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Original article

Behavioral responses underpinning resistance and susceptibility of honeybees to *Tropilaelaps mercedesae*

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Abstract – Behavioral responses of *Apis cerana*, *Apis dorsata*, and *Apis mellifera* to the ectoparasitic mite, *Tropilaelaps mercedesae*, were compared using two laboratory bioassays: cohorts of 50 caged worker bees and individual-caged worker bees, all of unknown ages. For the group bioassays, ten *T. mercedesae* were placed on the bodies of bees in each cohort. After 6 h, nearly 2/3 of the mites placed on *A. cerana* had fallen from the bees onto sticky traps that were placed under the cages, compared to only about 1/3 for *A. dorsata* and *A. mellifera*. The majority of fallen mites fell within 24 h from *A. cerana* (93.3±2.3%), 36 h from *A. dorsata* (92.2± 1.9%), and 48 h from *A. mellifera* (91.3±1.4%). Higher proportions of injured mites were observed among the mites that fell from *A. cerana* (38.3±12.9%) and *A. dorsata* (33.9±17.4%) than among those that fell from *A. mellifera* (19.5±7.2%). The rapid fall of mites from the bodies of *A. cerana* may be due to a combination of auto-grooming and rapid body shaking of the bees. In individual bee assays, where individual bees were challenged with one female *T. mercedesae*, *A. cerana* and *A. dorsata* exhibited faster behavioral responses to the presence of mites than did *A. mellifera* (39.4±13.2, 44.9±19.2, and 188.4±63.9 s, respectively). Phoretic *T. mercedesae* were mostly observed attaching to the propodeum/petiole region of all three bee species, although some mites also occupied the wing base area of *A. dorsata* and *A. mellifera*.

grooming behavior / *Apis cerana* / *Apis dorsata* / *Apis mellifera* / *Tropilaelaps mercedesae*

1. INTRODUCTION

Mites, in the genus *Tropilaelaps*, are primary brood ectoparasites of the giant Asian honeybees (*Apis dorsata* and *Apis laboriosa*) (Delfinado-Baker et al. 1989), although they are not considered to be serious pests of these bee species. This is primarily due to their inability to feed on adult bees (Koeniger and Musaffar 1988). Hence, the mites can only survive in colonies with the presence of brood in which they readily feed and reproduce while inside the cells. In vitro studies have shown that *Tropilaelaps mercedesae* (previously mistaken for *Tropilaelaps clareae*) lives only ca. 1–3 days on adult bees (Kitprasert 1984; Woyke 1984; Koeniger and Musaffar 1988; Rinderer et al. 1994). In addition, natural migrations of giant honeybees produce long broodless periods, which reduce mite infestation rates (Wongsiri et al. 1989). Brood nest hygienic behavior and other behaviors such as shimmering (Butler

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1954) or body shaking (Sakagami 1960) have also been shown to severely reduce *Tropilaelaps* populations in *A. dorsata* colonies (Burgett et al. 1990). It is also likely that there are other behavioral mechanisms of resistance to *Tropilaelaps* in giant honeybees which have yet to be identified.

Tropilaelaps spp. infest *Apis mellifera* imported to Asia with devastating effect. Earlier reports identified *T. clareae* to be the most serious parasite of *A. mellifera* colonies in Asia (Burgett et al. 1983). However, with the identification of new *Tropilaelaps* species, *T. mercedesae* is now known to be the most widely spread *Tropilaelaps* species causing mortality of *A. mellifera* colonies (Anderson and Morgan 2007). *T. mercedesae* infestation of *A. mellifera* occurs in regions well outside the distribution of giant honeybees, and hence, the mite is regarded as an emerging global threat to this bee species (Anderson and Morgan 2007). The once considered most serious threat to *A. mellifera*, *T. clareae*, is now known to only cause losses of *A. mellifera* colonies in the Philippines (Cervancia 1993), while the remaining two species in the genus, *Tropilaelaps koenigerum* and *Tropilaelaps thaii*, are considered harmless to *A. mellifera* (Anderson and Morgan 2007).

In Asia, *Tropilaelaps* spp. have also been collected from colonies of *Apis cerana* and *Apis florea*. Individuals of *T. mercedesae* (which at the time was thought to be *T. clareae*) have been found in *A. cerana* colonies in Pakistan, Myanmar, and Thailand (Delfinado-Baker 1982; Wongsiri et al. 1989) and, in India, associated with adult bees of *A. florea* (Kapil and Aggarwal 1987). Nevertheless, *T. koenigerum* has been found reproducing on the brood of *A. cerana* in India (Abrol and Putatunda 1995) and, recently, one adult female *T. mercedesae* was found reproducing on *A. cerana* brood in Thailand (Anderson and Morgan 2007). Although these latter observations span a wide geographical range, *Tropilaelaps* is only rarely found on *A. cerana* and normally those infestations are not associated with mite reproduction.

The low incidence of *T. mercedesae* infestations in *A. cerana* colonies in locations that are cohabited with giant honeybees suggests that these honeybees have a strong resistance to this parasite. However, no explanation of the resistance mechanism has been offered. It is possible that behavioral mechanisms underpin the resistance of *A. cerana* to *T. mercedesae*. The objectives of this study were to determine the mite removal time of *T. mercedesae* on worker bees of three honeybee species (*A. cerana*, *A. dorsata*, and *A. mellifera*), and to determine if behavioral responses mediate their resistance to *T. mercedesae*.

2. MATERIALS AND METHODS

2.1. Source and genotypic identification of *Tropilaelaps* mites

Tropilaelaps mites used in our studies were obtained from sealed brood of *A. mellifera* in an apiary located at the Bee Protection Center, Chiang Mai University, Chiang Mai, Thailand. Their identity was confirmed by DNA analysis. For this, genomic DNA was extracted from each of ten mites as described by Boonham et al. (2002). The entire ITS1-5.8S-ITS2 gene region was amplified using an ITS4 primer (5′-TCCTCCGCTTATTGATATGC-3′) and an ITS5 primer (5′-GGAAGTAAAAGTCG TAACAAGG-3′) in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) (White et al. 1990). The mixture was initially denatured at 94°C for 4 min and then processed with 30 amplification cycles, each consisting of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C. The reactions were completed by a final extension step for 10 min at 72°C. This step was modified from the methods of Anderson and Morgan (2007). The PCR products were separated on a 1.5% agarose gel by electrophoresis. PCR bands were purified by using NucleoSpin® Exact II (Germany). When compared in the GenBank database using the BioEdit program (Hall 1999), the DNA sequences (320–520 bp) derived from our samples (accession number HM748312-HM748316) showed a 99% similarity to sequences from *T. mercedesae* collected from Sri

Lanka (EF025472.1) and Palawan, Philippines (EF025471.1) and hence were confirmed as *T. mercedesae* (Anderson and Morgan 2007).

2.2. Experiment 1. Evaluation of the responses to *T. mercedesae* using groups of caged bees

The cages used to house bees in this experiment were plastic containers (18×8×4 cm), each with a single hole (diameter=1.3 cm) on the top covered with a screen mesh (8 mesh) and with the entire bottom removed and replaced with mesh that prevented bees from escaping but allowed mites to pass through. This mesh also served as ventilation for the bees. A sheet of cardboard, coated with a mixture of petroleum jelly and vegetable oil (1:1), was attached to the bottom of each cage under the screen mesh to trap mites that fell from the caged bees.

We compared the responses of *A. cerana*, *A. dorsata*, and *A. mellifera* to *T. mercedesae* that were introduced into the cages. A total of 11 colonies (three *A. cerana*, three *A. dorsata*, and five *A. mellifera*) were used as sources for the test bees. From all of the five *A. mellifera* and two of the *A. cerana* colonies, 50 worker bees of unknown ages were randomly collected from the brood nest of their parent colonies. Additionally, worker bees from the entrance of one feral colony of *A. cerana* were used. The *A. mellifera* colonies used as a source for the test bees were different from the colonies used as the source for mites. *A. dorsata* worker bees were collected from three different colonies in the provinces of Phayao and Chiang Mai by scooping bees from the curtain of each nest using an insect net. In order to facilitate introduction of bees into the cages, all test bees (all three species) were anesthetized with carbon dioxide.

For each of the 11 honeybee colonies, 6 cages (each cage represented one replicate) were established for a total of 66 cages (*A. cerana*=18, *A. dorsata*=18, and *A. mellifera*=30). Each cage received ten adult female *Tropilaelaps* of unknown age. To increase the possibility that all inoculum mites were females, mites were collected from newly sealed bee larvae to white-eyed pupae. Using a fine brush (size, number 0), the collected mites were transferred into a Petri dish with bee pupae until enough mites (≥10) were collected to inoculate one cage of bees. Individual mites were then introduced through the cage hole onto bees using the brush. The cages were then placed inside an incubator at 30°C and 40% relative humidity. The number of mites that fell to the sticky trap attached to the bottom of the cages was monitored at 6, 12 h, and then every 12 h until no additional mites fell. All fallen mites were collected and immediately examined under a dissecting microscope for the presence or absence of injuries. Sex of the mites was also confirmed at this time. Following 24–48 h, when no further fallen mites were observed, each cage was placed in a plastic bag and frozen to kill the bees and any remaining mites. Bees were then washed with water to recover phoretic mites left on the bees in order to validate grooming efficiency. The plastic bags were also inspected for the presence of mites. After washing, individual bees were further examined visually under a microscope to ensure that no mites remained. Observations were made during the period February to April 2010.

Behavioral responses of caged honeybees toward the introduced *Tropilaelaps* were observed using the "scan sampling" technique (Martin and Bateson 2007) for each observation period. In brief, cages of bees were scanned for ca. 3 min for the occurrence of grooming behavioral activities by using a video camera (Sanyo VPC-TH1, Vietnam) set up 5 cm from the side of the cage. Eight observations for *A. cerana*, 3 for *A. dorsata*, and 16 for *A. mellifera* were recorded. Visual observation on the response of bees was also made during mite inoculation.

In order to verify whether or not body shaking in *A. cerana* was triggered by the presence of *Tropilaelaps* or the presence of an "observer" while recording, two groups of *A. cerana* (three colonies used as sources of test bees) were established: mite-inoculated (five cages) and not mite-inoculated (five cages) as the control. Bees' responses to the mites were recorded after 6, 12, and 24 h post-inoculation for ca. 5 min. Mites that fell were also collected and examined for injuries under a dissecting microscope. All bees were washed with water and also examined individually after 24 h for the presence or absence of phoretic mites. Control cages were also recorded to determine if the presence of an observer elicited body shaking behavior.

2.3. Experiment 2. Individual bee responses toward *T. mercedesae* and attachment sites of the mites

Worker bees from a single colony of *A. cerana*, and *A. mellifera* (both used in experiment 1) were collected in front of the hive entrances using an insect net. Since the *A. dorsata* colony used in experiment 1 absconded, *A. dorsata* worker bees were collected from a new nest with the aid of an insect net. Bees of each species were placed in separate holding cages (described in Section 2.2) and fed honey prior to being exposed to *T. mercedesae*. For each species, 20 bees were randomly selected and placed individually into small Petri dishes (diameter=50 mm). Ten bees were individually inoculated with one mite each using a fine brush, while the other ten bees served as controls (touched with the brush without mite inoculation). Female *Tropilaelaps* were collected from either emerging brood or tan-bodied pupae of one highly infested *A. mellifera* colony, different from the one used to source *A. mellifera* test bees. Behavioral responses of both groups toward the introduced *Tropilaelaps* were recorded simultaneously for 10 min using two video cameras (Sanyo VPC-TH1, Vietnam and Panasonic HDC-HS250, Japan).

After mite inoculation, grooming activities (related or unrelated to the presence of mite) were observed and counted within a 10-min period. Latency or time from mite introduction to either the onset of the first occurrence of grooming activity or mite drop (which probably reflects undetected grooming) was also measured (Martin and Bateson 2007). Successful removal of a mite was defined as the ability of the bee to rid itself of the mite (regardless of the mite remounting the bee) within the 10-min period. All mites that fell from the bee were examined under a dissecting microscope for the presence or absence of injuries. Observations on the attachment sites of the inoculated mites on the bees were also noted microscopically and visually during this time. At the end of each observation, each bee was examined under a dissecting microscope to determine the final attachment site of the inoculated mite.

2.4. Data analyses

Data on the cumulative proportion of mites that dropped from bees through time were first transformed using an arcsine–square root transformation to approximate normality and then subjected to analysis of variance (ANOVA) for repeated measures by using PROC MIXED (SAS version 9.2, SAS Institute 2008). Since a significant interaction between honeybee species and time of observation was detected, a post hoc slice test was performed to determine the effect of honeybee species on the proportion of mites that had fallen during each observational period. The effect of honeybee species on the proportion of injured mites (calculated as the number of injured mites divided by total mites that fell) was determined using a one-factor ANOVA. The proportions of injured mites were transformed using an arcsine–square root. Data on grooming activities approximated normality, so data were not transformed. A two-factor ANOVA was used to determine the effects of honeybee species and the presence or absence (brush-touched only) of *T. mercedesae* on their occurrence. There was no interaction detected; thus, no posttest was necessary. Grooming response time was transformed using a square root transformation to approximate normality. To determine the effect of honeybee species on the grooming response time toward an introduced mite, a one-factor ANOVA was used. Means were separated with Bonferroni-adjusted *t* tests.

3. RESULTS

3.1. Experiment 1. Evaluation of the responses of three honeybees to *T. mercedesae* using groups of caged worker bees

3.1.1. Cumulative proportion of fallen T. mercedesae

Analysis of the number of *T. mercedesae* that fell to the cage floors showed a significant interaction between honeybee species and time of observation ($F=16.38$; $df=10, 378$; $P<0.0001$). Almost 2/3 of the inoculated mites had fallen from the caged *A. cerana* after 6 h (Table I). In contrast, only about 1/3 of the mites fell from the caged *A. dorsata* and *A. mellifera* during the same time period. A similar

Table I. Cumulative proportion (mean±SE) of *T. mercedesae* that fell from cages of three different honeybee species through time and condition of fallen mites.

	Apis cerana n=18 cages; 180 mites	*Apis dorsata* n=18 cages; 180 mites	*Apis mellifera* n=30 cages; 300 mites
Time of observation (h)			
6	61.1±1.6a	38.9±4.5b	34.0±2.2b
12	82.2±2.4a	63.9±2.8b	52.3±1.8b
24	93.3±2.3a	87.2±1.4a	66.0±1.9b
36	–	92.2±1.9a	82.0±1.5b
48	–	–	91.3±1.4
Condition of mites			
Uninjured	61.7±4.1b	65.9±3.0b	80.4±1.3a
Injured	38.3±12.9a	33.9±17.4a	19.5±7.2b

For each time of observation and mite condition, means for the different honeybee species followed by different letters are significantly different at $P<0.05$.

trend was observed after 12 h of observation. After 24 h, similarly high proportions of mites fell from caged *A. cerana* and *A. dorsata* workers, compared with low proportions from the caged *A. mellifera*. No fallen mites were recorded from *A. cerana* after 36 h. At this time, a higher proportion of mites had fallen from the caged *A. dorsata* than fell from caged *A. mellifera* (t=3.81, P=0.0002). Overall, it took only 24 h before most mites (>90%) had fallen from caged *A. cerana*, and this was 12 and 24 h faster than for *A. dorsata* and *A. mellifera*, respectively. At the end of the experiment, a similar proportion of mites was not recovered for all honeybee species (Tukey's test, $P>0.05$) (*A. cerana*=6.7±0.9%, *A. dorsata*=7.8±0.8%, and *A. mellifera*=7.7±0.7%). No mites were recovered from the bee washes or inspections of individual bees. A similar trend was observed in the mite-inoculated vs. not mite-inoculated assay wherein 56±8% of the mites had fallen within 6 h, 84±10% within12 h, and 92±7.5% within 24 h.

3.1.2. *Injury to phoretic* T. mercedesae

Inspections of the phoretic mites that fell showed that most were apparently uninjured for all three honeybee species. However, the number of injured mites was influenced by honeybee species (F=15.61; df=2, 63; $P<0.0001$). The proportion of injured *Tropilaelaps* that fell from caged *A. cerana* was similar to that of *A. dorsata* yet significantly higher than for caged *A. mellifera* (Table I). Regardless of the time of observation and mites' condition (injured or not), all mites collected were dead. Results of the mite-inoculated vs. not mite-inoculated assay also showed similar trends (uninjured= 60.7±7.5%, injured=39.3±4%).

Injuries observed on fallen mites were located on different parts of the mites' body (Table II), with the majority of damage occurring on the legs. The palpus and body shield showed the least damage.

3.1.3. *Description of the behavioral responses of three honeybee species to introduced* T. mercedesae

We report patterns of behavioral responses of bees (infested or not) based on examining 8, 3, and 16 video recordings for *A. cerana*, *A. dorsata*, and *A. mellifera*, respectively. A behavior common to all three honeybee species was auto-grooming. The grooming bees raised their abdomens slightly and brushed their abdomens with their hind legs, or rubbed their hind

Table II. Percentage of total injuries inflicted on *T. mercedesae* by three honeybee species.

Honeybee species	Body part					
	Leg 1	Leg 2	Leg 3	Leg 4	Palp	Body shield
A. mellifera	47.1	25.0	17.6	5.9	1.5	2.9
A. dorsata	33.0	20.5	10.2	30.7	4.5	1.1
A. cerana	50.0	16.7	15.3	11.1	4.2	2.8

legs together. Most of the time, the bees twisted or pivoted their abdomens while grooming. The bees also used both fore- and mid legs to groom the head and thorax. While auto-grooming, *A. cerana*, unlike *A. mellifera* or *A. dorsata*, also engaged in "body shaking". For this, the *A. cerana* workers moved their bodies rapidly in a side-to-side movement (10.47±1.41 Hz, n=10). During this body shaking, the bees shook their bodies repetitively.

Groups of *A. cerana* inoculated with *T. mercedesae* and those that were not inoculated with mites also displayed common auto-grooming activities described above. However, body shaking was observed in 6 out of 11 video recordings. In the control group, no body shaking was observed at any of the observation times (6, 12, or 24 h; n=10 videos). We also observed *A. cerana* (two out of eight recordings) and *A. mellifera* (3 out of 16 recordings) displaying allo-grooming against *T. mercedesae*. No allo-grooming was observed with *A. dorsata*. *A. mellifera* was the only honeybee species that displayed "Nasanov-scenting" behavior which was observed in 7 out of the 16 video recordings analyzed.

3.2. Experiment 2. Evaluation of the responses of three honeybee species to *T. mercedesae* using individual worker bees

3.2.1. Latency to the onset of grooming activities by three honeybee species

Analysis showed that when individual bees were presented with *T. mercedesae*, the latency, or time from mite introduction to either the onset of the first of grooming activity or mite drop, varied among species of honeybees (F=4.87; df=2, 25; P=0.016). *A. cerana* (39.4±13.2 s) and *A. dorsata* (44.9±19.2 s) responded to the presence of *T. mercedesae* more quickly than did the *A. mellifera* (188.4±63.9 s).

3.2.2. Behavioral responses and attachment sites

The auto-grooming activities described in Section 3.1.3 using groups of caged worker bees were also observed in the single-bee bioassay. There were no apparent differences in the details of the grooming responses of the three honeybee species. However, we observed a significant (F=10.38; df=2, 54; P=0.0002) interaction between honeybee species and treatment (mite vs. being touched with a brush; Fig. 1) for the number of grooming activities performed. When presented with a mite, *A. dorsata* and *A. cerana* equally groomed more than did *A. mellifera*. But when touched with a brush, *A. mellifera* displayed more grooming activities than *A. cerana* and *A. dorsata*, even with the absence of a mite threat. However, we did not observe any body shaking using individual *A. cerana* worker bees. Similarly, no Nasanov scenting was observed in *A. mellifera*.

T. mercedesae used several attachment sites on all the three honeybee species. The majority of the phoretic mites moved constantly from one site to another as the infested bees groomed. The mites were either around the head/head–thorax region, on the scutellum with their opisthosoma protruding, on the dorsal or lateral

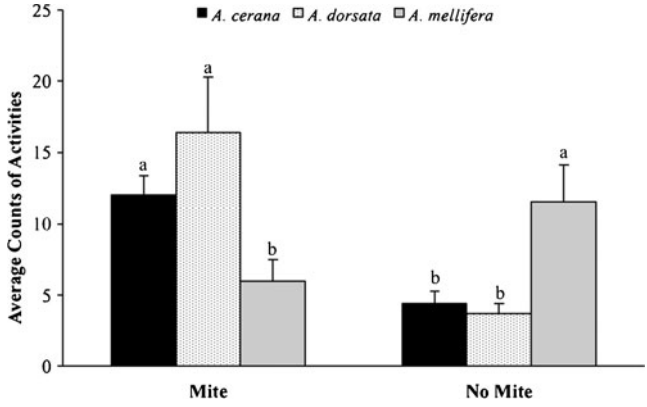

Figure 1. Number (mean±SE) of grooming activities as affected by honeybee species within a 10-min observation period. For each inoculation type, *bars with different letters* are significantly different at $P<0.05$.

side of thorax attached to a cluster of setae, near the wing base, between the base of forelegs (ventral) with opisthosoma protruding, near the base of a leg, femur of the leg, or between the thorax and abdomen (propodeal tergite) with the head pointing toward the petiole or on the concave dorsum of the abdomen. However, at the end of the experiment, about 61.5% and 50% of the mites were found in the petiole region of *A. cerana* and *A.mellifera*, respectively. On *A. dorsata*, the mites were found at several attachment sites, but more mites were found near the base of the wings (28.6%) and petiole region (28.6%).

4. DISCUSSION

Our results showed substantial differences in the survival of *T. mercedesae* when artificially placed on *A. cerana*, *A. dorsata*, and *A. mellifera* worker bees, which was probably related to worker bee grooming behavior. The shortest survival was observed on *A. cerana* (24 h). Mite survival on *A. dorsata*, which is the indigenous host of *Tropilaelaps*, was longer (36 h), while mites survived longest on *A. mellifera* (48 h). In Pakistan, Koeniger and Musaffar (1988) reported a similar survival (27 h) of *T. clareae* (which was most likely *T. mercedesae*, as *T. clareae* has been shown to be restricted to the Philippines) on *A. cerana*. However, they found that survival was shortest on *A. mellifera* (25 h) and longest on *A. dorsata* (57 h), which contrasts sharply with our results. This discrepancy may be due to the differences in the genotypes of the three species of honeybees studied. Radloff et al. (2010) documented that *A. cerana* from Pakistan belongs to the morphocluster Northern *cerana*, while *A. cerana* of Northern Thailand is of the Indochinese *cerana* type. Differences in the methodology used may have also contributed to this disparity. In this study, we used 50 bees per cage and kept them in a 30°C incubator, while Koeniger and Musaffar (1988) used 20 bees per cage kept at 26°C. The number of bees per cage and temperature may have had a significant effect on the bees' ability to groom.

So far, reproduction of *T. mercedesae* has only been observed in one capped brood cell of *A. cerana* (Anderson and Morgan 2007). This rare observation may be due to the bees' resistance to this brood parasite, which may also explain their poor survival on adult bees in this study. The poor survival of *T. mercedesae* on *A. cerana* may be due to a suite of defense strategies displayed by this honeybee species. For example, in our studies *A. cerana* performed apparently intense and long autogrooming episodes when exposed to *T. mercedesae*. In addition, *A. cerana* performed body

shaking (with an intensity that should suffice to dislodge phoretic mites), which is normally displayed by guard bees as a defense mechanism to bee-hawking by *Vespa velutina* (Tan et al. 2005; Tan et al. 2010). Body shaking has also been observed in *Apis nuluensis* (Koeniger et al. 1996) and *A. dorsata* (Kastberger et al. 1998) against *Vespa* spp. and other predators. It is unlikely that the body shaking observed in our experiments was triggered by the presence of an "observer" while recording since no body shaking was observed in either the uninoculated cages (group bioassay) or the single-bee bioassay (mite-inoculated and brush-touched only) in which one to two observers were present. Since we also did not observe body shaking in any of the mite-inoculated individual *A. cerana*, this observation suggests the importance of body shaking as a "signal or warning" of the presence of intruders in a social setting. However, groups of *A. dorsata* worker bees did not respond similarly to the presence of *T. mercedesae* in this study, although they are known to engage in the behavior in a different context. We were not able to confirm whether a bee that displayed body shaking was infested with *Tropilaelaps*. They may not all have been infested since not all of them displayed intermittent auto-grooming. Perhaps body shaking may also be a response to the actions of infested bees nearby. The body shaking and wing activation of infested bees may have produced a sound or vibration that triggered other bees to also engage in these actions. This hypothesis may explain our observations of the simultaneous body shaking of bees which were not in direct contact.

Similarly, while we observed Nasanov scenting [a behavior observed in *A. nuluensis* (Koeniger et al. 1996) and in *A. dorsata* against predatory wasps (Kastberger et al. 1998)] in *A. mellifera* workers caged in groups, no Nasanov scenting was observed in the experiment using single bees. Thus, it is also possible that this behavior is only expressed in a group setting. Whether or not this behavior was performed in response to the presence of mites is unclear with our available data.

Tropilaelaps have a different body structure from that of *Varroa* mites which prevents them from concealing themselves between the sternites of the bees (Rath 1999). In this study, we observed different attachment sites for *T. mercedesae* while being phoretic on adult bees. However, the propodeum/petiole region (concave area of the dorsal abdomen) seemed to provide the most protection for the mite especially in *A. cerana* and *A. mellifera*. Similar observations were reported by Woyke (1984) and Ritter and Schneider-Ritter (1988) for *A. mellifera*. Although the mites infesting *A. dorsata* also used the petiole region, a similar proportion of them were found near the base of the wings at the end of the experiment. A body action (twisting of the abdomen while a mite is attached at the propodeal tergite of the thorax) may have caused mites to be irritated and move to the more exposed wing base area. The mites in this area moved around as the bees performed auto-grooming movements and appeared to be vulnerable to grooming.

Clearly, fallen mites result from a variety of causes. The mites may be brushed off during auto-grooming. Body shaking and wing beating may throw them from the bees. Body twisting may expose them to other grooming actions. Despite having a small sample size, the results of our single-bee experiment showed successful mite removal by *A. cerana* and *A. dorsata* only: four out of ten mites with *A. cerana* (one injured, one without obvious physical injury but not able to walk, and two uninjured and able to walk), and three out of ten with *A. dorsata* (one with injured legs and two uninjured). One successful mite removal in *A. dorsata* was caused by the beating of the wings. In contrast to the findings of Peng et al. (1987) and Büchler et al. (1992) with *Varroa jacobsoni*, allo-grooming did not significantly contribute to the resistance of *A. cerana* to *T. mercedesae*.

In our study, about 1/3 of the fallen mites were injured in both *A. cerana* and *A. dorsata* trials and only about 1/5 in *A. mellifera* trials. Higher proportions of uninjured than injured mites had also been reported by several researchers who studied grooming behavior in

Varroa mites (Peng et al. 1987; Büchler et al. 1992; Aumeier 2001). With *Varroa* mites, a high proportion (ranging from 30% to 50%) of groomed mites had damaged legs (Ruttner and Hanel 1992; Rosenkranz et al. 1997; Corrêa-Marques et al. 2002), and injuries to the idiosoma were relatively rare (about 1–2%) (Ruttner and Hanel 1992). Our observations on the types of injuries agreed with these findings.

Our results suggest that the three species of honeybees in the study had varied degrees of behavioral resistance to *Tropilaelaps* in the phoretic stage. *A. cerana* appeared to have the highest behavioral resistance owing to its body shaking in response to the presence of mites. Body shaking may be an effective response to *Varroa* which serves as a preadaptation and is still more effective against *Tropilaelaps* owing to its morphology. *Tropilaelaps* has a different morphology from that of *Varroa* and has probably not coevolved with the behavior. *A. dorsata*, the natural host of *Tropilaelaps*, also showed a high level of behavioral resistance to *Tropilaelaps*. However, since the mite has coevolved with *A. dorsata*, it is more successful on *A. dorsata* than it is on *A. cerana*. Although *A. mellifera* has not been exposed to parasitic brood mites until its introduction to Asia, it does display auto-grooming behavior in response to them. This is probably a preadaptation as a result of its coevolution with *Acarapis* mites. The success of *A. mellifera* auto-grooming is less than that of the two Asian bees. However, because *A. mellifera* was able to remove about 50% of the mites after 12 h and injured at least 1/5 of the recovered mites, a selection program may result in a stock of *A. mellifera* that is resistant to *T. mercedesae*. *A. mellifera* varies in its resistance to *Acarapis woodi* with European *A. mellifera* having more resistance than North American *A. mellifera* (de Guzman et al. 1998; de Guzman et al. 2002). Lineages of *A. mellifera* that show increased resistance to *A. woodi* may be more resistant to *Tropilaelaps*.

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Réponses comportementales à la base d'une résistance et d'une susceptibilité des abeilles à *Tropilaelaps mercedesae*

Comportement de toilettage / *Apis cerana* / *Apis dorsata* / *Apis mellifera* / Acari

Verhaltensreaktionen im Zusammenhang mit einer Resistenz bzw. Anfälligkeit von Honigbienen gegenüber *T. mercedesae*

Grooming Verhalten / *Apis cerana* / *Apis dorsata* / *Apis mellifera* / Acari

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