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ORIGINAL RESEARCH ARTICLE

Interactions of *Tropilaelaps mercedesae*, honey bee viruses and immune response in *Apis mellifera*

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Tropilaelaps mites are the major health threat to *Apis mellifera* colonies in Asia because of their widespread occurrence, rapid population growth and potential ability to transfer bee viruses. Honey bee immune responses in the presence of feeding mites may occur in response to mite feeding, to the presence of viruses, or to both. In this study, the mRNA expression levels were measured for three antimicrobial peptide encoding genes (*abaecin*, *apidaecin* and *hymenoptaecin*) and a phagocytosis receptor gene (*eater*) in worker brood infested with different numbers of actively feeding *T. mercedesae*. Also, all samples were measured for the amount of acute bee paralysis virus (ABPV), black queen cell virus (BQCV), deformed wing virus (DWV), Kashmir bee virus (KBV) and sacbrood virus (SBV). Using an artificial mite inoculation protocol, the analysis showed that *apidaecin* was significantly down-regulated when tan-bodied pupae were infested with 1–2 mites and when capping of the cells of newly sealed larvae were opened and closed without mite inoculation (o/c) as compared to the control group (undisturbed brood, no mite inoculation). Reduced transcription levels of the *eater* gene were also recorded in the o/c group. However, an up-regulation of *apidaecin* and *eater* genes was observed in highly infested pupae when compared to o/c group. This occurrence is perhaps due to an adaptive response of the bees to higher mite infestations by up-regulating their immune expression. No significant expression differences were detected for *abaecin* and *hymenoptaecin* and the viruses ABPV, KBV and SBV were not detected. However, 86.7% of the pupae were infected with DWV, 83.3% were infected with BQCV and 73% were infected by both of these viruses. In addition, the *Tropilaelaps*-inoculated pupae showed higher levels and incidence of DWV compared to uninfested pupae. The presence of these two honey bee viruses was not related to the number of *T. mercedesae* infesting the pupae. Also, the presence of variable levels of DWV and low levels of BQCV did not provoke any expression differences for any of the targeted genes. Overall, this research indicates that feeding by *Tropilaelaps* mites produces an immune response, that the level of viruses did not produce a correlated immune response by the four genes tested and that *Tropilaelaps* may be a potential vector of DWV but not to a high degree. The data indicated that the major impact of *Tropilaelaps* infestation is caused by the mite itself.

Interacciones de *Tropilaelaps mercedesae*, virus de las abejas de la miel, y la respuesta inmune en *Apis mellifera*

Loa ácaros *Tropilaelaps* son la principal amenaza para la salud de las colonias de *Apis mellifera* en Asia debido a su presencia generalizada, el crecimiento acelerado de la población y la capacidad potencial de transferir virus de las abejas. La respuesta inmune de la abeja de la miel en presencia de ácaros alimentándose puede ocurrir como respuesta a la alimentación de los ácaros de alimentación, a la presencia de virus, o a ambos. En este estudio, se midieron los niveles de expresión de ARNm de tres genes antimicrobianos codificantes de péptidos (abaecin, apidaecin, hymenoptaecin) y un gen del receptor de la fagocitosis (*eater*) en cría de obreras infestada con diferentes números de *T. mercedesae* en fase de alimentación activa. Además, se midieron en todas las muestras la cantidad de virus de la parálisis aguda de las abejas (ABPV), virus de la celda real negra (BQCV), virus de las alas deformadas (DWV), el virus Cachemira de abejas (KBV) y el virus de la cría ensacada (SBV). Mediante el uso de un protocolo de inoculación artificial del ácaro, el análisis mostró que apidaecin fue significativamente sub-regulada cuando la pupa en fase oscura se infestó con 1-2 ácaros y cuando la limitación de las células de las larvas recién sellada se abrió y cuando el sellado de las celdas de las larvas operculadas recientemente se abrieron y cerraron sin inoculación del ácaro (a / c) en comparación con el grupo control (cría sin perturbaciones, sin la inoculación de ácaros). También se registró una reducción de los niveles de transcripción del gen *eater* en el grupo a / c. Sin embargo, se observó una sobre regulación de los genes apidaecin y eater en pupas altamente infestadas, en comparación con el grupo a / c. Este hecho es quizás debido a una respuesta adaptativa de las abejas a infestaciones de ácaros más altas mediante la sobre regulación de su expresión inmune. No se detectaron diferencias significativas de expresión para abaecin y hymenoptaecin y no se detectaron los virus ABPV, KBV y SBV. Sin embargo, el 86,7% de las pupas estaban infectadas con DWV, el 83,3% con BQCV y el 73% con ambos virus. Además, las pupas inoculadas con *Tropilaelaps* mostraron niveles más altos e incidencia de DWV en comparación con pupas no infestadas. La presencia de estos dos virus de abejas de miel no estuvo relacionada con el número de *T. mercedesae* infestando las pupas. Además, la presencia de niveles variables de DWV y bajos niveles de BQCV no provocó ninguna expresión diferencial para cualquiera de los genes específicos. En general, esta investigación indica que

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la alimentación por los ácaros *Tropilaelaps* produce una respuesta inmune, que el nivel de virus no produjo una respuesta inmune correlacionada con los cuatro genes probados y que *Tropilaelaps* puede ser un vector potencial de DWV pero no a un nivel elevado. Los datos indicaron que el mayor impacto de la infestación por ácaros *Tropilaelaps* es causado por el propio ácaro.

Keywords: *Tropilaelaps mercedesae*; *Apis mellifera*; immune response; antimicrobial peptides; honey bee virus

Introduction

Worldwide, parasites and pathogens present serious threats to the health of the European honey bee (*Apis mellifera*). In Asia, *A. mellifera* is commonly infested with two different genera of parasitic mites (*Varroa* spp. and *Tropilaelaps* spp.) (Anderson & Morgan, 2007; Anderson & Roberts, 2013; Anderson & Trueman, 2000). Although these mites have similar life cycles (Kapil & Aggarwal, 1987, 1989), the populations of *Tropilaelaps* spp. are generally higher than populations of *varroa* in *A. mellifera* colonies (Anderson & Morgan, 2007; De Jong, De Jong, & Goncalves 1982; Oldroyd & Wongsiri, 2006). Hence, in Asia, *Tropilaelaps* mites inflict more serious damage to *A. mellifera* colonies than varroa (Burgett, Akratanakul, & Morse, 1983; Burgett, Rossignol, & Kitprasert, 1990; Koeniger & Musaffar, 1988; Laigo & Morse, 1968; Otis & Kralj, 2001).

The puncture wounds caused by the feeding activities of *V. destructor* provide entry for bee diseases (Chen & Siede, 2007). Several bee viruses have been reported to be associated with varroa mite infestation (De Miranda et al., 2013; Tentcheva et al., 2004). Deformed wing virus (DWV) is the most common virus associated with varroa infestation causing wing deformities and decreased body size of infected adult bees (Chen & Siede, 2007). These symptoms are part of a phenomenon termed parasitic mite syndrome (Shimanuki, Calderone, & Knox, 1994). Like *varroa, Tropilaelaps* feeds and reproduces within capped brood cells causing abnormalities and sometimes death to bees (Burgett et al., 1983). Recently, the presence of *T. mercedesae* in *A. mellifera* colonies was reported to be associated with the infection of DWV in China (Forsgren, De Miranda, Isaksson, Wei, & Fries, 2009). Further, virus replication has also been documented in DWV-positive *T. mercedesae* together with their infested host bees (Dainat, Ken, Berthoud, & Neumann, 2009). Therefore, *T. mercedesae* may be considered to be a biological vector of DWV.

Honey bees mount both humoral and cellular immune responses at wound sites caused by *V. destructor* (Casteels-Josson, Zhang, Capaci, Casteels, & Tempst, 1994; Kanbar & Engels, 2003). Yang and Cox-Foster (2005) reported that varroa caused immunosuppression in parasitised *A. mellifera* although other studies have suggested that immuno-suppression is inconsistent (e.g., Gregory Evans, Rinderer, & De Guzman, 2005). Although DWV-positive varroa mites have been collected from Thai *A. mellifera* colonies (Chantawannakul, Ward, Boonham, & Brown, 2006), no studies have been done to determine the presence of DWV in *T. mercedesae* in Thailand. Additionally, the immune response of *A. mellifera* towards *Tropilaelaps* parasitism and to the different honey bee viruses this mite may vector remains unknown. Therefore, this study was conducted to compare the levels of *A. mellifera* honey bee immune-peptide transcripts between unparasitised honey bees and those parasitised with different numbers of *T. mercedesae* in a Thai population of *A. mellifera*. The presence of different honey bee viruses and their potential effects on the expression of immune genes was also investigated.

Materials and methods

Experimental design

The test colony (*A. mellifera*) used in this study was apparently mite-free with no observable bacterial or fungal diseases. Age-controlled brood was obtained by caging the queen (24 h) with a push-in wire screen (8 mesh) on an empty comb. The test section of brood consisted of 20 rows with 20 brood cells per row. Inoculum female *Tropilaelaps* were collected from the newly sealed worker brood of a highly infested colony. Adult female mites were individually introduced into newly sealed larvae in the test section of brood using a transfer technique established for varroa (Garrido & Rosenkranz, 2003; Kirrane et al., 2011). Briefly, this technique involved using a minute pin to create a small opening at the edge of the cell capping of a newly sealed larvae, through which individual *Tropilaelaps* were transferred using the tip of an insect brush. The tip of the insect brush was moistened with a small amount of honey to facilitate mite inoculation by slowing the movement of the mite being introduced. The capping was pressed back quickly using the insect brush to reseal the opening. Cohort brood cells were randomly assigned to one of four groups: (a) brood inoculated with one mite; (b) brood inoculated with two mites; (c) brood with capping opened and closed without mite inoculation; and (d) undisturbed brood cells (control). At the time of mite inoculation, the positions of the inoculated brood were mapped on a transparency sheet. Prior to returning the frame into the host colony, the test brood section was covered with a screen wire push-in cage (8 mesh) to prevent worker bees from removing the inoculated brood. Seven days after mite inoculation, brood cells were examined for the presence or absence of mites. All tan-bodied pupae and the mites infesting them were collected separately and were placed in a −80 °C freezer until RNA extraction.

Since some introduced foundress mites reproduced inside the brood cells, infested pupae were re-grouped

Table 1. Sequences of the tested immune genes, TaqMan primers and probes for Real-Time Quantitative RT-PCR.

Primer	Target	5′ to 3′ sequence	Reference
Am actin-F	Reference gene	TTGTATGCCAACACTGTCCTTT	Simone et al. (2009)
Am actin-R		TGGCGCGATGATCTTAATTT	
Abaecin-F	Antimicrobial peptide	CAGCATTCGCATACGTACCA	Evans (2006)
Abaecin-R		GACCAGGAAACGTTGGAAAC	
AmEater-F	Receptor	CATTTGCCAACCTGTTTGT	Simone et al. (2009)
AmEater-R		ATCCATTGGTGCAATTTGG	
ApidNT-F	Antimicrobial peptide	TTTTGCCTTAGCAATTCTTGTTG	Simone et al. (2009)
ApidNT-R		GTAGGTCGAGTAGGCGGATCT	
Hymenopt-F	Antimicrobial peptide	CTCTTCTGTGCCGTTGCATA	Evans (2006)
Hymenopt-R		GCGTCTCCTGTCATTCCATT	
KBV83-F	Kashmir bee virus (KBV)	ACCAGGAAGTATTCCCATGGTAAG	Chantawannakul et al. (2006)
KBV161-R		TGGAGCTATGGTTCCGTTCAG	
KBV109-T		CCGCAGATAAC TTAGGACATCAATCACA	Chantawannakul et al. (2006)
APV95-F	Acute bee paralysis virus (ABPV)	TCCTATATCGACGACGAAAGACAA	
APV159-R		GCGCTTTAATTCCATCCAATTGA	Chantawannakul et al. (2006)
APV121-T		TTTCCCCGGACTTGAC	
DWV958-F	Deformed wing virus (DWV)	CCTGGACAAGGTCTCGGTAGAA	Chantawannakul et al. (2006)
DWV9711-R		ATTCAGGACCCCACCCAAAT	
DWV9627-T		CATGCTCGAGGATTGGGTCGTCGT	Chantawannakul et al. (2006)
BQCV8195-F	Black queen cell virus (BQCV)	GGTGCGGGAGATGATATGGA	
BQCV8265-R		GCCGTCTGAGATGCATGAATAC	Chantawannakul et al. (2006)
BQCV8217-T		TTTCCATCTTTATCGGTACGCCGCC	
SBV311-F	Sacbrood virus (SBV)	AAGTTGGAGGCGCGYATTTG	Chantawannakul et al. (2006)
SBV380-R		CAAATGTCTTCTTACD AGAAGYAAGGATTG	
SBV331-T		CGGAGTGGAAAGAT	Chantawannakul et al. (2006)
Tropilaelaps278-F	ITS1–5.8S-ITS2 *r*RNA gene	CTTAGTCCTTAAATCG ATGAAAAACATTG	
Tropilaelaps379-R		TCCATGCTGAAATTTTCATTCAA	
Tropilaelaps319-T		ATTGATGTGAGTTGT GAAATTTTGTGAGCATTGT	

Notes: F, forward primer; R, reverse primer; T, probe. Probes consist of oligonucleotides with a 5′ reporter dye (FAM, 6-carboxy-Xuorescein) and a 3′ quencher (TAMRA, tetra-methylcarboxyrhodamine.

for PCR analysis based on the number of active feeding mites (foundress, young female and male adults and nymphs) present in each pupal cell. Eggs were not considered. The test groups were as follows: (a) pupae infested with 1–2 mites; (b) pupae infested with 3–4 mites; (c) pupae infested 5–6 mites; (d) pupae infested 7–8 mites; (e) pupae where the wax cappings of the cells were opened and closed without mite inoculation (o/c); and (f) pupae from undisturbed cells (control). Since cell capping were deliberately opened to introduce mites, the o/c group served as a control check to determine whether the manipulation of cell capping alone also triggered a response. Ten pupae were sampled for each group. None of the test pupae were infested with varroa mites.

RNA extraction and cDNA synthesis

Total RNA was extracted from individual pupae and their corresponding *Tropilaelaps* foundress using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA was removed using DNAse I by incubation at 37 °C for 1 h followed by 10 min at 75 °C. First-strand cDNA was generated from approximately 2 g total RNA using 50 U Superscript III (Invitrogen, Carlsbad, CA), 2.5 nmol DNTP mix and 2.5 nmol oligo $(dT)_{20}$. The synthesis was carried out at 50 °C for 50 min followed by 5 min at 85 °C and 20 min at 37 °C after added RNAse H (Invitrogen).

Quantitative PCR amplification for immune genes

The cDNA products were amplified with an IQ5 Real-Time PCR thermal cycler (Bio-Rad) using EXPRESS SYBR GreenER™ qPCR SuperMix Universal (Invitrogen). Gene-specific primers are listed in Table 1. The thermal programme for all reactions was 95 ° for 2 min followed by 40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min 20 s. Fluorescence signal was measured in every cycle during the annealing step to quantify product amount. At the end of the PCR reaction, a melt-curve dissociation analysis was conducted to confirm product size.

The results were expressed as the threshold cycle (C_t) value, which illustrated the number of cycles needed to generate a fluorescent signal greater than a predefined threshold. Relative gene expression of the number of infested mites was determined by normalising the C_t

value of the reference gene (*-actin*) to the target gene (ΔC_t) for each sample. The expression was calculated as ($C_{t\ control} - C_{t\ target}$) (Evans et al., 2006; Evans et al., 2013).

Quantitative PCR amplification for viruses present in bee and mite samples

All individual pupae and foundress mites were amplified for virus detection and quantification. The cDNA products were amplified using FastStart TaqMan Probe Master (Roche) on an IQ5 Real-Time PCR thermal cycler (Bio-Rad) with each specific primer and TaqMan probe set as listed in Table 1. Plates were cycled using established system conditions (48 °C for 30 min, 95 °C for 10 min and 40 cycles of 60 °C for 1 min plus 95 °C for 15 s) as described by Chantawannakul et al. (2006).

Statistical analysis

Transcription levels were normalised using SPSS version 17.0 for Windows (SPSS Inc., 2008). Data on target gene transcription levels for pupae exposed to different numbers of mites were analysed using one-way ANOVA. Where differences were found, the means were compared using a Tukey-HSD with a 95% confidence. Pearson's correlation coefficient was used to determine relationships between the levels of DWV in pupae and in the harvested mites (SAS Institute, 2008).

Results

Expression of *apidaecin* was significantly ($F = 3.10$, df = 5, $P = .016$) down-regulated in pupae that had their cell capping manipulated (o/c) and in pupae infested with 1–2 mites when compared to the control treatment (undisturbed brood cells) (Figure 1). The immunity gene *eater* was also significantly ($F = 4.349$, df = 5, $P = .002$) down-regulated in the o/c group as compared to the control group (Figure 2). However, the transcription levels of both *apidaecin* and *eater* genes numerically increased in highly infested groups as compared to the o/c group. There were no statistical differences among the transcript levels for any of the groups for the immune genes *abaecin* ($F = 1.109$, df = 5, $P = .366$) and *hymenoptaecin* ($F = 1.485$, df = 5, $P = .210$) even though numerical increases of transcription levels were observed.

We do not understand why the o/c manipulation resulted in a down-regulation. However, if we accept this down-regulation as a baseline observation for all mite treatment groups and reanalyse the data using o/c as the control, the results differ somewhat. ANOVA results indicate that pupae with 7–8 mite produced more *apidaecin* than o/c pupae ($F = 2.843$, df = 4, $P = .035$) but not the other pupae with lower numbers of infesting mites (Figure 1). Production of *eater* was also higher in the 3–4 and 7–8 mite groups than in o/c group ($F = 3.545$, df = 4, $P = .013$) (Figure 2). However, this procedure did not alter the results for *abaecin* ($F = 1.338$, df = 4, $P = .271$) or *hymenoptaecin* ($F = 1.679$, df = 4, $P = .171$). Regression analyses of transcription levels with numbers of active mites identified significant relationships for *apidaecin* ($r = .428$, $P = .002$), *eater* ($r = .299$, $P = .035$) and *hymenoptaecin* ($r = .305$, $P = .031$). No correlation was detected between the transcription level and number of active mites for *abaecin* ($r = .213$, $P = .138$).

Quantitative viral analysis of bee pupae and the adult mites infesting them showed that all samples were negative for three honey bee viruses: acute bee paralysis virus , Kashmir bee virus and sacbrood virus. Additionally, mites were negative for black queen cell virus and only had DWV. However, 86.7% (52 of 60) pupae samples were found positive with variable levels of DWV ($C_t = 30.08 \pm 6.27$) and 83.3% (50 of 60 pupae) were found positive with low levels of BQCV ($C_t = 35.78 \pm 1.46$). About 73% of the test pupae were concurrently infected with DWV and BQCV. The incidence and levels of BQCV did not differ among all test groups. We also found that *Tropilaelaps* infested pupae ($C_t = 26.76 \pm .78$) have significantly higher level of DWV than uninfested pupae ($C_t = 36.05 \pm 1.00$) ($F = 53.43$, df = 1, $P < .001$). Importantly, no correlation between DWV levels in pupae and expression levels of the four target genes was detected [*abaecin* ($r = .018$, $P = .89$); *apidaecin* ($r = .021$, $P = .87$); *hymenoptaecin* ($r = -.122$, $P = .35$); and *eater* ($r = .042$, $P = .74$)].

When foundress *Tropilaelaps* retrieved from the infested brood cells were analysed, 42% were infected with DWV (17 of 40). Of those that were infected, significantly lower levels ($t = 4.34$, $P = .0001$) of DWV ($C_t = 36.48 \pm 3.04$) were detected as compared to their corresponding bees ($C_t = 28.00 \pm 7.46$). The individual levels of DWV in all the mites retrieved from pupal cells did not vary among the test groups ($F = 1.91$, df = 3, $P = .146$) and did not influence the levels of DWV in their pupal hosts ($r = .115$, $P = .478$).

When comparing the proportion of bee pupae and their corresponding *T. mercedesae* with or without DWV, the majority of samples fell into two main groups: (1) DWV-positive bees infested with DWV-positive mites and (2) DWV-positive bees infested with DWV-negative mites. The remaining samples fell into two additional yet minor groups: (3) DWV-negative bees infested with DWV-positive mites and (4) DWV-negative bees infested with DWV-negative mites (Table 2). Based on the frequency of infection of DWV in bees and mites, the association of infections in Table 2 indicates that bees with infected mites are more often infected than bees without infected mites ($\chi^2 = 6.7$, df = 3, $P = .009$).

Discussion

Of the four genes tested, only *apidaecin* and *eater* displayed differential expression levels among treatments

Table 2. Percentages of bee pupae and their corresponding *T. mercedesae* with or without DWV. Chi-square analyses test independence of association of infected bees and infected mites.

	Control group		Mite group			
Bee and mite status	Manipulated	Unmanipulated	1–2	3–4	5–6	7–8
DWV-positive bee	90	100	–	–	–	–
DWV-negative bee	10	0	–	–	–	–
DWV-positive bee and mite	–	–	50	50	30	20
DWV-positive bee, DWV-negative mite	–	–	20	50	70	40
DWV-negative bee, DWV-positive mite	–	–	10	0	0	10
DWV-negative bee and mite	–	–	20 ($\chi^2 = 45.6$, $P < .0001$)	0	0	30 ($\chi^2 = 75.3$, $P < .0001$)

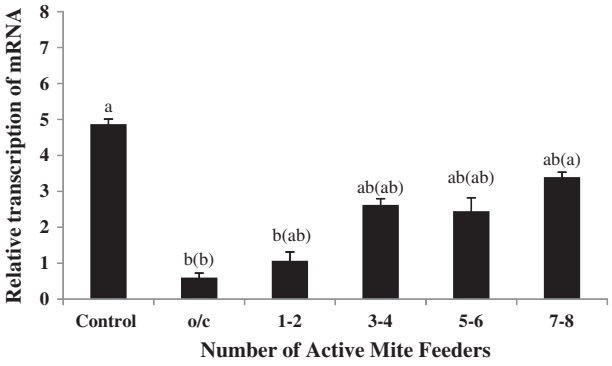

Figure 1. Expression levels (mean ± SE) for pupae exposed to different numbers of *T. mercedesae* for the *apidaecin* gene.
Notes: Bars with different letters are significantly different (ANOVA, $P < .05$). Letters above the bars inside () show the differences in expression levels when reanalysed without the control. Control – undisturbed cells without mite inoculation, o/c – small opening made along the edge of cell capping and then pressed closed without mite inoculation.

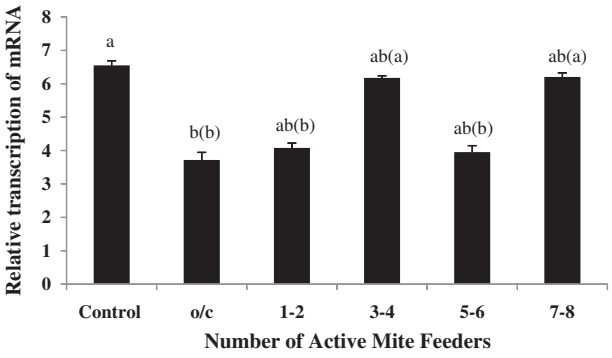

Figure 2. Expression levels (mean ± SE) for pupae exposed to different numbers of *T. mercedesae* for the *eater* gene.
Notes: Bars with different letters are significantly different (ANOVA, $P < .05$). Letters above the bars inside () show the differences in expression levels when reanalysed without the control. Control – undisturbed cells without mite inoculation, o/c – small opening made along the edge of cell capping and then pressed closed without mite inoculation.

although these genes and *hymenoptaecin* all showed higher transcript levels with increasing mite numbers. The expression of the immunity-related gene *apidaecin* showed an initial down-regulation with the o/c treatment and with lower numbers of feeding mites but an increase in expression as mite numbers increased (Figure 1). It is also previously reported that higher numbers of varroa mite feeding could induce more immune responses (Gregory et al., 2005). However, we did not observe the suppression of immune response in *A. mellifera* by *Tropilaelaps* in our experiments, similar to those observed in varroa (Gregory et al., 2005; Yang & Cox-Foster, 2005).

A reduced amount of transcription for the *eater* gene was also recorded in the o/c group compared to the control group (Figure 2). *Eater* also was down-regulated with low mite numbers and transcription levels numerically increased as mite numbers increased. *Eater* regulates a major receptor and plays an important role in recognition and phagocytosis in *Drosophila* (Ertürk-Hasdemir & Silverman, 2005; Kocks et al., 2005). The infested pupae might enlist both humoral (*apidaecin*) and cellular (*eater*) responses to protect themselves from microbial infection through wounds derived from mite feeding.

It is interesting to note that both *apidaecin* and *eater* showed a significant down-regulation in response to the manipulation of cell cappings (Figures 1 and 2). Currently, we have no explanation for this down-regulation in response to the creation of a small opening in the cell capping, but we assume that this down-regulation is similar for all pupae in cells that were opened.

No significant differences were noted in the transcription levels for the gene *abaecin* or the gene *hymenoptaecin* although *hymenoptaecin* did show a positive ($r = .31$) regression with numbers of infesting mites. These weak or non-responses might be due to these genes having specific and different functions as immune-related genes (Evans et al., 2006). Both *abaecin* and *hymenoptaecin* have been shown to increase expression in response to microbially challenged honey bees (Casteels, Ampe, Jacobs, & Tempst, 1993; Casteels

et al., 1990) and *Bombus terrestris* (Erler, Popp, & Lattorff, 2011). Zhang, Liu, Zhang, and Han (2010) observed similar low transcription levels of the immune-related genes *phenol oxidase* and *defensin* in both varroa-infested and uninfested *A. mellifera*. Alternately, these weak or non-responses may be a result of the immune suppression ability of the mite which may act more strongly for these genes.

In addition to the expression of the four immune-related genes, we also examined the viral levels for each pupal bee sample and corresponding mites. Research with varroa has shown that these parasites enhance the prevalence and distribution of viruses (Chen & Seide, 2007; Martin et al., 2012). Although we found higher DWV levels in infested than uninfested pupae, viral replication in either pupae or mites was not determined in this study. However, the results clearly showed that the mite could carry DWV genetic material, and DWV replication has been reported to occur in *Tropilaelaps* (Dainat et al., 2009). This suggests that the mite is a potential vector of DWV. Nevertheless, there were more cases of pupae with DWV, and the corresponding mites having no DWV than there were of the mites having DWV and the bee pupae not having the virus (Table 2). These results are similar to past research that also showed the presence of DWV in the honey bee hosts and the absence of the virus in their matching *T. mercedesae* (Forsgren et al., 2009). These observations indicate virus transmission to pupae is not dependent upon mites. Although bee viruses can be detected in a single varroa mite (Chantawannakul et al. 2006), no BQCV was detected in any of the *Tropilaelaps* infesting the bees that were infected with BQCV. This suggests that *Tropilaelaps* mites do not act as a BQCV vector and that the BQCV may not be able to infect the mites.

The impact that the mite-virus complex has on the overall health and survivability of honey bee colonies is still not fully understood. Bee viruses are routinely found in apparently healthy colonies (Martin et al., 2012). In our study, the variety of viruses is less than found in studies involving *A. mellifera* in varroa in other countries (Sanpa & Chantawannakul, 2009). Yet, the *Tropilaelaps* mite in and of itself is clearly able to destroy honey bee colonies. These contrasts suggest that viral infections vectored by the varroa mite may be more serious than viral infections vectored by *Tropilaelaps*. Certainly, no viruses other than DWV could be found in *Tropilaelaps*. This observation is supported by previous research in Northern Thailand where it was observed that in colonies where both *varroa* and *Tropilaelaps* were present, multiple types of viral infections could be found in comparison with colonies that were only infested with *Tropilaelaps* that had fewer types of viral infections (Sanpa & Chantawannakul, 2009).

This research focused on the expression of four well-known insect immunity genes. For a more complete picture of the immune response of *A. mellifera* to *T. mercedesae* infestation, additional genes should be tested. For example, Nazzi et al. (2012) used RNA-seq with a gene list of 166 genes from Evans et al. (2006) to compare the transcriptional profile of bees collected from colonies with high and low *varroa* infestations. The results of Nazzi et al. (2012) focused on immunity-related genes and they identified the down-regulation of 19 immune genes and the up-regulation on six immune genes in response to infestation. Still, it is difficult to describe all possible factors which trigger honey bee immune responses after being challenged with *Tropilaelaps*. However, timing of the expression of genes may be an important factor when trying to identify the importance of an expression result (Kucharski & Maleszka, 2005; Ragland, Egan, Feder, Berlocher, & Hahn, 2011; Tarver, Xuguo, & Scharf, 2010). Perhaps the extraction of the tan-bodied pupae was too late to detect the immediate immunity response of *Apis* to *Tropilaelaps* or parasite infestation. The tan-bodied pupae life stage was chosen because of past similar research and the potential to compare results (Evans, 2004; Navajas et al., 2008). Thus, studies of earlier developmental stages closer to mite invasion or cell capping (when the first feeding of the foundress mite occurs) may help identify early responder genes.

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