1 and 12 days post-emergence in both of gut and remaining abdomen tissues. However, queens showed decreased expression when aged 6 days old. In a recent study, Alaux et al. (2011) reported that the vitellogenin titer increased about 58 % in honey bee queens when infected with *N. ceranae*. In contrast, Autúnez et al. (2009) demonstrated that the mRNA levels of *vitellogenin* in *N. ceranae* infected worker bees significantly decreased after 7 days of infection. Vitellogenin is a female-specific glucolipoprotein yolk precursor synthesized in the fat body of abdomen (Byrne et al. 1989). It plays an important function in immunity and longevity of worker honey bees (Amdam et al. 2004). Therefore, it is not conclusive that vitellogenin is affected by *N. ceranae* infection in queens. Other factors may contribute to the expression of the *vitellogenin* gene.

Differences in the transcript levels of genes were clearly observed between the gut and remaining abdomen. Our results showed that the expression of *eater*, *apidaecin*, and *vitellogenin* in the gut was lower than that from the remaining abdomen. Not surprisingly, since the fat body distributes throughout the abdomen and it is the site of antimicrobial peptides synthesis. The results showed that *eater* transcripts were decreased in the gut tissue and there were elevated in the abdomen part of older queens. Contrary to *eater* expression, the mRNA levels of *apidaecin* in the gut of queens were increased in older queens. This indicates that younger queens produce lower *apidaecin* levels than older queens and may help explain the increased susceptibility of younger queens to *N. ceranae*. In this study, we showed that *vitellogenin* expression in the gut and abdomen did not differ between queens aged 6, 18, and 24 days old post-emergence, except in queens aged 12 days where there was a significant vitellogenin production compared to the others. In honey bee queens, vitellogenin synthesis starts 60 h before adult emergence and vitellogenin titers stays high thereafter (Barchuk et al. 2002). Moreover, Corona et al. (2007) have reported that older queens have higher *vitellogenin* transcript levels than do young queens. Surprisingly, queens aged 12 days old had the highest *vitellogenin*

expression in our study. We hypothesize that high *vitellogenin* level may result from immunity, hormone signaling, and/or oxidative stress. Taken together, we suggest that *vitellogenin* expression in queens does not vary greatly between ages 6 to 24 days of age.

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OPEN ACCESS

Effets de l'âge de l'hôte sur la sensibilité à l'infection et sur l'expression d'un gène immunitaire chez les reines d'abeille (*Apis mellifera*) inoculées avec *Nosema ceranae*

Abeille / reine / peptides anti-microbiens / supercédure

Einfluss des Wirtsalters auf die Infektionsanfälligkeit und die Expression von Immungenen bei Honigbienenköniginnen (*Apis mellifera*), die mit *Nosema ceranae* infiziert wurden

***Nosema ceranae* / Honigbienenkönigin / *Apis mellifera* / Umweiselung / antimikrobielle Peptide**

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