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The ectoparasitic mite *Tropilaelaps mercedesae* reduces western honey bee, *Apis mellifera*, longevity and emergence weight, and promotes Deformed wing virus infections

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ABSTRACT

Historically an ectoparasite of the native Giant honey bee *Apis dorsata*, the mite *Tropilaelaps mercedesae* has switched hosts to the introduced western honey bee *Apis mellifera* throughout much of Asia. Few data regarding lethal and sub-lethal effects of *T. mercedesae* on *A. mellifera* exist, despite its similarity to the devastating mite *Varroa destructor*. Here we artificially infested worker brood of *A. mellifera* with *T. mercedesae* to investigate lethal (longevity) and sub-lethal (emergence weight, Deformed wing virus (DWV) levels and clinical symptoms of DWV) effects of the mite on its new host. The data show that *T. mercedesae* infestation significantly reduced host longevity and emergence weight, and promoted both DWV levels and associated clinical symptoms. Our results suggest that *T. mercedesae* is a potentially important parasite to the economically important *A. mellifera* honey bee.

1. Introduction

Global trade of managed western honey bees, *Apis mellifera*, from its native Eurasian and African ranges has provided bountiful opportunities for novel host-parasite relationships (Mutinelli, 2011; Neumann et al., 2016). This is especially true for Southeast Asia, which hosts at least eight indigenous honey bee species (Crane, 1999) in addition to the introduced *A. mellifera* (Moritz et al., 2005). *Tropilaelaps mercedesae*, an indigenous and ubiquitous parasitic mite of the giant honey bee *Apis dorsata* (Laigo and Morse, 1968), is an example of one species that has exploited sympatric *A. mellifera* populations across much of Asia since introduction of the latter (Anderson and Morgan, 2007). Similar to the more well studied mites *Varroa* spp. that historically parasitized the eastern honey bee *Apis cerana* and now also exploit *A. mellifera* (*Varroa destructor*: Anderson and Trueman, 2000; *V. jacobsoni*, Roberts et al., 2015), *T. mercedesae* exploits adult hosts, but is primarily a brood parasite (Kapil and Aggarwal, 1987; Sammataro et al., 2000; Oldroyd and Wongsiri, 2006; Rosenkranz et al., 2010). Fertile female mites oviposit within cells that contain developing honey bees; progeny subsequently hatch and pierce the cuticle of the host to feed on hemolymph (Burgett et al., 1983; Woyke, 1987c). Comparative studies of *T. mercedesae* and *V. destructor* parasitizing *A. mellifera* in Asia have demonstrated that the former produces a higher number of offspring and requires a shorter phoretic period on adult hosts, thereby promoting relatively higher population levels within colonies that could potentially have devastating consequences (Woyke, 1987a,b,c; Sammataro et al., 2000; Garrido and Rosenkranz, 2003). Indeed, anecdotal reports from beekeepers suggest that *T. mercedesae* can have a severe impact on local *A. mellifera* colonies (Buawangpong et al., 2015).

Lethal and sub-lethal effects of parasitism by *V. destructor* on *A. mellifera* are well-documented (Rosenkranz et al., 2010). Globally, it is considered to be the single most important biotic stressor to colonies of European *A. mellifera* honey bee subspecies (Dietemann et al., 2012; Neumann et al., 2012); feeding on haemolymph results in anaemia, the vectoring of viruses, host immune suppression, reduced host longevity (De Jong et al., 1982; De Jong and De Jong, 1983; Yue and Genersch, 2005; Chen and Siede,

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2007; Yang and Cox-Foster, 2007) and ultimately colony mortality (Neumann and Carreck, 2010). Conversely, relatively few information exists about the effects of *T. mercedesae* parasitism on *A. mellifera*. Recent studies suggest that similar to *V. destructor*, *T. mercedesae* can vector honey bee parasites such as Deformed wing virus (DWV) (Dainat et al., 2009; Forsgren et al., 2009) and can influence host immune responses (Khongphinitbunjong et al., 2015b), but no data currently exist regarding common lethal and sub-lethal measures of mite parasitism. In light of anecdotal reports by beekeepers in Asia that *T. mercedesae* might be more devastating to colonies of *A. mellifera* than *V. destructor*, data on the effects of this mite are urgently required considering the potential for this parasite to expand its range due to movement of bees and their products. Here, we investigated possible lethal (longevity) and sub-lethal (emergence weight, DWV infection levels and clinical symptoms) effects of *T. mercedesae* on *A. mellifera*.

2. Materials and methods

2.1. Experimental design

This experiment was conducted at Chiang Mai University, Chiang Mai, Thailand during October and November 2013 using local *A. mellifera* western honey bees of mixed European origin and *T. mercedesae* mites; the latter was confirmed by molecular analyses according to Khongphinitbunjong et al. (2012).

Three queenright *A. mellifera* colonies were used in this study; all had non-patchy-brood frames, low brood infestation levels of *T. mercedesae* (0.29 ± 0.17%) and *V. destructor* (0%), respectively (estimated following Pettis et al., 2013), and no visible clinical symptoms of other diseases (e.g. chalkbrood). Acaricide treatments were not applied to the experimental colonies within three months of the study to facilitate sufficient *T. mercedesae* numbers. Adult female *T. mercedesae* mites were obtained by uncapping newly sealed worker brood of highly infested colonies (Kirrane et al., 2011; Khongphinitbunjong et al., 2013). Age cohorts of bees for the experiment were obtained by confining queens in each colony on an empty brood comb for 24 h using a standard cage frame (Williams et al., 2013). Within six hours of capping, *T. mercedesae* were introduced into age cohort brood cells using an established artificial infestation method that inserted a single mature female mite via a small incision (~1 mm) created at the base of the cell capping (Garrido and Rosenkranz, 2003; Dietemann et al., 2013; Khongphinitbunjong et al., 2013). Cohort brood cells in each colony were randomly assigned to one of three groups (n = 40 per group per colony) that consisted of: (1.) cells undisturbed (C/C = controls); (2.) cells with an incision but without an introduced mite (I/C = controls for effect of experimental manipulation); and 3.) cells with an incision and with an introduced mite (I/T = mite treatment). The position of each experimental cell was mapped on a transparent sheet to enable future re-examination. After the experimental treatments were performed, test frames were maintained at 34 °C and 70% relative humidity following standard protocols (Williams et al., 2012) in a growth chamber. Each pupa was removed from its cell ~10–11 days after inoculation during the grey thorax stage to ensure that all the mother mites and progeny could be collected (Anderson and Roberts, 2013; Khongphinitbunjong et al., 2015b). Pupae were transferred to modified 1.5 ml ventilated microcentrifuge tubes and maintained under previously described conditions to allow proper development to maturity. Within 24 h of removal from the cells, each individual was weighed and examined for deformed wings according to Williams et al. (2009). Each bee was subsequently marked with a worker Opalith platelet (Neumann et al., 2003), and transferred to plastic hoarding cages (Retschnig et al., 2014a,b). For each treatment and colony, two cages were used (n = 20 bees per cage). Each cage was provided sucrose solution *ad libitum* (1 water: 1 sugar), and again maintained at previously described growth chamber conditions. Cages were observed daily until all bees had died; dead bees were individually collected and kept at −20 °C before RNA extraction.

2.2. Deformed wing virus analyses

Clinical symptoms of DWV are easily diagnosed in the field, and include deformed wings and shortened abdomens (Bailey and Ball, 1991). A total of 70 bees that died in cages at Day 2 ± 1 not exhibiting (C/C = 15, I/C = 15, I/T = 13) or exhibiting (C/C = 2, I/C = 5, I/T = 20) clinical symptoms were analyzed for DWV levels according to Khongphinitbunjong et al. (2015a,b). These dead bees provided a 'snap-shot' using a suitable sample size of individuals collected within a compressed time period in order to prevent extended parasite replication from influencing results (Huang and Solter, 2013; Williams et al., 2014). Total RNA was extracted from individual bees using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration (ng/μl) and purity (A260/280) of total RNA was determined using a spectrophotometer (BioDrop™). Reverse transcription reactions for cDNA synthesis were performed using an iScript™ cDNA Synthesis Kit (Biorad). The presence of DWV virus was determined by qPCR using IQ5 Real-Time PCR thermal cycler (Bio-Rad). Amplification was performed in 20 μl reaction volumes using SsoFast EvaGreen (BioRad), consisting of 10 μl SSo SYBER mix, 1 μl of 10 μM of each primer [DWV(AY292384.1) and *β-actin* (Simone-Finstrom et al., 2009)], 7 μl of nuclease free water and 1 μl cDNA. Reactions were run at 95 °C for 30 s, 40 cycles of 95 °C for 1 s, 59 °C for 5 s followed by a melt-curve dissociation analysis. All reactions included three replicates. The qPCR data were expressed as the threshold cycle (Ct) value and were determined by normalizing the Ct value of the reference gene (*β-actin*) to the target genes (ΔCt). To compare DWV levels across treatments, the qPCR data were interpreted using the $2^{-\Delta\Delta Ct}$ method (Livak and Schnittgen, 2001; Chaimanee et al., 2012; Boncristiani et al., 2013). The treatment group with the lowest viral level was used as the calibrator and the levels of viruses in all other groups were expressed as n-fold differences relative to the calibrator (Chen et al., 2005).

2.3. Statistical analyses

Statistical analyses were performed using JMP (version 11.0) and SPSS (version 17.0). Figures visualizing data were created using Microsoft Excel (Office version 2010), except for longevity which was created using SPSS (version 17.0). Some data were not normally distributed; emergence weight and longevity data were therefore square-root transformed, whereas proportional deformed wing data were arcsine square-root transformed. Emergence mass, wing deformity, and DWV levels were analyzed using a mixed model that incorporated colony as a random factor. When differences were found, means were compared using Tukey-HSD (emergence weight, proportions of bees with deformed wings and DWV levels for all individuals) and Student's *t* test (DWV levels considering clinical symptoms) with 95% confidence. Differences in bee longevity among treatments were compared using a Kaplan–Meier survival analysis; Log Rank multiple comparison tests were used to determine difference between specific treatments.

3. Results

Overall, 84% of the introduced female mites reproduced; the average number of progeny was 1.86 ± 0.12. No *V. destructor* mites were found in any cells of the experimental brood frames. Bees