Effects of *Varroa destructor* on temperature and humidity conditions and expression of energy metabolism genes in infested honeybee colonies

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ABSTRACT. *Varroa destructor* mites pose an increasing global threat to the apicultural industry and agricultural ecology; however, the issue of whether certain environmental factors reflect the level of mite infection is far from resolved. Here, a wireless sensor network (WSN) system was used to examine how *V. destructor*, which has vital impacts on honeybee (*Apis mellifera*) health and survival, affects the temperature and humidity of honeybee hives in a field experiment. This approach may facilitate early identification of *V. destructor* in hives, and thus enable timely remedial action. Using quantitative PCR, we also evaluated the expression of two genes, adipokinetic hormone (AKH)

and adipokinetic hormone receptor (AKHR).The results showed that temperature in highly infested broods was higher than that in broods with low infestation. Moreover, mite infection in honeybee colonies was positively correlated with temperature but negatively correlated with humidity ($P < 0.05$). Similar to previous observations, quantitative analysis suggested that the expression levels of AKH and AKHR from honeybees with low infection were significantly higher than those from bees with high infection ($P < 0.01$). These results showed that the expression levels of these genes in colonies with high mite infestation were closely associated with changes in hive temperature and humidity. This study demonstrates that *Varroa* infection not only causes changes in temperature inside honeybee colonies, but also affects the expression of honeybee energy metabolism genes.

Key words: *Varroa destructor*; Temperature; Differential expression; Energy metabolism

INTRODUCTION

Honeybees (*Apis mellifera*) are among the most important pollinators of plants, and provide pollination services for agricultural crops and ecosystems. However, the loss of honeybee colonies is increasingly being reported due to a poorly known phenomenon referred to as colony collapse disorder (CCD),in which the worker bees suddenly disappear (van Engelsdorp et al., 2008). Since it was first identified in 2006 in the USA (Biesmeijer et al., 2006), CCD has been reported from many regions of the world, and the subsequent decrease in the number of honeybee colonies in the following years attracted increasing attention from the apicultural community and agricultural ecologists. Furthermore, several stressors related to CCD were subsequently identified and evaluated, including viruses (Cox-Foster et al., 2007), bacteria (Johnson et al., 2009), fungi (Higes et al., 2007) as well as pesticides (Tremolada et al., 2010). Among these pathogens, *Varroa destructor* has been detected in 98% of CCD-affected colonies and is considered a significant factor correlated with CCD (Locke et al., 2012).

V. destructor lives in the cells of honeybee colonies and feeds on the hemolymph of developing honeybees, resulting in losses among both adults and brood (Nazzi and Conte, 2015). *V. destructor* is not only an ectoparasite of honeybees but is also an effective vector for viruses such as Israeli acute paralysis virus (IAPV) (Di Prisco et al., 2011). The number of viral copies in honeybees has been found to be strongly correlated with the density of *V. destructor* within a colony (Locke et al., 2012). Recent studies have demonstrated that the presence of *V. destructor* is a strong predictor of winter reduction in honeybee populations in Europe and the USA, and that combined with viruses, this parasite can accelerate the death of honeybees (Dainat et al., 2012). Hemolymph feeding by *V. destructor* has been demonstrated to induce immunosuppression in honeybees, which can lead to increases in the titers of pre-existing infections (Yang and Cos-Foster, 2007). Vector-borne transmission often results in more virulent infections in terms of evolutionary epidemiology. In particular, honeybees infected with *V. destructor* exhibit decreased weight and lifespan, and an increased incidence of undeveloped honeybees and related phenotypes such as shortened abdomen, discoloration, and deformed wings (Katherine et al., 2012). Experimental evidence shows that *V. destructor*

reduces the flight duration and homing ability of honeybees (Kralj and Fuchs, 2006). With these facts in mind, several measures have been taken by beekeepers and scientists in an effort to control *V. destructor*, including the use of biotechnological, genetic, and chemical agents (Rinderer et al., 1997).

However, it remains very important to be able to diagnose and detect mite infection before deciding to control it using appropriate measures. To monitor the spread of the Asian honeybee mite, a simulation model was used to evaluate the rate of spread before it broke out (Sanson, 2007). Furthermore, a glass vial bioassay procedure was developed to monitor field populations of *V. destructor* for insecticide resistance, and this procedure was successful in controlling the resistance of *V. destructor*, thereby providing the basis for a management strategy (Kanga et al., 2010). In addition, certain physical methods are available for determining whether honeybees are infected by *V. destructor*. Because of the characteristics of the external parasite, they are easily found on the body surface of adults, larvae, and pupae. Other features used to distinguish infected honeybees, include shriveled wings, which are frequently seen in emerging or old bees, patchy brood patterns, and reddish mites on the bees or honeycombs. Further common methods used to diagnose mite infection involve calculating the number of mites dropped onto the bottom board of bee hives or calculating the number of mites in a certain number of honeybees. However, although these methods have been considered to provide evidence for the level of mite infection, they might lead to delays in the prevention and treatment of infection and lead to the loss of both individual bees and entire colonies.

An additional challenge to diagnosing and monitoring the extent of mite infection in honeybees is that the effects of mite infection are not always observable. Recent studies have shown that the development of information technology provides new opportunities for monitoring pathogens, particularly for insects such as honeybees and ants in nests or cave habitats. A detection system using video signals and image processing techniques was developed on the basis of a semi-automatic approach within honeybee brood cells, which attained 90.98% efficiency in monitoring the movement of mites (Víctor et al., 2013). Subsequently, radio frequency identification (RFID) has made available new tracking equipment with a more accurate approach for measuring research subjects studied by scientists. RFID systems were first used to monitor the behavior of bees and ants in 2003, and have provided a feasible means of following individuals when they emerge from or are outside a colony (Streit et al., 2003). Subsequently, Li et al. (2013) constructed an RFID system that monitored honeybee foraging behaviors and homing ability in real-time. In studies of honeybee ecology, RFID has been used to examine the effect of sub-lethal doses of insecticides on individual forager bees and their homing ability (Schneider et al., 2012). In addition, an imaging LIDAR instrument placed near a bee hive has been used to monitor honeybee behavior (Hoffman et al., 2007), and an automatic tracking and identification system based on RFID has been used to recognize bees and ants (Streit et al., 2003).

All of this work represents significant progress in the study of honeybee biology and behavior. However, to gain more knowledge about bee biology and bee pathogens, we need to identify a way to monitor the stability and health status of honeybees in real-time. With the aim determining mite infection in honeybees, we used a wireless sense network (WSN) in the present study to monitor temperature and humidity changes within colonies, and analyzed the expression levels of two energy metabolism genes. Our data provide evidence that *V. destructor* infection not only raises temperature inside the honeybee hive but also induces high energy consumption in honeybees.

MATERIAL AND METHODS

System for monitoring temperature and humidity changes

The temperature and humidity monitoring system consisted of three main subsystems - a data acquisition system, a data processing system, and a data transport system - as described previously (Xiao et al., 2014) (Figure 1). The data transport system was produced by China Unicom's CDMA services, which provided a wireless bidirectional channel between data acquisition and processing. The data recorded by the sensors were transmitted to a remote information server through China Unicom's CDMA services. Data acquisition was transmitted by WiFi wireless LAN, and the TCP/IP protocol was used for computer communication. Accordingly, users will be able to monitor temperature and humidity parameter values via the Internet. To test the stability of the signal and the appropriate distance of the sensor node of this system, we conducted pre-experiments three times before we monitored the temperature and humidity in a honeybee hive.

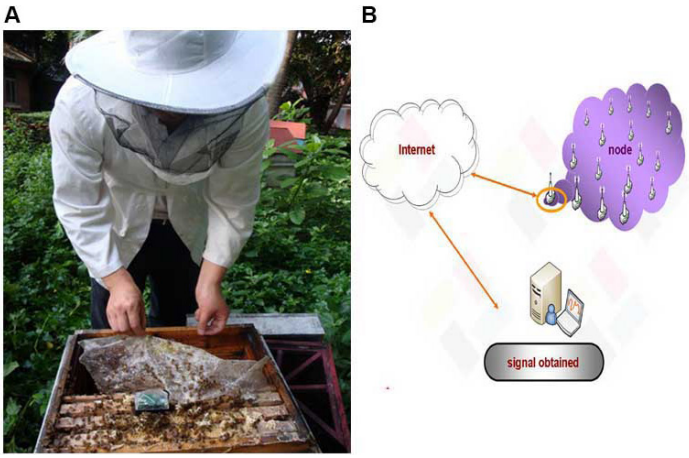

Figure 1. The wireless sensor network system that was used to monitor temperature and humidity. **A.** Wireless sensor node placed within the honeybee colonies. **B.** Wireless sensor network system used to collect data from the sensor nodes within the colonies.

Selection of equipment and nodes

The basic equipment of the monitoring system consists of two parts. The first is a sensor node with an antenna positioned at the center of honeybee hives, which collects information on temperature and humidity for 60 min. This system was used to collect data three times over three consecutive days in three consecutive months. The second part stores and analyzes the data. The WSN system and sensor nodes were purchased from the Wireless Drag Communication Company (Chengdu, China), and the CC2430 sensor node and IAR software were specifically adapted for this system. All wireless sensors were assumed to operational if all lights were on when the monitoring started. All monitored data were transformed into average values.

Experimental honeybee colonies

Honeybee colonies were selected from a single apiary, which is the property of the Institute of Apicultural Research, located in northern Guangdong Province (23.06°N, 113.15°E) of China. The queens were bred and selected from the same batch. Honeybee colonies with high and low mite infestation were selected for monitoring temperature and humidity using the wireless sensor system, and six colonies were selected with standard 10 frames. The populations and proportion of the three honey bee castes (queens, workers, and drones) were identical. Three colonies were maintained under control conditions by treating with an acaricide, whereas the other three colonies were untreated. The colonies with low infestation but not the colonies with high infestation were treated in accordance with the method of Nazzi et al. (2012).The two groups were 2 km apart from each other. The colonies had been maintained using standard beekeeping practices and had been closely observed by a professional bee inspector in the spring and autumn of each year. The level of mite infection in adult bees was estimated in three consecutive months. The numbers of ectoparasitic mites in colonies were assessed by examining 1000 bees, using the protocol described by Nazzi et al. (2012). This experiment was performed three times per month in the months of July, August, and September of 2011.

RNA extraction and RT-PCR

Honeybee samples (~30 bees each) collected from a single colony were pooled and homogenized with a Geno/grinder homogenizer (Metuchen, NJ, USA) in a 15-mL sterile tube using TRI reagent (Sigma-Aldrich), according to the manufacturer instructions. Total RNA was dissolved in 20 μL sterile water and stored at -80°C until used. The quantity and purity of the RNA sample were measured using a NanoDrop spectrophotometer (Thermo Scientific, Pittsburgh, PA, USA).

To rule out interference from other pathogens, the presence of common honeybee pathogens in the colonies used in this study was monitored using the RT-PCR methods described in a previous study (Hou et al., 2014).These pathogens included viruses such as acute bee paralysis virus (ABPV), black queen cell virus (BQCV), DWV, *Varroa destructor virus*-1 (VDV-1), chronic bee paralysis virus (CBPV), IAPV, and sacbrood virus (SBV); bacteria (American foulbrood and European foulbrood); fungi (*Nosema ceranae* and *Nosema apis*); and other parasites such as tracheal mites. First-strand cDNA was synthesized from total RNA (4 μg) using the Maxima Reverse Transcriptase Kit (Thermo Scientific, Pittsburgh, PA, USA), following the manufacturer protocol. PCR amplification was performed with forward and reverse primers of the target pathogens as previously described (Hou et al., 2014). For diagnostic PCR amplification of honeybee pathogens such as bacteria, fungi (*Nosema*), *V. destructor* mites, and viruses, we used GoTaq (Promega, USA). Amplification was carried out using a GenePro thermal cycler (BioER) as follows:5 min at 94°C; 34 successive cycles of 30 s at 94°C, 30 s at 55°C, and 72°C for 1 min; and a final extension of 10 min at 72°C. Each PCR reaction included one negative control. The PCR products were examined by electrophoresison 1% agarose gels containing 0.5 mg/mL ethidium bromide. Positive identification was confirmed by sequencing the PCR products.

Quantitative real-time PCR

Fat bodies were collected from the abdomens of live honeybees with forceps and quickly frozen in liquid nitrogen for analysis of adipokinetic hormone (AKH) and adipokinetic hormone receptor (AKHR). Real-time PCR was carried out in a Rotor-Gene 6000 instrument (Corbett Rotor-Gene, USA), using a SYBR Green-based Kapa Fast qPCR kit (KapaBiosystems, USA), in combination with cDNA synthesized via RT-PCR according to the manufacturer instructions. The primer sequences used for amplification of the target transcripts were as follows: for AKH, 5'-CGTAAGCTTCGACCAAGTTTTT-3' and 5'-CATTCGACAACTCCGATCCT-3'; for AKHR, 5'-ATAATCACCACCACGGGATT-3' and 5'-GACCTTCGTTGAATCGCATA-3' (Wang et al., 2012). The forward and reverse primers of the housekeeping gene (RPL8) were 5'-TGGATGTTCAACAGGGTTCATA-3' and 5'-CTGGTGGTGGACGTATTGATAA-3', respectively. The reaction conditions were 10 min at 95°C, followed by 40 cycles of 10 s at 95°C, 15 s at 60°C, and 20 s at 72°C. CT values measured using a standard curve prepared from 10-fold serial dilutions of target DNA were used to show the titers of the target genes (Hou et al., 2014). Data were analyzed using the standard curve method. We confirmed that the qRT-PCR products were not contaminated by DNA or primer dimers by observing negative control samples and melting curves.

Data analysis

The average values per colony were used in all statistical analyses. The effect of mite infection on the temperature and humidity in each of the six colonies was analyzed using a *t*-test with Origin 8.0 software. Gene expression data were log transformed to approximate normality as described previously (Wang et al., 2012). An LSD test was used to compare the expression levels of AKH and AKHR between the honeybees with high and low mite infection. The variance components are expressed as standard deviations.

RESULTS

Screening of pathogens

The results of a survey for common pathogens are summarized in Table 1. All pathogens in colonies in this study were preliminarily identified as common honeybee pathogens according to standard methods based on biology and behavioral observation (Hou et al., 2014). Screening of common honeybee pathogens, including ABPV, BQCV, DWV, VDV, CBPV, IAPV, SBV, American foulbrood, European foulbrood, *N. ceranae*, and *N. apis*, was performed as described previously (Chen et al., 2008; Hou et al., 2014) to rule out contamination by other pathogens. As shown in Table 1, all tested samples from the experimental colonies were negative for the monitored pathogens, with the exception of DWV in colonies1 and 2. This was more likely caused by mite infection due to the capacity for mite-transmitted DWV, as previously reported (Zioni et al., 2011).

In order to verify the differences caused by the level of mite infection, we assessed the number of mites from different colonies. As shown in Figure 2, the number in colonies with high infestation steadily increased to its highest level at the time of the final measurement, whereas infestation remained at a low level in the colonies with low infestation.

Table 1. Pathogen incidence detected in experimental colonies. The + and –signs represent positive and negative samples in the detection results, respectively. DWV, VDV, IAPV, ABPV, BQCV, CBPV, SBV, AFB, and EFB indicate deformed wing virus, *Varroa destructor* virus, Israeli acute paralysis virus, black queen cell virus, chronic bee paralysis virus, sacbrood virus, American foulbrood, and European foulbrood, respectively. *N. apis* and *N. ceranae* represent *Nosema apis* and *Nosema ceranae*, respectively. The groups with high and low mite infestation are represented as 1, 2, and 3 and 4, 5, and 6, respectively.

Colony	DWV	VDV	IAPV	ABPV	BQCV	CBPV	SBV	AFB	EFB	*N. apis*	*N. ceranae*
1	+	-	-	-	-	-	-	-	-	-	-
2	+	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-

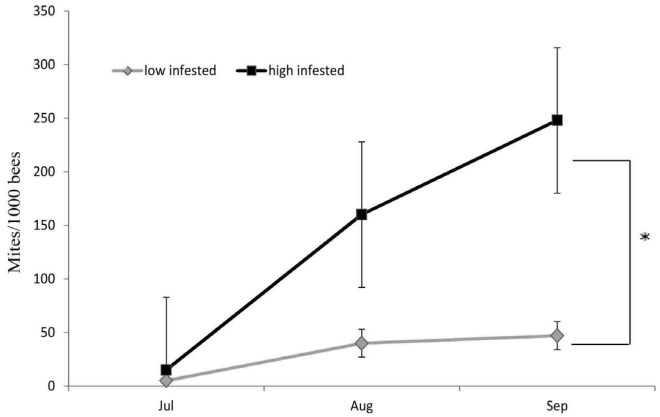

Figure 2. Number of mites per 1000 bees in low- and high-infested colonies. Significantly different mean values are denoted with asterisks (*P ≤ 0.05).

Monitoring temperature and humidity

The wireless sensors nodes were placed in the middle of combs of experimental honeybee hives with both high and low *V. destructor* infestation (Figure 1A). The wireless sensor system was constructed to collect temperature data according to the ZigBee protocol (Xiao et al., 2014) (Figure 1B). To further examine the effect of temperature change on humidity, we also simultaneously monitored the humidity within honeybee hives. Although the colonies were randomly allocated to the groups of high and low mite infestation, the initial monitoring of temperature and humidity was different. The temperature was significantly higher in the mite-infected group than in the group with low infection (Figure 3A). Although there was a small drop in temperature over time in the high-infected group, it was still higher than that in the low-infected group. The general temperature trend was relatively stable in both experimental groups, suggesting that the difference was induced by higher mite infection. In contrast, the humidity in the colonies with high mite infestation was lower than that in the colonies with low mite infestation, although the values tended to be very close after monitoring for 60 min (Figure 3B). This indicates that the differences in humidity between the colonies with high and low mite infestation were caused by temperature change.

The relationships between the data collected by monitoring and the mites were assessed through correlation analysis using original average data. This analysis suggested that the temperature in the honeybee colonies and mite infection were highly correlated (Pearson correlation coefficient = 0.9328), demonstrating that infection by *V. destructor* is positively correlated with temperature. The initial temperatures of the colonies with high and low mite infestation were 34.69° and 33.05°C, and the final temperatures were 34.73° and 32.99°C, respectively (Figure 3A), which represents a significant difference in temperature between colonies with low and high mite infestation (t = 81.18, d.f. = 3, P = 0.00004). In contrast, the humidity of colonies with high mite infestation was lower than that of colonies with low infestation. The results showed an obvious initial difference between the high and low infestation groups (73.87 and 77.73%, respectively), although the final humidity in colonies of the two groups was very close. The average humidity of the two groups was apparently different during the monitoring period (t = -3.27, d.f. = 3, P = 0.04); however, we found no obvious differences between temperature and humidity in the low-infested colonies.

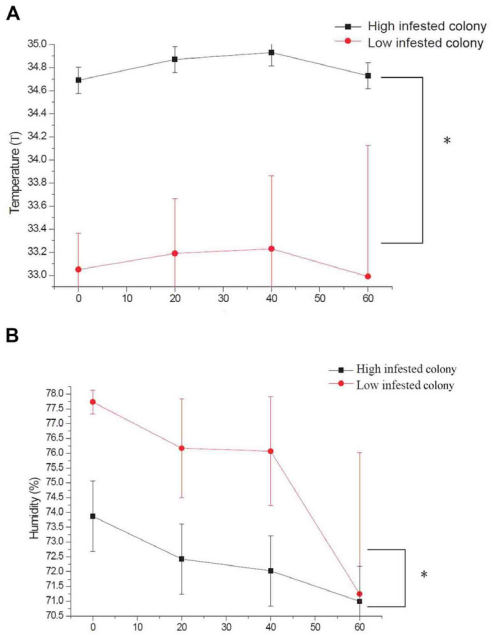

Figure 3. Comparison of temperature and humidity in honeybee colonies with high and low *Varroa destructor* infections. **A.** Real-time temperature change. **B.** Real-time humidity change. The X-axis represents time in minutes. Asterisks denote significant differences (*P ≤ 0.05).

Detection of energy consumption

To further verify the difference induced by mite infection, we examined the expression of two energy-related genes, AKH and AKHR. Transcript levels were measured on the basis of copy numbers from the QPCR experiment. As shown in Figure 4, the energy-related genes of honeybees were significantly affected by mite infection (P < 0.05). The expression levels of AKH were much higher in the low-infected group than in the high-infected group (Figure 4A),

suggesting that this is caused by the raised temperature due to mite infection. Likewise, the level of AKHR was considerably higher in the low-infected group than in high-infected group (Figure 4B). The significant differences we detected between the groups with high and low mite infestation further suggested that mite infection resulted in higher energy consumption by the honeybees.

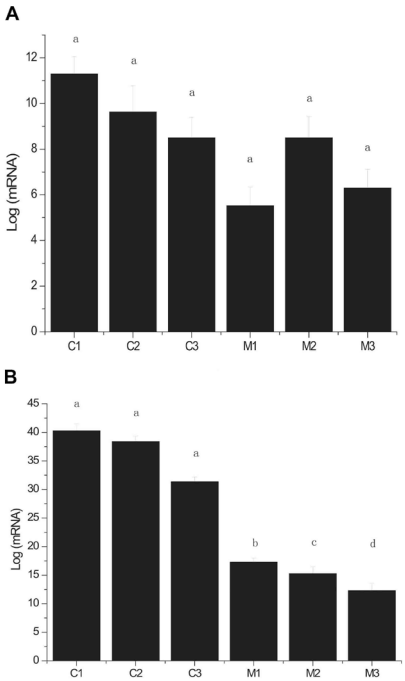

Figure 4. Effect of mite infection on metabolism-associated honeybee genes. Shown are the relative expression levels of adipokinetic hormone (AKH) (**A**) and adipokinetic hormone receptor (AKHR) (**B**). C and M represent experimental honeybee colonies with low and high mite infection, respectively. The bars with different letters represent significant differences ($P < 0.05$).

DISCUSSION

V. destructor is the most destructive parasite of honeybee, often causing significant disability in individual honeybees and even the collapse of entire colonies (Meikle and Diaz, 2012). Despite decades of study on the control of these mites, the situation is still on-going and, if anything, is growing worse because of two possible obstacles to formulating an effective control strategy: 1) most beekeepers will simply treat honeybee colonies as and when they find mites or notice abnormal appearances in honeybees based on previous experience; and 2) it is usually too late to control the mites when beekeepers find them in their honeybee colonies. In addition, the reproduction of female mites in capped brood cells interferes with the probability of detection and subsequent treatment (Harbo and Harris, 2005). Accordingly, monitoring should become a necessary measure to detect the mites before they have a chance to spread rapidly and widely.

The WSN monitoring system described in this study can be used to investigate the effects that could be caused by pathogens. The results showed that the WSN system can monitor the changes in temperature and humidity of honeybee colonies in real-time and indicate that temperature and humidity can, to a certain degree, be regarded as indicators of mite infection within honeybee colonies. Wireless communication and telemetric systems are feasible for on-line data collection tasks and animal monitoring in the field (Hwang et al., 2010), and greatly extend our ability to remotely monitor and control the physical environment. In addition, WSN is a wireless communication network that can self-organize and interact directly with the physical world. It can produce information that alerts to potential risks such as frost damage or toxic gases (Hwang et al., 2010).

However, WSN has rarely been applied to honeybee studies. As the number of honeybee colonies around the world decreases, some attempts have been made to identify the causes in a more accurate way (Abramson et al., 1997). Our data show that we can determine whether the colonies are infected by mites by observing the temperature and humidity inside honeybee hives. These results clearly indicate that temperature and humidity can be considered as indicators that reflect mite infection. In agreement with previous findings (Ma et al., 2014), our survey confirms that *V. destructor* infection can induce changes in the temperature and humidity of a honeybee colony and indicates that mite infection can be monitored using the WSN system without the need to inspect the bottom board or use an artificial checking comb. These findings are similar to those reported by Zhao et al., who studied the relationship between temperature and the number of broods infected by chalkbrood, and suggested that the number of infected broods increased with rising temperature in the honeybee colony (Zhao et al., 2007). It is not surprising that we observed colony temperatures being maintained at 33°C under normal conditions; however, because in practice different methods are used to measure temperature (Chen, 1989), this can give rise to discrepant measurements. Although the optimal honeybee hive temperature is approximately 33°C, honeybees can survive at 34° or 35°C (Gao, 2002). Significantly, however, the experimental data showed that 34° or 35°C is more beneficial to the reproduction of mites, although they will be adversely affected if the temperature is below 33°C (Gao, 2002). This explains why mite infection could induce a temperature rise in the honeybee colony. Alternatively, this might also indicate that honeybees mount a type of defensive response that functions to decrease the temperature, producing environmental conditions unfavorable to mite reproduction. This is in line with observations that regions with relatively lower average temperatures have lower colony losses (vanEngelsdorp et al., 2008), and is confirmed in a study by Gao, who demonstrated that the average number of mites surviving at 35°C is 2.4 but only 0.9 at 33°C by artificial measurement system (Gao, 2002). Interestingly, heating to remove mites has been used for more than 20 years in most regions of Europe (Harbo et al., 2000). In this context, honeybees are known to exhibit a variety of behavioral traits associated with social interactions. One of these, the hygienic behavior, involves the fanning of wings as a behavior to impair the survival and reproductive success of *V. destructor* (Rosenkranz et al., 2010). Nevertheless, wing fanning will consume honeybee energy and increase the temperature.

The important result of the present work is the finding that *V. destructor* affected the energy metabolism of honeybees. In honeybees from colonies with high mite infestation, the levels of AKH and AKHR, which play important roles in energy metabolism (Wang et al., 2012), are lower than in the bees from colonies with high infestation. Although our results are consistent with the previous findings that pathogens affect energy metabolism (Wang et

al., 2012), the measured amount differs, which is probably attributable to the use of different housekeeping genes in the different analyses. From a physiological perspective, these results may explain why a honeybee colony is readily subject to parasites and loss in winter in the USA or Europe. First, the mites accelerate death by inducing higher energy consumption in honeybees. Second, the energy supply available to honey bees is sometimes not sufficient to sustain survival, particularly in winter due to the lack of nectar. Changes in energy metabolism will occur if there are changes in the sum of other environmental heat exchanges (Moffatt, 2001). The resulting increase in passive heat loss must be made up in some way for bees to maintain a constant temperature. Thus, there must be an increase in energy metabolism if no decrease occurs in other factors causing the loss.

This observation indirectly supports the fact that there is a significant correlation between the densities of mite infection and environmental factors (temperature and humidity), which were induced to fluctuate because of accelerated honeybee behavior (Bonoan et al., 2014). The honeybees have to generate more energy to shiver in order to eliminate parasitic mites, which induces more bees to fan within or outside the bee hive, thereby leading to an increase in temperature and a decrease in humidity in the honeybee hive.

Of special interest are the relationships between mites and temperature and other factors because temperature is an important factor in the development of honeybee eggs, larvae, and pupae. These relationships not only reflect indirectly on the health and energy consumption of honeybee colonies but also provide evidence for the level of pathogen infection (Bujok et al., 2002). In particular, the development of mite populations within the honeybee colony will increase the opportunities for pathogen transmission and lead to breakouts of overt infections of viruses or other diseases, resulting in the loss of individual bees or even the collapse of entire colonies. *V. destructor* is relatively common in honeybee colonies, and by monitoring this mite it is possible to determine the threshold levels of mites that might indicate the necessity for treatment or an evaluation of treatment efficacy, as well as confirming the presence of infection in the honeybee colony in time. Monitoring will help us to understand how honeybees cope with these problems and, more importantly, how the colonies respond physiologically to these diseases. This will also provide evidence for further establishing the relationships among environmental factors, energy consumption, and behavior.

Although this monitoring lays the foundations, further investigations are needed, including studies of mite infection levels when temperature and humidity are changed, and how mite infection affects the energy metabolism of honeybees. Our studies provide approaches for monitoring the temperature in honeybee colonies with high mite infestation. Although these findings do not precisely reflect the level of honeybee infection by mites, they do provide new information for the detection of the mite infection in real-time and pave the way for extensive studies on the prevention and control of mite infestation.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Abramson CI, Aquino IS, Azeredo GA and Price JM (1997). Some preliminary studies on the ability of africanized honey bees (*Apis mettifera* L.) to tolerate cold temperatures when placed inside a refrigerator. *Psychol. Rep*. 81: 707-718. http://10.2466/pr0.1997.81.3.707

Biesmeijer J, Roberts SPM, Reemer M, Ohlemuller R, et al. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351-355. http://10.1126/science.1127863

Bonoan R, Goldman RR, Wong PY and Starks PT (2014). Vasculature of the hive: heat dissipation in the honey bee (*Apis mellifera*) hive. *Naturwissenschaften* 101: 459-465. http://10.1007/s00114-014-1174-2

Bujok B, Kleinhenz M, Fuchs S and Tautz J (2002). Hot spots in the bee hive. *Naturwissenschaften* 89: 299-301. http://10.1007/s00114-002-0338-7

Chen FS (1989). The effect of different temperature on the development of Varroa mites. *Apicult. China* 1: 10-11.

Chen Y, Evans JD, Smith IB and Pettis JS (2008). Nosema ceranae is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. *J. Invertebr. Pathol.* 97: 186-188 http://dx.doi.org/10.1016/j.jip.2007.07.010.

Cox-Foster DL, Conlan S, Holmes EC, Palacios G, et al. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318: 283-287. http://10.1126/science.1146498

Dainat B, Evans JD, Chen YP, Gauthier L, et al. (2012). Predictive markers of honey bee colony collapse. *PLoS One* 7: e32151. http://10.1371/journal.pone.0032151

Elizondo VA, Bricerio MT, Travieso CM and Alonso BA (2013). Video Monitoring of a mite in honeybee cells. *Adv. Mat. Res.* 2: 1107-1113.

Hoffman DS, Nehrir AR, Repasky KS, Shaw JA, et al. (2007). Range-resolved optical detection of honeybees by use of wing-beat modulation of scattered light for locating land mines. *Appl. Opt.* 46: 3007-3012. http://dx.doi.org/10.1364/AO.46.003007

Di Prisco G, Pennacchio F, Caprio E, Boncristiani HF, et al. (2011). *Varroa destructor* is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. *J. Gener. Virol*. 1: 151-155. http://10.1099/vir.0.023853-0

Gao S (2002). The effect of temperature and humidity of bee hive on the Varroa mite. *Chin. J. Anim. Husb. Vet. Med.* 4: 46-47.

Harbo JR and Harri JW (2005). Suppressed mite reproduction explained by the behaviour of adult bees. *J. Apic. Res.* 44: 21-23. http://dx.doi.org/10.1080/00218839.2005.11101141

Higes M, García-Palencia P, Martín-Hernández R and Meana A (2007). Experimental infection of *Apis mellifera* honeybees with Nosema ceranae (Microsporidia). *J. Invertebr. Pathol.* 94: 211-217 http://dx.doi.org/10.1016/j.jip.2006.11.001.

Hou CS, Rivkin H, Slabezki Y and Chejanovsky N (2014). Dynamics of the presence of Israeli acute paralysis virus in honey bee colonies with colony collapse disorder. *Viruses* 6: 2012-2027. http://10.1007/s11033-014-3706-4

Hwang J, Shin C and Yoe H (2010). Study on an agricultural environment monitoring server system using wireless sensor networks. *Sensors* 10, 11189-11211. http://10.3390/s101211189

Hoffman DR (2000). Heating adult honey bees to remove Varroa Jacobsoni. *J. Apic. Res.* 39: 181-184. http://dx.doi.org/10.1080/00218839.2000.11101041

Johnson RM, Evans JD, Robinson GE and Berenbaum MR (2009). Changes in transcript abundance relating to colony collapse disorder in honey bees (*Apis mellifera*). *Proc. Natl. Acad. Sci*. 35: 14790-14796. http://10.1073/pnas.0906970106

Kanga LH, Adamcayk J, Marshall K and Cox R (2010). Monitoring for resistance to organophosphorus and pyrethroid insecticides in Varroa mite populations. *J. Econ. Entomol*. 5: 1797-1782. http://10.1603/EC10064

Katherine AA, Eduardo S, Rodrigo V, Stephanie W, et al. (2012). How Varroa parasitism affects the immunological and nutritional status of the honey bee, *Apis mellifera*. *Insects* 3: 601-606. http://10.3390/insects3030601

Kralj J and Fuchs S (2006). Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. *Apidologie* 37: 577-587. http://dx.doi.org/10.1051/apido:2006040

Li ZG, Chen YP, Zhang SW, Chen SL, et al. (2013). Viral infection affects sucrose responsiveness and homing ability of forager honey bee, *Apis mellifera* L. *PLoS One* e77354. http://10.1371/journal.pone.0077354

Locke B, Forsgren E, Fries I and de Miranda JR (2012). Acaricide treatment affects viral dynamics in *Varroa destructor*-infested honey bee colonies via both host physiology and mite control. *Appl. Environ. Microbiol.* 1: 227-235. http://10.1128/aem.06094-11

Ma L, Chen H, Hu K and Zhu Y (2014). Hierarchical artificial bee colony algorithm for RFID network planning optimization. *Sci. World J.* 2014: 941532. http://dx.doi.org/10.1155/2014/941532

Meikle WG and Diaz R (2012). Factors affecting pupation success of the small hive beetle, *Aethina tumida. J. Insect Sci.* 12: 118-121. http://dx.doi.org/10.1673/031.012.11801

Moffatt L (2001). Metabolic rate and thermal stability during honeybee foraging at different reward rates. *J. Exp. Biol.* 204: 759-766.

Nazzi F and Conte YL (2015). Ecology of *Varroa destructor*, the Major Ectoparasite of the Western Honey Bee, *Apis mellifera*. *Ann. Rev. Entomol*. 61: 417-432. http://10.1146/annurev-ento-010715-023731

Nazzi F, Brown SP, Annoscia D, Del Piccolo F, et al. (2012). Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathog*. 8: e1002735. http://10.1371/journal.ppat.1002735

Rinderer TE, Kuznetsov VN, Danka RG and Delatte GT (1997). An importation of potentially Varroa-resistant honey bees from far-eastern Russia. *Am. Bee J.* 137: 787-790.

Rosenkranz P, Aumeier P and Ziegelmann B (2010). Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.* 103 (Suppl 1): S96-S119 http://dx.doi.org/10.1016/j.jip.2009.07.016.

Sanson RL (2007). Simulation modelling as a tool for evaluating surveillance programmes for detection of the Asian honeybee mite (*Varroa destructor*) in the South Island of New Zealand. *N. Z. Vet. J.* 55: 273-279. http://dx.doi.org/10.1080/00480169.2007.36781

Schneider CW, Tautz J, Grünewald B and Fuchs S (2012). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS One* 7: e30023. http://10.1371/journal.pone.0030023

Streit S, Bock F, Pirk CWW and Tautz J (2003). Automatic life-long monitoring of individual insect behaviour now possible. *Zoology* 3: 169-171. http://http://dx.doi.org/10.1078/0944-2006-00113

Tremolada P, Mazzoleni M, Saliu F and Colombo M (2010). Field Trial for Evaluating the Effects on Honeybees of Corn Sown Using Cruiser® and Celest xl® Treated Seeds. *B. Environ. Contam. Tox*. 3: 229-234. http://10.1007/s00128-010-0066-1

van Engelsdorp D, Hayes JJ, Underwood R and Pettis J (2008). A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008. *PLoS One* 3: e4071. http://10.1371/journal.pone.0004071

Wang Y, Brent CS, Fennern E and Amdam GV (2012). Gustatory perception and fat body energy metabolism are jointly affected by vitellogenin and juvenile hormone in honey bees. *PLoS Genet*. 8: e1002779. http://10.1371/journal.pgen.1002779

Xiao XQ, Zhu TY, Qi L, Moga L, et al. (2014). MS-BWME: a wireless real-time monitoring system for brine well mining equipment. *Sensors* 14: 19877-19896. http://10.3390/s141019877

Yang XL and Cos-Foster D (2007). Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge. *Parasitology* 3: 405-408. http://10.1017/S0031182006000710

Zhao WZ, Wang M, Chen Q, Cao LF, et al. (2007). Study on temperature and humidity of honeybee colonies infected by chalkbrood disease. *J. Bee* 4: 24-28.

Zioni N, Soroker V and Chejanovsky N (2011). Replication of *Varroa destructor* virus 1 (VDV-1) and a *Varroa destructor* virus 1-deformed wing virus recombinant (VDV-1-DWV) in the head of the honey bee. *Virology* 417: 106-112 http://dx.doi.org/10.1016/j.virol.2011.05.009.