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Animal behaviour

Varroa destructor changes its cuticular hydrocarbons to mimic new hosts

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Varroa destructor (*Vd*) is a honeybee ectoparasite. Its original host is the Asian honeybee, *Apis cerana*, but it has also become a severe, global threat to the European honeybee, *Apis mellifera*. Previous studies have shown that *Varroa* can mimic a host's cuticular hydrocarbons (HC), enabling the parasite to escape the hygienic behaviour of the host honeybees. By transferring mites between the two honeybee species, we further demonstrate that *Vd* is able to mimic the cuticular HC of a novel host species when artificially transferred to this new host. Mites originally from *A. cerana* are more efficient than mites from *A. mellifera* in mimicking HC of both *A. cerana* and *A. mellifera*. This remarkable adaptability may explain their relatively recent host-shift from *A. cerana* to *A. mellifera*.

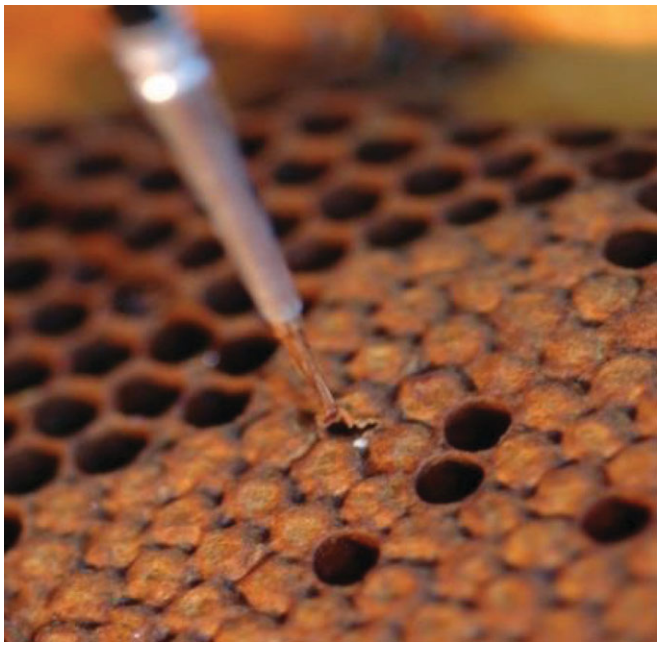
1. Introduction

Chemical mimicry has been described as playing a major role in the infiltration of parasites into insect societies [1]. In non-social arthropods, chemical mimicry can be highly sophisticated: bolas spiders, for example, emit three chemicals mimicking a sex pheromone to attract their moth prey [2]. Chemical mimicry of social insects by parasites is more demanding, because social insects have much more sophisticated chemical communication [3]. By varying proportions of their chemical components, social insects can recognize nest-mates and determine larval age [4,5]. Even in such complex communication systems, however, parasites are still able to exploit their host's communication codes to evade detection [6,7].

The mite *Varroa destructor* (*Vd*) is an acarian ectoparasite of honeybees, and its original host was the Asian honeybee (*Apis cerana*, *Ac*). *Vd* jumped host to the western honeybee (*Apis mellifera*, *Am*) in the 1940–1950s and has since become the largest threat to *Am* worldwide. *Ac* colonies do not die partly because bees are able to detect and destroy mites on adult bees (grooming behaviour) [8] and inside capped brood cells (*Varroa* hygienic behaviour) [9]. By contrast, such behaviours are limited in *Am* bees and their colonies die within 2–3 years if mites are not chemically controlled.

One important feature of *Vd* is its ability to mimic host hydrocarbons (HC) to reduce host detection. A previous study has shown that *Vd* changes its cuticular HC according to host development stages, such that the parasite chemically matches its host's current developmental stage best [6], and another shows that *Vd* are also able to mimic small colony differences in *Am* [7]. In this study, we further test the hypothesis that *Vd* can change its HC to match that of a different host species.

(*a*)



(*b*) bees: B

bee species \ receiving mites from	*mellifera*	*cerana*
mellifera B*M	BMM	BCM
cerana B*C	BMC	BCC

Varroa: V

original host \ host after transfer	*mellifera*	*cerana*
mellifera VM*	VMM	VMC
cerana VC*	VCM	VCC

Figure 1. (*a*) *Varroa destructor* originally from *Apis cerana* or *Apis mellifera* drone brood were transferred into *Ac* and *Am* worker brood using a paint brush, and (*b*) codes for the bees and mites used in the cross-foster experiment. (Online version in colour.)

2. Material and methods

(a) Interspecific mite transfer

Interspecific mite transfer was carried out at the Honeybee Research Institute, Jiangxi Agricultural University, Nanchang, China (28.46° N, 115.49° E). Recently capped (within 6 h) brood cells were each opened by perforating the cap with an insect pin. Then a mite was carefully transferred into a cell using a fine brush, and the cell sealed with melted beeswax. After mite transfer, the frame of infested brood was incubated at 35°C and 50% relative humidity. Eight days later, the host pupa and mite(s) in the cell were carefully extracted from each cell. We had four groups of mites depending on their former and present hosts, and four groups of bees depending on their species and where the mites came from, with a total of eight treatments (figure 1; electronic supplementary material, S1).

(b) Chemical analysis of cuticular hydrocarbons

Each bee pupa (B) and its mite (V), and daughter mites if any (D), were separately extracted by rinsing each with hexane (liquid chromatography grade) inside a glass vial for 10 min; extracts were shipped to France for chemical analyses. Six to 11 gas chromatograms were obtained for each developmental stage of *Varroa* (V or D) and bee pupae (B). After individual gas chromatography (GC) analysis, extracts were pooled for analysis by gas chromatography coupled to mass spectrometry (GC-MS) to identify the different components of the extracts. The relative proportions of cuticular compounds of the different categories of mites and bee pupae were analysed using a principal component analysis (PCA; electronic supplementary material, S2).

(c) Data processing and computing between-groups chemical similarities

To compare compositions of cuticular compounds, Mahalanobis' distances to all pairs of groups were determined for the mites and bees for all treatments. The first 12 principal components of the PCA were used; they accounted for 89.7% of the total variance. A clustering algorithm was then applied to the Mahalanobis' distance matrix. The table of correlation ratios was built up to determine, for all variables, their discriminant values for the various groups. The correlation ratio of a quantitative variable is the ratio of the between-group variance to the total variance of the quantitative variable. The higher the ratio, the more discriminant the variable is (electronic supplementary material, S3).

3. Results

(a) Chemical and principal component analyses

Fifty compounds previously identified [6,10] were found in both *A. cerana* and *A. mellifera* in this study (electronic supplementary material, table S1). GC and GC-MS analyses show that the compounds discriminating the two bee species were mainly unsaturated HC and *n*-alkanes.

The PCA based on the two first components (PC1 and PC2) separated not only bees from *Varroa*, but also the two host bee species (figure 2; electronic supplementary material, S4). Mites formed two clusters based solely on their new hosts. Mites from the original host *cerana*, VCM (or DCM) and VCC (or DCC), were closer to the host profile than mites from the *mellifera* host, VMM/DMM and VMC/DMC (figure 2). Some mites had 'perfect' mimicry because they were inside their host circles, with three VCC and four VCM showing this ability (figure 2). Daughter mites (D**) did not show better mimicry than their mothers (V**) to either hosts. Reproductive status of transferred mites is indicated in the electronic supplementary material, S5.

(b) Similarity index

A Wilk's test showed significant differences among the eight groups. Mahalanobis distance analysis showed that similarity indices differed between pairs of groups (table 1; electronic supplementary material, figure S1) and reflected the proximity of individuals.

The highest similarity (i.e. lowest indices) was for bees BCC and BMC, with BMM and BCM having lower similarity. After those two pairs, the closest groups were always with

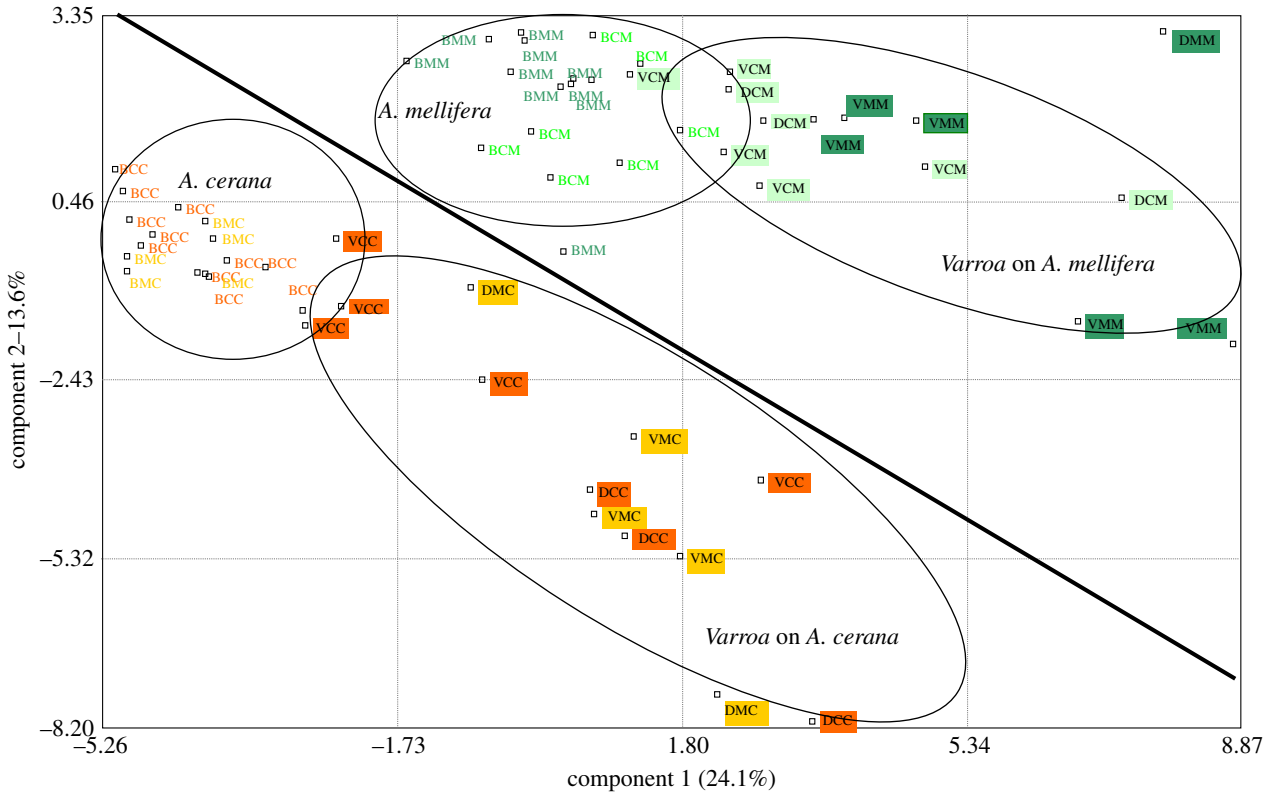


Figure 2. Separation of bees and mites based on the two first axes (37.7% of the total variation) of principal component analysis on their hydrocarbons. Colours are related to the host (yellow/orange for *Apis cerana*; green for *Apis mellifera*). Coding for the eight treatments is presented in figure 1. (Online version in colour.)

Table 1. Pairwise Mahalanobis' distances between the eight groups of individuals, from the first 12 principal components coordinates. BCC and BMC are the closest 'related' (most similar), i.e. *A. cerana* was least affected by the origin of mites (whether originally from *cerana* or *mellifera*). BMM and BCM are closely related but branch distance was longer than that of BCC and BMC. VCM then clusters with BMM/BMC first, suggesting that *Varroa* originally from *cerana* and now on *mellifera* are more similar to the *mellifera* bees; these three as a group then are more similar to VMM, *Varroa* originally from *mellifera* and still on *mellifera*. BCC/BMC relate to VCC more closely, similar to above, suggesting that *Varroa* from *cerana* originally and still on *cerana* mimicked the best their host *cerana* bees; these three then clustered with VMC, *Varroa* originally from *mellifera* and now on *cerana*. Finally, the top four groups (**M) and the bottom four groups (**C) then form two common clusters, suggesting that the second host playing a much more important role than the original host, and *Varroa* and their secondary host showing a higher similarity.)

	BMM	BCM	BMC	BCC	VMM	VMC	VCM
BCM	4.73						
BMC	18.01	18.27					
BCC	17.34	17.62	0.81				
VMM	14.04	12.46	18.66	18.25			
VMC	26.27	25.87	13.05	13.38	20.65		
VCM	8.77	9.35	20.10	19.43	14.38	27.19	
VCC	21.99	21.73	8.12	8.20	18.98	9.15	22.28

the *Varroa* mites hosted by *cerana* (VCC and VCM), followed by the *Varroa* mites hosted by *mellifera* (VMM and VMC) with a slightly higher index. The two host bees (*mellifera* versus *cerana*) were well separated, but the highest index (lowest similarity) was between the two types of organisms, bees versus *Varroa* mites (see table 1 for indexes).

4. Discussion

This study shows that *Vd* cuticular HC patterns are similar to those of its honeybee hosts, *Am* and *Ac*. This agrees with the previous findings that mites were more similar to their immediate host's developmental stage [6,7,11], but our study used different host species to challenge the mite's ability to modify its HC.

Because mites clustered according to their new hosts, but not according to their original hosts, we assumed that these mites changed their HC profiles after being transferred to the new host. This is supported by the results from control group mites that did not change hosts (*mellifera* to *mellifera* or *cerana* to *cerana*). *Ac* originated mites had more similar HC profiles to their new hosts than *Am* originated mites to their new hosts (figure 2), suggesting that *Ac* mites are better mimickers.

Previous analysis has indicated mitochondrial polymorphism in *Varroa* [12]. As was published in that study, *Varroa* from *Am* were determined to be Korean haplotype 2 (K2) and those from *Ac* were Korean haplotype 3 (K3). This suggests that the haplotype K3 might be more closely related to the original *Varroa jacobsoni*, which only reproduces in *A. cerana*.

The ability of *Ac* or some *Am* strains (e.g. *Varroa* sensitive hygiene, VSH) to detect and remove *Vd* [8,9,13,14] creates selection pressure for *Vd* to mimic their hosts. Conversely, bees are under selection pressure for more sensitive olfaction to detect *Vd* for mite removal. Increased olfactory related gene expression is found in VSH bees and *Vd*-resistant French bees [14–16].

Our results show that the parasite always mimics host cuticular components independently of mite origin, but that mimicking seemed more effective for a *Varroa* originating from an *Ac* colony, perhaps because of a longer coevolutionary period with *Ac* than *Am*. Both mite haplotypes show an ability to mimic host cuticular HC, enabling them to successfully parasitize a new host species. This remarkable ability may explain their relatively recent, successful host-shift from *Ac* to *Am*.

A recent study [7] showed that mites are not actively mimicking their hosts, instead by passively using host materials via contact. Our data here suggest that, at least for mites from *Ac*, some active processes might be involved because they mimicked their hosts better. Regardless, this ability may have played a critical role in *Vd*'s ability to shift host species [17]. Our results give a clear illustration of an arms race between a parasite and its host based on chemical mimicry and its detection.

Data accessibility. Raw data deposited at: http://dx.doi.org/10.5061/dryad.1bf13.

Authors' contributions. Z.Y.H., Y.L.C and Z.J.Z. designed and performed mite transfer experiments. A.G.B and J.P.C did chemical and PCA analyses. M.R. performed the statistical analyses. A.G.B, Y.L.C., Z.J.Z, J.P.C. and M.R. wrote a first draft. Z.Y.H, A.G.B. and Y.L.C. finalized the manuscript. All authors gave final approval for publication.

Competing interests. We declare we have no competing interests.

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