EVIDENCE FOR PASSIVE CHEMICAL CAMOUFLAGE IN THE PARASITIC MITE *Varroa destructor*

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Abstract - Social insect colonies provide a stable and safe environment for their members. Despite colonies been heavily guarded, parasites have evolved numerous strategies to invade and inhabit these hostile places. Two common strategies are chemical mimicry via biosynthesis of the hosts' odour or chemical camouflage were compounds are acquired straight from the host. The ectoparasitic mite *Varroa destructor* feeds on the heamolymph of its honeybee host *Apis mellifera* and uses chemical mimicry to remain undetected as it lives on the adult host during its phoretic phase or while reproducing on the honeybee brood.. During the mite life cycle it switches between host adults and brood, which requires it to adjust its profile to mimic the very different odours of honeybee brood and adults. In a series of transfer experiments using adult bees and pupae, we tested whether *V. destructor* does this by synthesising compounds or using chemical camouflage. We show that *V. destructor* required direct access to the host cuticle to mimic its odour and was unable to synthesise host-specific compounds itself. Mites use chemical camouflage to mimic the host odour, even when dead, indicating a passive physico-chemical mechanism of the parasite cuticle. The chemical profile of *V. destructor* was adjusted within three to nine hours after switching hosts, demonstrating that passive camouflage is a highly efficient, fast and flexible way for the mite's to adapt to a new host's profile when moving between different host life stages, or host colonies.

INTRODUCTION

Colonies of social insects are a popular target of parasites, as they represent a stable micro-climate, as well as, a concentrated source of food and other resources (Wilson 1971; Hölldobler and Wilson 1990). The main challenge for these parasites is to overcome the intricate system of defence that social insects have evolved to defend their colonies against such invaders. The parasite needs to enter the colony unscathed and remain unharmed whilst trying to exploit its resources. Cuticular hydrocarbons (CHCs), excreted as part of the insect's cuticular lipid layer, are used by many insects to recognise and identify individuals around them (Howard and Blomquist 2005). In social insects CHCs are species- (Martin and Drijfhout 2009) and colony-specific (Martin et al. 2013), and colony members compare their own CHC profiles to those around them to detect potential nest invaders; the first line of colony defence.

Many social parasites have evolved strategies to evade this chemical recognition system (Dettner and Liepert 1994; Lenoir et al. 2001; Bagnères and Lorenzi 2010) either through biosynthesis of host CHCs (chemical mimicry *sensu stricto*: Akino et al. 1999; Howard et al. 1982, 1990; Lenoir et al. 1997; Lenoir et al., 2001), or by reducing their own CHC profile to undetectable levels (chemical insignificance: wasps: Lorenzi and Bagnères 2002; parasitoids: Kroiss et al. 2009), using a combination of both (Jeral et al. 1997; Uboni et al. 2012) or acquiring compounds straight from the host (chemical camouflage: beetles: Vander Meer and Wojcik 1982; parasitoid: Akino et al. 1999; spiders: von Beeren and Witte 2012). Cini et al. (2011) showed that it is CHCs in particular rather than other, co-occurring compounds that are important in the detection of the parasite by the host.

Many parasites interact with their host inside the nest and use chemical mimicry to be perceived and treated as if they were a member of the colony (Lenoir et al. 2001). Some parasites mimic a particular caste (Akino and Yamaoka 1998) or gender (Hojo et al. 2009) of their host to increase acceptance and trick the host into feeding them. Nevertheless, very little is known about the chemical strategies used by parasites that exclusively feed and reproduce on their hosts. The ectoparasitic mite *Varroa destructor* is a major pest of the European honey bee *Apis mellifera* and has a very close relationship with its host. This mite uses 'drifting' bees to infest new colonies and, once inside the

colony, it switches to nurse bees to increase its likelihood of reaching the brood area, where it reproduces (Kraus et al. 1986). To reproduce the mite moves from the adult nurse bee onto a final staged larva. Once the brood cell has been sealed the mite emerges from beneath the larva and starts its reproductive cycle feeding on the developing pupa. When the pupae emerges from the brood cell as adult bee, the foundress mite and her mature offspring are also released. The males soon perish but the females enter their phoretic phase, feeding on the haemolymph of the adults (Rosenkranz et al., 2010). There are no behavioural interactions between the mite and its host, but instead *V. destructor* blends in with the bee's body chemistry to become chemically invisible to the host as the mite is carried around the colony (Nation et al. 1992). Honeybee CHC profiles vary depending on the age and gender of the bee (Nation et al. 1992, Arnold 2000, Aumeier et al. 2002, Kather et al. 2010) and results published by Nation et al. (1992) and Martin et al. (2001) suggest that *V. destructor* adjusts its mimicry to match these differences as it switches hosts, which indicates that the mite does not use chemical insignificance to remain undetected in the host colony.

Here we investigated the mechanism through which *V. destructor* is able to mimic the CHC profile of its host and tested whether the mite uses chemical camouflage or biosynthesis to achieve its chemical mimicry. Because of the high death rate of bees associated with radio labelling of CHCs in *A. mellifera* (Gary Blomquist and Falko Drijfhout, *pers. comm.*), we used the speed at which *V. destructor* adjusts its mimicry to a new host as an indicator for whether any changes in CHC profile were due to biosynthesis of compounds (true chemical mimicry) or the mite transferring compounds straight from the host (chemical camouflage). Studies have shown that once biosynthesis of new compounds has started it takes one to several days for these compounds to reach the cuticle (de Renobales et al. 1988, Ichinose and Lenoir 2009). Therefore, we assumed that if *V. destructor* synthesised compounds itself it would take at least one day to detect any significant change in the mite's CHC profile. However, if the mite transferred host compounds onto its own cuticle, we can expect to see changes in the mite's CHC profile within a few hours of exposing it to the new host. Fortunately, the CHC profiles of adult bees are rich in alkenes whereas pupae profiles are rich in methylalkanes, thus allowing easy quantification of changes in the mite's cuticular chemistry when mites are moved

between these two honeybee life stages. We therefore, measured the speed at which the mite was able to adjust its profile to that of its new host.

In addition, we predicted that if *V. destructor* used chemical camouflage, it would need direct physical contact with the host's cuticular lipid layer to be able to transfer it onto its own cuticle. However, if mites synthesised their CHC, they may be able to adjust their profiles without direct cuticular contact with the host or access to host's hemolymph. Therefore, we conducted a series of additional experiments where we again moved mites from adult bees onto pupae, but this time we restricted their access to the host cuticular lipid layer or the host hemolymph or both to test if this affected the ability of the mites to adjust their profile.

Finally if *V. destructor* uses chemical camouflage to mimic its host, how is this achieved given that several other parasites actively groom their host to transfer compounds but such behaviour is not seen in *V. destructor*. To investigate this we transferred dead mites that had their own CHC profile removed chemically onto bee and fly pupae and observed if mites could still acquire the pupa's CHC profile.

METHODS

Sample Collection - For all experiments, mites were collected from adult bees (i.e. nurse bees) by removing brood frames from a hive and covering the bees on the frame with icing sugar. Brood frames were returned to the hive and a Varroa board was inserted at the bottom of the hive to catch the fallen mites. These were removed from the board using a fine moist brush, which was cleaned with water and dried after each mite. Mites were gently wiped to remove excess sugar and placed in Eppendorf tubes. As expected, mites collected this way had a CHC profile very similar to that of adult (nurse) bees (Fig. 1) and were used as a starting point for all treatment groups.

Adult bees and pupae of the same hive were collected prior to sugaring to allow for mite-bee comparison later on. The pupae collected were of the white-eye stage i.e. 3-4 days after the cell had been sealed. All treatment groups were kept at 34°C and 70% humidity. During experiments, mites were kept in 0.6ml microcentrifuge tubes that had been prepped with a wet cotton ball at the bottom to maximise humidity inside the vial and a hole was created in the lid to allow for oxygen exchange. Each treatment group

contained eight mites per replicate and ten replicates per group. At the end of the experiment, mites were stored at -20°C for chemical analysis later. Only mites that were still alive at the end of the experiment were used for chemical analysis (refer to Table 1 for the total number of mites per treatment group that were used for chemical analysis). The CHC profiles of bee pupae were also quantified to control for any changes in mite CHC chemistry caused by inter-pupae differences rather than due to a treatment effect.

Speed of host CHC acquisition - The first experiment investigated the speed at which *V. destructor* is able to adjust its chemical mimicry to that of a new host. Mites were transferred from adult bees (alkene rich) onto pupae (methylalkanes rich), and kept on the pupae for 20 mins, 1 hr, 3 hrs, 9 hrs or 27 hrs to measure the speed at which *V. destructor* acquires a new host profile. A total of 460 mites were collected, of which 60 were frozen straight away to serve as starting point (0 hrs control). The remaining 400 mites were transferred to microcentrifuge tubes for the various time periods, with one pupa added to each tube.

Limited access to host cuticular lipid layer - The second experiment tested whether *V. destructor* needs access to the host cuticular lipid layer or access to the host hemolymph to adjust its mimicry. Mites were moved from adult bees to pupae and limited in their access to either a) the host's lipid layer or b) the host's hemolymph and lipid layer. A total of 400 mites were transferred to adult bees kept in bee cages and allowed to feed for 24 hrs to control for any effects of starvation during the experiment. After 24 hrs, the mites were transferred to microcentrifuge tubes which had been prepped with four rows of holes along the sides of the tube. This allowed mites to perceive the smell of any pupae placed immediately around the vial without allowing direct access to these pupae. The tubes were then placed in a petri dish, with 2-3 tubes per dish.

Four treatment groups were created: 1) Mites were kept on one pupa per tube to imitate a natural situation (control group), where mites have access to the host's cuticular lipid layer and its hemolymph. 2) Mites were able to smell the host but were denied access to its hemolymph and cuticular lipid layer. In this group, pupae were placed around the (perforated) tubes which prevented the mites touching the pupae. 3) Mites had

access to host hemolymph but not the host cuticular lipid layer. To achieve this, the mites were kept on a pupa that had been stripped off its cuticular lipid layer by washing the pupae in high-performance liquid chromatography (HPLC) grade hexane for 3 min. and leaving it to dry on a clean glass plate for 30 min. We had already confirmed by GC-MS that all CHCs had successfully been removed. Because the washed pupae were now odourless, unwashed pupae were placed around the microcentrifuge tubes containing the washed pupae and mites to ensure that the pupaa odour was present. In the tubes the mites still fed on the washed pupa and there were no obvious differences in mite behaviour between this treatment group and the control group. 4) The final mite group was kept in full isolation with no pupae added to or placed around the tube, to serve as negative control (full isolation from the host). All treatment groups were left for 18 hours before mites and pupae were retrieved and frozen prior to chemical analysis.

There was clear behavioural evidence that mites were able to perceive the odour of the pupae placed around the tubes, as mites started to accumulate around the holes along the tube wall, as soon as pupae were placed in the petri dish. This behaviour was not observed in the isolated mite group, where no pupae were placed around the tube.

Limited access to host hemolymph - To create a scenario in which mites had access to the host's cuticular lipid layer but could not feed on the host hemolymph, we maintained *V. destructor* on mature fly pupae (*Calliphora vomitoria*) that had been stripped off their own CHCs (washed in HPLC grade hexane for 3 min.) and then spiked the fly pupae with the odour of a bee pupae. Each fly pupa was covered with the extract of one bee pupa (washed in HPLC grade hexane for 15 min.) by gradually dripping the extract onto the fly's cuticle and leaving it to dry over a 30 min. period. (Fig. 1b). The cuticle of a mature fly pupae is too thick for the mite to penetrate; hence mites can only access the artificial lipid layer but not the hemolymph. For this experiment a total of 220 mites were collected, of which 60 were frozen straight away to serve as starting points. 80 mites were kept on fly pupae and the remaining 80 were kept on bee pupae (positive control) in microcentrifuge tubes prepped with a wet cotton ball and one hole in the lid as described above. All treatment groups were left for 18 hours before mites and pupae were retrieved and frozen prior to chemical analysis.

Active versus passive transfer of host CHCs – To investigate whether *V. destructor* uses passive CHC transfer as an alternative strategy to actively grooming the host, we tested whether dead mites were still able to mimic a host odour. Mites were killed by freezing at -20°C for 1 hr. They were then defrosted for 10 mins, then stripped of their CHCs by immersing them in HPLC grade hexane for 3 min. and leaving them to dry for 30 min. 80 of these dead mites were transferred to petri dishes containing white-eye pupae. The dead mites were placed onto pupae with their legs touching the host cuticle to imitate the natural position of the mite on the host. As positive control, 80 live mites were transferred to petri dishes containing white-eye pupae. Mites were again left for 18 hrs and frozen for chemical analysis. A sub-set of washed mites tested by GC-MS confirmed that all CHCs had been successfully removed from the dead mites prior to the transfer onto the host.

Chemical and Statistical Analysis - Samples were extracted in HPLC hexane containing a C_{20} standard (1mg/100ml hexane). Each bee sample was extracted in 0.5 ml hexane, and mite samples were extracted in pools of six mites in 300µl hexane per sample. Samples were left at room temperature for 15 min., before transferring 30 µl of extract to a glass insert and left to evaporate overnight.

Samples were re-suspended in 30µl hexane and analysed on an HP6890GC (equipped with an HP-5MS column; length: 30m; ID: 0.25mm; film thickness: 0.25µm) connected to an HP5973 MSD (quadrupole mass spectrometer with 70-eV electron impact ionization). Samples were injected in the splitless mode. The oven was programmed from 70ºC to 200ºC at 40ºC/min and then from 200ºC to 320ºC at 25ºC/min and, finally, held for 5 min at 350ºC. The carrier gas helium was used at a constant flow rate of 1.0ml min-1. Compounds were identified using standard MS databases, diagnostic ions and Kovats indices.

The peak area of each compound was determined by manual integration of each total ion chromatogram (TIC), which was then translated into actual concentration (mg/ml hexane) using the peak of the C_{20} standard. Compounds which on average contributed less than 1% to the overall chemical profile (i.e. n-alkanes + alkenes +

methylalkanes) were excluded from the analysis. Compounds were grouped into three main chemical classes: *n*-alkanes, alkenes and methylalkanes.

Because the CHC profiles of adult bees and bee pupae mainly differ in their alkene:methylalkane ratio, we focused our analysis on these two CHC families and excluded the *n*-alkanes. For the speed of host CHC acquisition, a regression analysis was conducted to investigate whether the alkene:methylalkane ratio changed significantly during the 27- hour-period after the mite had been placed onto the new host. For all other experiments, one-way *ANOVA*'s and *post-hoc* Tukey tests were carried out to test for significant changes in the alkene:methylalkane ratio between treatment groups. The assumptions associated with doing an ANOVA test were checked prior to doing the analysis. All tests were performed using the statistical software R (v 2.81).

RESULTS

Speed of host CHC acquisition - As expected, the CHC profiles of honey bee pupae had a high methylalkane: alkene ratio that was reversed in the adult bees (Fig. 2a). Hence, mites collected from adult bees, at the beginning of the experiment, had high concentrations of alkenes but low levels of methylalkane. When mites were then transferred onto a pupa, their methylalkane levels increased followed by a drop in alkene concentration over the 27 hrs during which mites were exposed to the new host. This led to a significant drop in the alkene: methylalkane ratio (Fig. 2b) over time, which levelled off after 9 hours of exposure to the host (Polynomial Regression: $y=1.5 - 3.795 x + 3.265 x2$, $F=26$, d.f. = 2, 52, $p < 0.0001$). A significant increase in mite methylalkane concentration was already visible after the first 20 min. of being on the new host (ANOVA, $F=4$, d.f.=5,49, $p<0.001$), which was followed by a second increase after 3 hrs (ANOVA, $F=4$, d.f.=5,49, $p<0.004$) (Fig.2b). Alkene concentration remained constant for the first 3 hours and then dropped significantly (ANOVA, $F=10$, d.f.=5,49, $p<0.001$).

Limited access to host cuticular lipid layer - The treatment groups of the second experiment differed significantly in their alkene: methyalkane ratio (ANOVA, F=30.31,

d.f.=4,43, $p < 0.001$) (Fig. 3a). As expected, mites that were kept on (unwashed) bee pupae, and hence had access to both host hemolymph and the host's cuticular lipid layer, had significantly higher levels of methylalkane compared to mites kept on adult bees (*post-hoc* Tukey test: $p < 0.001$). The second mite group (Odour), which was able to smell the host through the perforated tube but had no direct physical access to host hemolymph or cuticle, was unable to increase their methylalkane levels so their alkene: methylalkane ratio remained the same as mites kept in full isolation (*post-hoc* Tukey test: $p = 0.92$). When mites only had access to host hemolymph by keeping them on washed pupae (Pupa + Odour – CHCs), these were also unable to increase their methylalkane concentration to match the CHC profile of the host. Instead their alkene: methylalkane ratio was again the same as mites kept in full isolation (*post-hoc* Tukey test: $p = 0.27$) (Fig 3a). When mites were isolated from the host pupae the concentration of all CHCs decreased (Fig. 3a). Therefore, access to host odour or host hemolymph alone was insufficient for mites to mimic their new host profile.

Limited access to host hemolymph - Mites were able to increase their methylalkane levels when given an artificial lipid layer (fly pupa spiked with the CHC profile of one bee pupa) even though access to host hemolymph was denied. Their alkene:methylalkane ratio was the same as that of mites kept on bee pupae (ANOVA, F=86.15, d.f.=2,22, $p < 0.001$; *post-hoc* Tukey test: $p = 0.31$) and significantly different from that of mites kept on adult bees (*post-hoc* Tukey test: $p < 0.001$) (Fig. 3b). This indicates that mites are able to adjust their chemical profile as long as they have direct access to the host's cuticular lipid layer, even if host hemolymph cannot be accessed.

Active versus passive transfer of host CHCs - Dead (washed) mites were still able to increase their methylalkane levels to the degree that they were significantly different in their alkene:methylalkane ratio compared to mites kept on adult bees (ANOVA, F=52.01, d.f.=2,22, $p < 0.001$; *post-hoc* Tukey test: $p < 0.001$) (Fig. 3c). Even though dead mites were similar in methylalkane concentration to live control mites (ANOVA, F=2.33, d.f.=1,16, $p = 0.15$), they had significantly higher levels of alkene, which led to a significant difference in alkene:methylalkane ratio in these two groups (*post-hoc* Tukey

test: $p < 0.001$). This disproportionate increase in alkene levels was not due to alkene residues left after washing mites, because washed mites that were analysed straight away (Control Mites) had no CHCs left on their cuticle after washing. The fact that dead mites were still able to adsorb host CHCs indicates that at least the uptake of host CHCs is a passive process.

DISCUSSION

The results indicate that *V. destructor* uses chemical camouflage to mimic the odour of its bee host. Without access to the host's cuticular lipid layer the mite was unable to adjust its mimicry to a new host, even when access to host hemolymph was provided. This finding suggests that *V. destructor* is unable to synthesize host-specific CHCs but instead there is a transfer of host CHCs from the host's cuticle to that of the mite; this even occurs when the mite is dead. This is further supported that changes in the mites profile was observed after just 20 mins. The rapid adsorption of a host's CHCs has been observed in a number of parasites and can be facilitated by an active process such as grooming the host (ants: Lenoir et al. 1997; Franks et al. 1990; spiders: von Beeren and Witte 2012; silverfish: von Beeren et al. 2011), in addition to any simple passive processes were close contact with the host cuticle is required (cockroaches: Everaerts et al. 1997; termites: Vauchot et al. 1998; beetles: Vander Meer and Wojcik 1982). For example, the myrmecophile 'shampoo' ant *Formicoxenus provancheri* grooms its host, *Myrmica alaskensis,* around 45% of its time inside the nest (Lenoir et al. 2001), while the myrmecophile beetle *Myrmecaphodius excavaticollis* passively adsorbs host CHCs without any obvious grooming behaviour (Vander Meer and Wojcik 1982). Further evidence for the passive transfer of CHCs has come from using artificially mixed colonies of *Reticulitermes* termites, where *R. santonensis* and *R. lucifugus grassei* acquired each other's CHCs without any allogrooming taking place (Vauchot et al. 1998). In both studies, *M. excavaticollis, R. santonensis* and *R. lucifugus grassei* are all able to take up host compounds even if dead individuals were used, thus excluding the possibility of biosynthesis or behavioural acquisition as relevant factors.

Our findings suggest that *V. destructor* also uses passive transfer (i.e., transfer of host CHCs without the parasite actively grooming or in any way interacting with the

342 host) of host CHCs to camouflage itself on the host, because dead mites were still able to
343 adsorb compounds when placed on the host. The mite's legs are too short to spread host
344 CHCs across its cuticle via grooming and, hence, passive CHC transfer is an efficient
345 strategy to facilitate the mite's chemical mimicry. When switching host stages, passive
346 chemical camouflage allows the mite CHC profile to rapidly adjust within a few hours to
347 match the chemical profile of its new host. So, when moving from an adult bee to a bee
348 pupa, the mite quickly adopts the profile of its new host. This process is aided by the fact
349 that the mite naturally seems to lose compounds when these are not provided through
350 contact with the host cuticular lipid layer, as was apparent when mites were kept in full
351 isolation. This was also observed in the myrmecophile beetle *Myrmecaphodius*
352 *excavaticollis* (Vander Meer and Wojcik 1982), as the termites *Reticulitermes*
353 *santonensis* and *R. lucifugus grassei* (Vauchot et al. 1998), were CHC transfer could
354 already be observed after just two hours of cohabitation, but acquired CHCs started to
355 decrease in concentration as soon as individuals were isolated from their host.
356 It was surprising that dead *V. destructor* mites preferentially adsorb alkenes
357 compared to live mites. Alkenes have a lower melting temperature in comparison to
358 methylalkanes (Gibbs 2002) and, thus, it is possible that these transfer more readily than
359 methylalkanes. If it was easier for mites to adsorb alkenes compared to methylalkanes,
360 this would have also been observed in the live mites unless the adjustment of the exact
361 alkene:methyalkane ratio is actively regulated by the mite. This result certainly requires
362 further investigation.
363 There is some evidence to suggest that the ability to passively adsorb CHCs may
364 be species-specific in arthropods. When *Reticulitermes santonensis* and *R. l. grassei* are
365 kept in (artificial) mixed colonies, the latter takes up more compounds of the former than
366 *vice versa* (Vauchot et al. 1998). A similar case was reported by Vienne et al. (1990 cited
367 in Vauchot et al., 1998), whereas *Formica selysi* acquired more allospecific CHC than
368 *Myrmica rubida* when these two ant species lived as a mixed colony. The fact that there
369 seems to be a species-specific ability of taking up CHCs indicates that there may be
370 differences in cuticular physiology or chemistry that allow some species to take up CHCs
371 more readily than others. If this occurs in non-parasitic arthropods, it is not surprising that
372 a range of parasitic arthropods have evolved the ability to soak up enough host CHCs to

achieve chemical camouflage. The physico-chemical mechanism that allows them to do this however remains elusive.

Our results confirmed the findings reported in Nation et al. (1992) that the chemical mimicry of *V. destructor* changes as the parasite switches hosts. The mite *V. destructor* is the first case known to us where a parasite frequently switches its camouflage as it moves between host stages. Because groups of individuals within an *A. mellifera* colony can vary significantly in their CHC profile, due to task (Kather et al. 2011) or life stage, a passive chemical camouflage is likely to be the best strategy to facilitate a quick adjustment in the parasite's camouflage to match the new host with minimal energetic cost. The mite also has a number of appendages such as suckers, hairs and its crab-like carapace (Rosenkranz et al., 2010) that allow it to hold on to the host during the transition time and, this way, these appendages buy the mite time until it is fully blended in with the host's CHC profile, which only takes a few hours. This study helps explain why *V. destructor* has become one of the most widespread and successful ecto-parasitic pests of honey bees.

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Table 1 Total number of mite samples (n) per treatment group across all experiments. For each mite sample, a pool of six mites were extracted and treated as one sample in the data analysis.

Experiment	Treatment Group	
Experiment 1 (Speed of host CHC acquisition)		**n**
	0 hrs	10
	20 min.	8
	1 hr	10
	3 hrs	8
	9 hrs	10
	27 hrs	9
Experiment 2 (Limited access to host cuticular lipid layer)		
	Mites (Adult Bee)	10
	Mites (Bee Pupa)	8
	Mites (Pupa + Odour – CHCs)	10
	Mites (Odour)	6
	Full Isolation	10
Experiment 3 (Limited access to host hemolymph)		
	Mites (Adult Bee)	6
	Mites (Fly Pupa + Bee Pupa CHCs)	10
	Mites (Bee Pupa)	9
Experiment 4 (Active vs. passive transfer)		
	Mites (Adult Bee)	10
	Dead Mites (Bee Pupa)	8
	Live Mites (Bee Pupa)	10

Fig. 1 CHC profiles of a) a honey bee pupa and b) a washed fly pupa spiked with the CHC extract of one bee pupa. The main CHCs are: 1) n-C_{23}, 2) $C_{25:1}$, 3) n-C_{25}, 4) 9-, 11-, 13-meC_{25}, 5) $C_{27:1}$, 6) n-C_{27}, 7) 9-, 11-, 13-meC_{27}, 8) $C_{29:1}$, 9) n-C_{29}, 10) 9-, 11-, 13-meC_{29}, 11) $C_{31:1}$, 12) n-C_{31}, 13) 9-, 11-, 13-meC_{31}, 14) $C_{33:1}$.

Fig. 2 Speed of chemical mimicry. Mites were collected from adult bees (time: 0hrs) and kept on pupae for different time intervals. Bee profiles are shown in panel a) and mite profiles in panel b). Mites acquired pupa-specific methylalkanes and lost adult-specific alkenes within 3-9 hours of exposure to the new host, leading to a drop in alkene-methylalkane ratio. Error bars show one standard deviation and sample sizes are given in Table 1.

Fig. 3 Alkene and methylalkane concentrations of mite CHC profiles across experiments 2 to 4. In each panel 'Adult bees' represents the starting CHC profile of mites i.e. kept on adult bees, and 'Bee pupae' is the positive control i.e. mites kept on untreated pupae. a) *experiment 2*; mites have access to the host cuticular layer. Pupa + Odour - CHC - are mites on washed bee pupae i.e. CHC removed but surrounded by unwashed pupae to provide host odour; Odour- are mites only exposed to pupae odour, and Full Isolation- is the negative control since mites were isolated from the host. b) *experiment 3*; excludes access to host haemolymph; Fly Pupa + Bee Pupa CHCs - are mites kept on fly pupae spiked with a bee pupa CHC profile. c) *experiment 4*; transfer of host CHCs was active or passive; Dead mites- their CHC profile removed and placed on living pupa, Live mites also had their CHC removed but not killed, while control mites had their CHC removed and were isolated from pupa and showed that chemical washing removed all the mites CHC. For each experiment, treatment groups differed in their alkene:methylalkane ratio if they have different letters, whereas groups with the same letter were similar in their alkene:methylalkane ratio. Error bars show the standard error and samples sizes are given in Table 1.

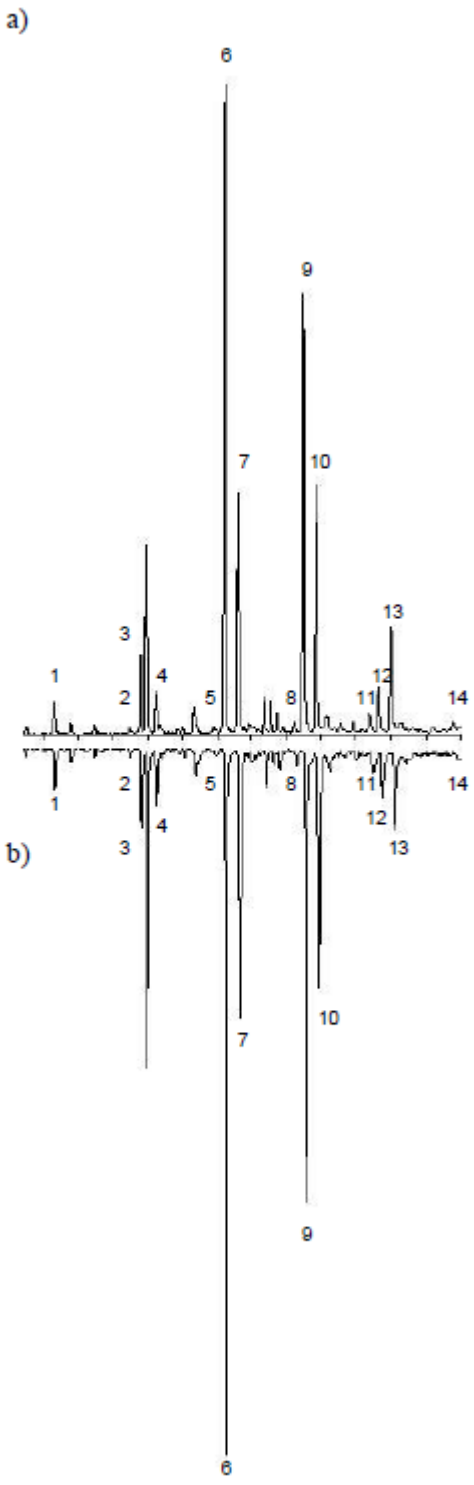

Fig. 1

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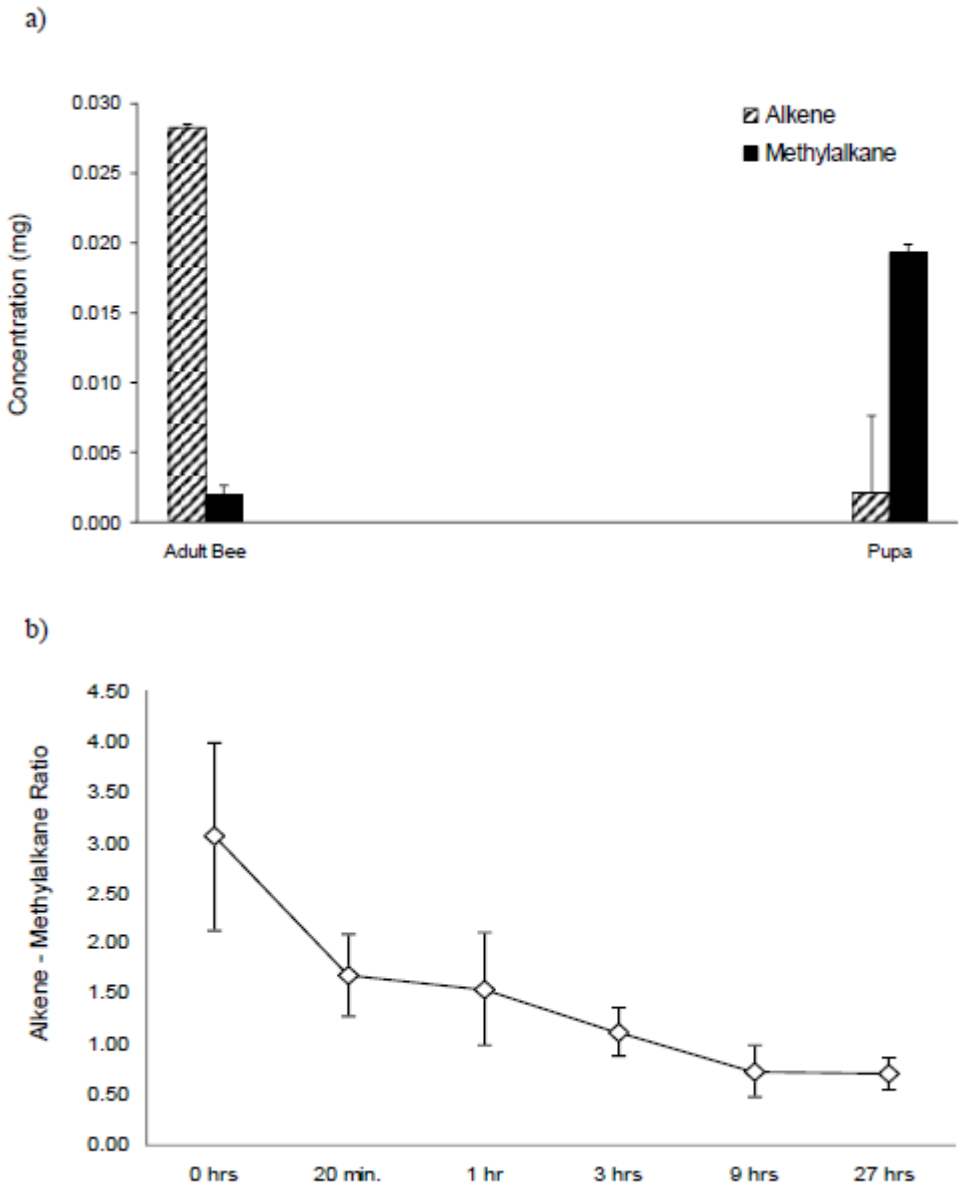

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604 Fig. 2

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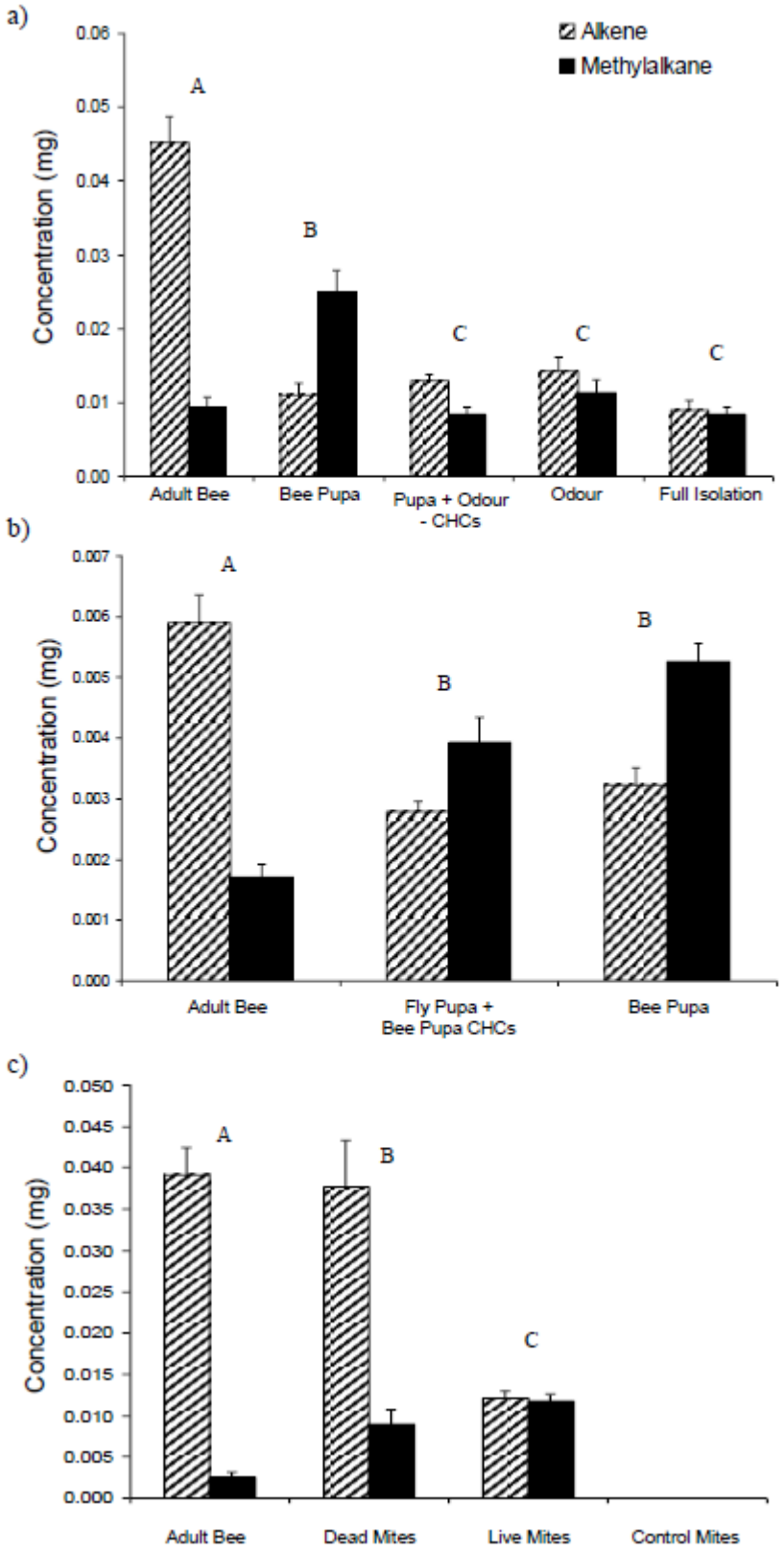
a)
Alkene
Methylalkane
Concentration (mg)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
A
B
C
C
C
Adult Bee
Bee Pupa
Pupa + Odour - CHCs
Odour
Full Isolation
b)
Concentration (mg)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
A
B
B
Adult Bee
Fly Pupa + Bee Pupa CHCs
Bee Pupa
c)
Concentration (mg)
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
A
B
C
Adult Bee
Dead Mites
Live Mites
Control Mites

610 Fig. 3