Bee Studies 15(2), 61-71
http://doi.org/10.51458/BSTD.2023.37

RESEARCH PAPER

Defensive Behaviors of the Central Highland Honeybees, *Apis mellifera bandasii* against *Varroa destructor* in Ethiopia

Alemayehu Gela[1,2]*, Araya Gebresilassie[1], Yitbarek Woldehawariat[1], Anagaw Atikem[1], Zewdu Ararso[2], Amssalu Bezabeh[2]

[1] Department of Zoological Sciences, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia
[2] Oromia Agricultural Research Institute, Holeta Bee Research Center, P. O. Box 22 Holeta, Ethiopia

Article History
Received 26 September 2023
Accepted 15 December 2023
First Online 22 December 2023

***Corresponding Author**
Tel.: 00251911678972
E-mail: alemayehu.bee@gmail.com

Keywords
Apis mellifera
Grooming behavior
Hygienic behavior
Varroa destructor
Infestation

Abstract

The parasitic mite *V. destructor* has caused long-lasting losses to the survival of European honeybee colonies. In contrast, African honeybees are likely capable of surviving the effects of this parasitic mite with varying defense mechanisms. This study provides insights into two defense behavioral traits, including hygienic and grooming behaviors of local honeybee, *Apis mellifera bandasii* colonies against *V. destructor* mite in Ethiopia. Hygienic behavior (HB) was evaluated using the standard pin-killed brood method by calculating the dead brood removal rates (%) at 24 and 48 hrs. While grooming behavior (GB) was assessed by measuring the number of daily fallen mites and the percentage of damaged mites. The results of hygienic behavior showed greater brood removal rates of 83.1±14.3% and 97.6±3.4% at 24 hrs and 48 hrs, respectively. There were strong negative correlations between the HB and Varroa infestation rates, indicating that HB has the potential to reduce the mite population in colonies. Grooming behavior also showed higher mean daily fallen mites per colony (16.3±10.2), of which about 80% of the total fallen mites (n=488) were damaged. Ten body damage categories were identified, with most damages inflicted on mites' legs, dorsal shield, and gnathosoma because of the GB. Our study suggests that combined hygienic and grooming behaviors could be used as effective defenses against *V. destructor* infestations in *A. m. bandasii* colonies. Therefore, future selective breeding programs should integrate these specific host defenses in order to produce sustainable colonies resistant to this parasitic mite.

Introduction

The ecto-parasitic mite, *Varroa destructor*, and the honeybee are the two interacting host-parasite arthropods that have become a subject of growing interest for many scientists around the world. Honeybees are the only sources of food for the varroa mite, and the mite's life cycle coincides with the development of honeybee pupae inside the brood cells (Yang et al., 2021; Rosenkranz et al., 2010). This parasitic mite primarily feeds on the fat bodies of the bees (Ramsey et al., 2019) while also spreading other bee pathogens like bee viruses, which ultimately lead to the highest rate of honeybee mortality (Le Conte et al., 2010; Hristov et al., 2020; Traynor et al., 2020). The feeding activity of the mite and its associated secondary infections can cause huge losses to the honeybee, *Apis mellifera,* and become a threat to colony survival and beekeeping productivity around the world (Flores et al., 2021; Noël et al., 2020; O'Shea-Wheller et al., 2022). In addition to reducing the potential beekeeping production, Varroa also has negative effects on the pollination capacity of a colony, which in turn significantly affects crop production for food security (Malfroy, 2015; Abrol and Sharma, 2013). The approach so far taken to control the mite, particularly using chemical treatments over the past 30 years did not completely solve the problem due to the spread of acaricide resistant mites and the risk factors associated with acaricide residues (Kablau et al., 2020; Plettner et al., 2017). As a result, *V. destructor* remains a complex invasive parasite, crippling the Western honeybee, *A. mellifera*, in the world for many generations (Nazzi and Le Conte, 2016; Traynor et al., 2020). In contrast, the mite has been considered a harmless pest to its native host, the native Eastern honeybee *(Apis cerana)* due to their naturally evolved defensive traits developed over a long evolutionary period (Peng et al., 1987; Rath, 1999; Boecking and Spivak, 1999). Thus, a balanced host-parasite relationship has been established between *A. cerana* and the *V. destructor* mite, revealing the potential resistance of *A. cerana* honeybees to the mite infestation (Grindrod and Martin, 2023; Grindrod and Martin, 2021).

Likewise, some *A. mellifera* colonies in Africa and African-derived populations have shown varying degrees of tolerance or resistance towards the infestation of Varroa mite without the interventions of beekeepers (Locke, 2016; Castilhos et al., 2023; Büchler et al., 2010). Particularly, African honeybee populations are likely to be less threatened by the impact of *V. destructor* compared to European honeybees (Muli et al., 2014; Tibatá et al., 2021; Nganso et al., 2017). Although the mite has continued its rapid spread across many African countries over the past 15 years, there has been no report on the visible colony losses linked to Varroa mite infestation in African continent (Allsopp et al., 1997; Begna, 2014; Chemurot et al., 2016, Dietemann et al., 2009; Fazier et al., 2010; Muli et al., 2014). The long co-existence of honeybee subspecies with the mite in the absence of chemical treatment is suggestive of the potential resistance or tolerance of the honeybee populations evolving natural adaptations against the Varroa mite. Behavioral defenses such as hygienic and grooming behaviors are important natural traits that enable resistant honeybee populations to survive and co-exist with the Varroa mites, particularly in Africa (Fazier et al., 2010; Muli et al., 2014).

Hygienic behavior involves targeting, opening and removal of diseased, injured, parasitized, or dead broods by the worker honeybees (Peng et al., 1987; Boecking and Spivak, 1999; Spivak and Reuter, 2001). In particular, when hygienic behavior targets the detection and removal of varroa infested brood cells, it is referred as "Varroa Sensitive Hygienic Behavior (VSH)" (Mondet et al., 2015). On the other hand, grooming behavior involves the potential dislodging and damage of ecto-parasites from the bodies of adult bees either by themselves or by their nest mates, where they reduce the population of parasites below the danger threshold level (Mondet et al., 2020; Aumeier, 2001, Russo et al., 2020). In fact, African honeybees in general have displayed strong hygienic and grooming behaviors than their European counterparts (Muli et al., 2014; Nganso et al., 2017). Nevertheless, the diverse bee species in Africa are likely to exhibit different responses tailored to combat the negative impact of the Varroa mite (Mondet et al., 2020; Fazier et al., 2010). Moreover, colonies within the same subfamily may respond differently to parasites and pathogens, due to different factors.

In Ethiopia, the occurrence of the Varroa mite was reported about a decade ago, and currently the mite has been widely distributed across all the geographic regions of the country with varying prevalence and infestation levels (Gela et al., 2023; Shegaw et al., 2022). However, there is no report indicating that the mite has induced colony losses or a pronounced impact on the apiculture industry in the country (Gratzer et al., 2021). Due to this fact, local beekeepers do not consider the mite as a serious pest, and do not use any treatment measures against the parasite. This phenomenon suggests that the local honeybee populations have evolved some sort of resistance or tolerance traits to maintain a stable host-parasite relationship against the aggressive infestation behaviors of this mite species. However, there is limited information that explains specific natural defensive mechanisms applied by honeybee subspecies of Ethiopia against *V. destructor* mite. In fact, Pirk et al. (2016) reviewed how the treatment-free beekeeping approach in Africa has allowed honeybees to develop some natural resistance traits or behavioral adaptations to combat the negative effects of different pests and pathogens. Gebremedhn et al. (2019) also reported the failure of female varroa mites to produce adult male progeny that suppresses the population growth of Varroa mite in *A. m. simensis* colonies for the first time in Ethiopia. However, this study was limited to a specific honeybee eco-type in the northern part of the country, and it may not represent the wider geographic population of honeybees in the country. A*. m. bandasii* is the most popular geographical race of honeybees spread in the central highlands of the Ethiopia, covering more than 90% of the highland areas (Mohammed, 2002). Begna et al. (2016) investigated the non-impact of Varroa mite on population dynamics, brood rearing, as well as foraging activities of *A. m. bandasii* colonies, suggesting their survival against the destructive nature of the parasitic mite. However, the specific tolerance or resistance mechanisms employed by these honeybee populations against the mite remain unclear. Therefore, this study was designed to determine whether the hygienic and grooming behaviors of *A. m. bandasii* could contribute for the defensive mechanisms against the *V. destructor* mite infestation. Understanding such natural defense behaviors will provide salient insights into future selective breeding program and enhance resistance traits in the local honeybee stocks.

Material and Methods

Study Location

The study was conducted from September 2021 to June 2022 in the laboratory and at apiary site of Holeta Bee Research Center, Oromia, Ethiopia located at 09º03'.24" N and 038º30'.72" E about 33 kilometers in the West direction of the capital city, Addis Ababa (altitude 2400 m a.s.l.). The climate of the study area is characterized by temperate to humid weather conditions with an average temperature of 14.15°C (ranging from 6.2°C - 22.1°C), annual rainfall of 1091.51 mm that varies between 800 and 1500 mm/year and a mean relative humidity of 60.6% (Mekonnon et al., 2015). The main vegetation types in the study area include *Guzotia* spp., *Acacia* spp., *Eucalyptus globulus, Vernonia amygdalina, Trifolium* spp., *Plantago* *lanceolata, Brassica carinata* and *Isoglossa laxa* (Fichtl and Adi, 1994).

Experimental Set Up and Honeybees

A total of 15 queen- right colonies (headed by naturally mated queens) of *A. m. bandasii,* the local honeybee race, were established in standard Langstroth hives in 2021, a year prior to the commencement of the experiment. All the experimental colonies originated from locally caught swarms and were standardized to have uniform conditions, including population strength, and they were checked for the presence of Varroa mites. At the beginning of the experiment, the infestation rate of the mite was determined using the standard method of detergent wash (Dietemann et al., 2013). Thereafter, the percentage of mite infestation rate (%) in each colony was expressed as the number of mites counted per 100 adult worker bees.

Evaluation of Hygienic Behavior (HB)

Hygienic behavior was assessed in all of the established colonies (N = 15) using the standard pin-killed brood assay method as described in Büchler et al. (2013). After selecting the section of bee comb containing caped young pupae cells (white-to purple-eyed stage), this section was punctured with a circular (5 cm, ID) polyvinyl chloride (PVC) pipe to demark the entire row of cells that surrounds approximately 164 cells (Fig. 1). The number of empty cells within each circular comb section was counted and recorded. Then, every capped pupa within the marked section of comb was pin-killed with a fine insect pin (entomological pin No-2) and the combs were placed back into the test colonies.

After 24 and 48 hrs, the frame with the comb sections was taken out again from the respective colonies to record the number of removed cells and the remaining dead broods at both consecutive periods. Moreover, the marked section of the comb was photographed for later count and confirmation, as indicated in the figure below (Fig. 1). Then, the number of fully removed pin-killed pupae cells from each test frame was recorded after 24 and 48 hours and expressed as the percentage of brood removal rate (%). Lastly, the total percentage of dead brood removal rate (%) was calculated according to (Kebede, 2006) as follow (Eq-1):

$$R = \frac{K-E-C}{T-E} x\ 100 \ldots\ldots (1)$$

Where; R = Percentage of dead brood removal rate (%) in time interval
K = Number of removed dead broods in time interval
C = No. of empty cells in the section before the test
E = No. of non-removed brood cells after the test
T = Total number of cells in the demarked brood section

Then, the colonies were classified into three groups: high, medium, and low hygienic colonies based on brood removal rate after 24 hrs. Consecutively, colonies with uncapped and removed dead broods of more than 90%, 60–90%, and less than 60% were classified as having high, medium, and low hygienic behaviors, respectively (Medina-Flores et al., 2014).

Evaluation of Grooming Behavior (GB)

Grooming behavior (fallen and damaged mites) was evaluated in ten selected colonies (N=10) with uniform colony population and strength using the standard method of estimating colony strengths (Delaplane et al., 2013). Five weak colonies were excluded from the experiment in order to minimize the biased effects of varying colony populations on grooming activity. Then, the original bottom boards of selected experimental colonies were removed and replaced with modified screened bottom boards following the procedure of Pettis and Shimanuki (1999). The screens were designed to allow only the passage of mites through them on the collecting trays, but not the bees. To intercept the falling mites, mite-collecting trays on the top side were covered with white cardboard and smeared with sticky, non-toxic petroleum jelly (Vaseline®). The trays were maintained in the hives, and the fallen mites were collected every 48 hrs from the cardboard papers for three consecutive days. On each data collection day, the slide board was removed, and fallen mites were collected, cleaned, and reintroduced into the bottom boards of the hive.

Subsequently, the collected fallen mites were counted and examined for body damage under a Zeiss Primo Star light microscope, Germany (Mg. Power 40X). Each examined mite was assigned as “damaged” or “undamaged” categories for the analysis. The proportion of damaged mites (%) in each colony was expressed by dividing the number of damaged mites by the total number of fallen mites at the end of the collection time (after 48 hrs). The damaged mites were also further grouped into different damage categories following previously established classifications of damaged mites (Corrêa-Marques et al., 2000).

Statistical Analyses

All statistical analyses were carried out using R-Software version 4.1.3 (R Core Team 2021). Data were checked for normality using the Shapiro-Wilk test, and they were normally distributed. A pairwise sample t-test was used to compare brood removal rates between 24 hrs and 48 hrs, as well the percentage of damaged and undamaged fallen mites. Linear model was used to estimate the Varroa infestation rate in relation to brood removal rates at 24 hours and 48 hours separately and compare the infestation rate in relation to the percentage of damaged and undamaged fallen mites separately. To determine the relationship between hygienic and grooming behavior of *A. m. bandasii* against Varroa infestation levels, the Pearson correlation test and a linear regression model were used. The average daily fallen mite count per colony was determined by dividing the total number of naturally fallen mites to the number of mite collection days following Strauss et al. (2015).

Results

Evaluation of Hygienic Behavior

The uncapping and removal percentage of pin-killed brood from the cells was used as an indicator of the level of hygienic behavior in experimental honeybee colonies. There was a variation in removing the dead broods from the cells among the experimental colonies. The mean removal rates of pin-killed broods were 83.1± 14.3% ranged from 57.6% to 98.1% after 24 hrs, and 97.6± 3.4% ranged from 89.4 to100% after 48 hrs (Fig. 2A, Table 1).

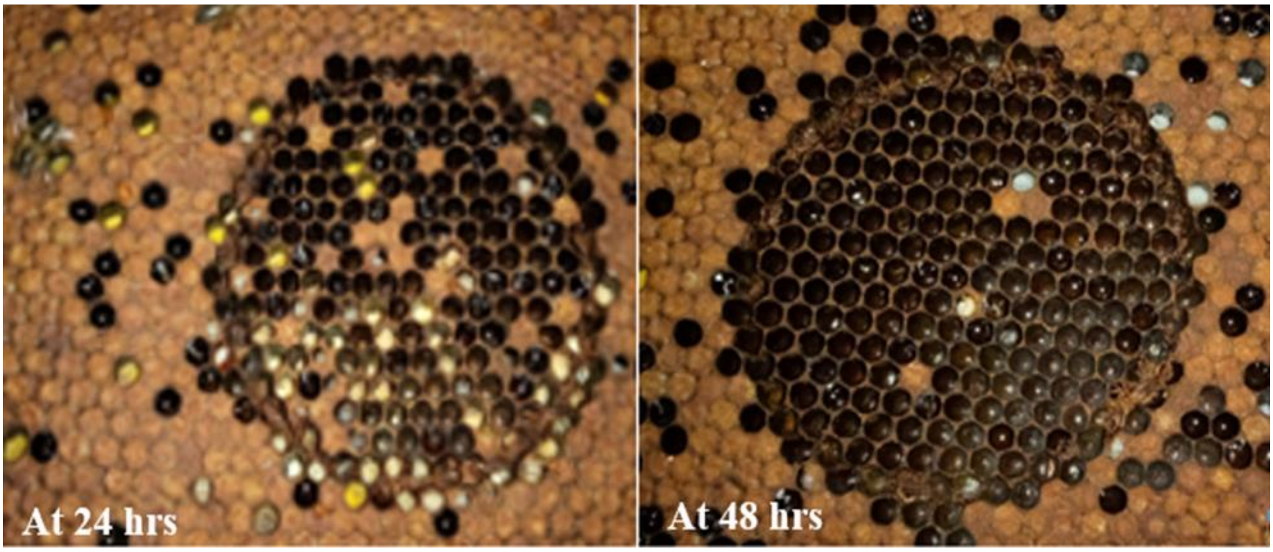

Figure 1. Sections of bee comb indicating the removal of pin-killed broods after 24 and 48 hours from *A. m. bandasii* colonies

Table 1. Description of the rates of pin-killed brood removal after 24 and 48 hours and the Varroa infestation rates, in *A. m. bandasii* colonies for the assessment of hygienic behavior

Description	N	Min	Max	Mean	Std. D
After 24 h	15	57.62	98.10	83.98	14.26
After 48 h	15	89.40	100.00	97.60	3.4
IR	15	0.16	13.43	4.06	3.78

Accordingly, 53.5% (8 of 15) of the colonies removed more than 90% pin-killed broods after 24 hrs, showing higher hygienic performances. While the other 40.0% (6 of 15) colonies removed about 60-90% of the dead broods, and were classified as medium HB. Yet, 6.5% of the colonies were able to remove less than 60% of the pin-killed broods, and classified as low HB (Fig. 2B).

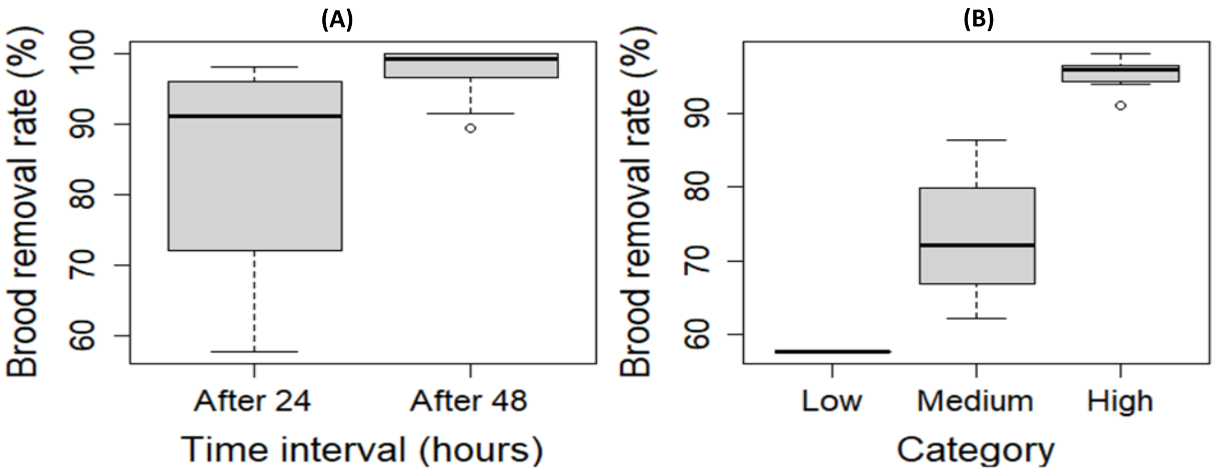

Figure 2. The mean percentage of pin-killed brood removal rates in time intervals (A) and the category of hygienic behavior for central highland honeybee, *A. m. bandasii* colonies (B)

There was negative correlation between the Varroa mite infestation rate (IR) and the proportion of pin-killed brood removal rate in honeybee colonies, both after 24 and 48 hrs of observations (Pearson correlation: r = -0.81, P = 0.001 and r = -0.483, P = 0.039, respectively) (Fig. 3A).

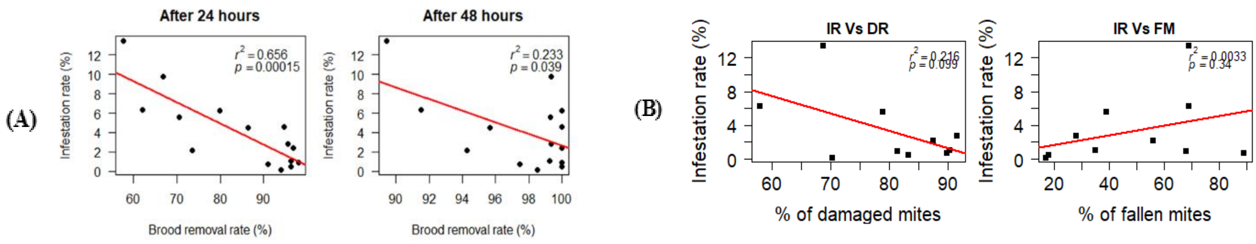

Figure 3. Correlations between Varroa mite infestation rate and brood removal rates across time intervals (A), as well as the correlations between Varroa mite infestation rate and the percentages of damaged mites and fallen mites (B) in the experimental colonies

Evaluation of Grooming Behavior

The results on the grooming behavior exhibited by the experimental colonies (N=10) in terms of fallen and damaged mites are depicted in Table 2 and Fig. 4B. Of the total 488 fallen mites collected from the bottom boards, 80.0±16.3% (Mean ± SD) were damaged, while 20±13.0% were undamaged, and these means were significantly different (t (df)= value of t; P < 0.001) (Fig. 4B). The average daily count fallen mites in experimental colonies was 16.3±10.2, ranging from 5.7±3.5 to 29.7±14.2. The higher number of daily fallen mites was recorded for colonies 6, 4 and 2, while colonies 3, 7 and 9 showed a lower number of daily fallen mite counts (Table 2).

Table 2. The mean daily count of fallen mites and the corresponding percentage of damaged mites within experimental honeybee colonies, A. *m. bandasii*

Colony code (N=10)	Daily fallen mites (Mean ± SD)	Damaged mites (Mean ± SD)	Undamaged mites (Mean ± SD)
C-1	13.0±11.4	78.9±9.2	21.1±1.8
C-2	23.0±8.2	57.9±22.0	42.1±2.1
C-3	5.7±3.5	70.4±17.9	29.6±0.4
C-4	23.0±7.8	68.8±18.6	31.2±4.1
C-5	22.7±8.7	81.4±10.2	18.6±11.3
C-6	29.7±14.2	89.9±3.8	10.1±6.2
C-7	6.0±3.6	83.3±20.8	16.7±1.7
C-8	11.7±3.2	90.3±10.9	9.7±6.4
C-9	9.3±4.2	91.7±14.4	8.3±0.7
C-10	18.7±18.7	87.5±8.5	12.5±9.1
Average	**16.3±10.23**	**80.0±16.3**	**20±13.0**
P-Value	***P < 0.001***	***P < 0.001***	

The findings indicate that there was no significant correlation between the percentage of damaged mites and infestation rate of mites (R = r^2=0.216, P= 0.099). Similarly, the association between the percentage of fallen mites and the mite infestation rate was not statistically significant (r^2 = 0.0033, P = 0.34; Fig 3B).

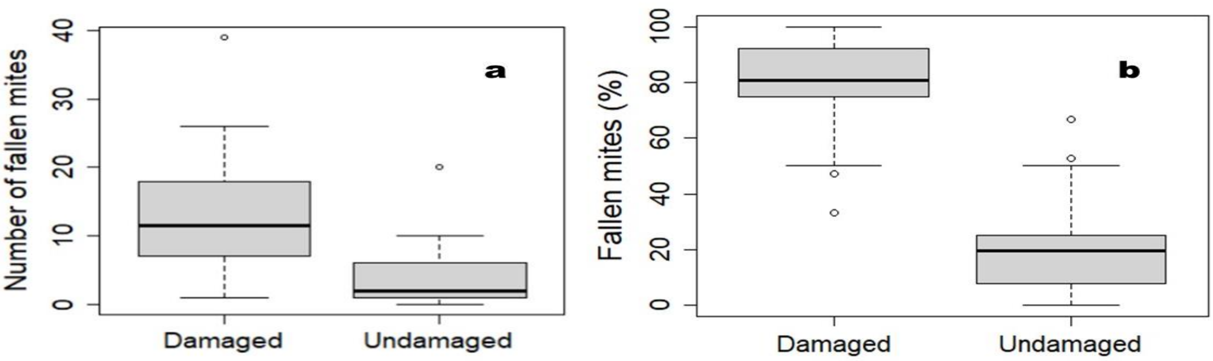

Figure 4. The average number of daily fallen mites (a) and the percentage distribution between damaged and undamaged mites (b) in local honeybees, *A. m. bandasii*

In this study, about 10 categories of damages were observed on the bodies of the mites (Fig. 5), and all injuries could be inflicted from the grooming activities of worker bees. Notably, three distinct new damage categories were observed in this study which include: carcass-empty dorsal shield + empty ventral shield; damaged legs + damaged gnathosoma + damaged shield; and hollow in the dorsal shield + damaged legs (Fig. 5D, 5H and 5J). The damaged legs (the complete or partial loss of one or more legs) (Fig. 5C) and damaged dorsal shields (Fig. 5J) were the most frequently observed (presented more than five times when examined under the microscope) body damages, with the proportion of mite injuries at 38.2% and 29.2%, respectively. Other damages represented in this assessment included combined injuries in the mite bodies and legs.

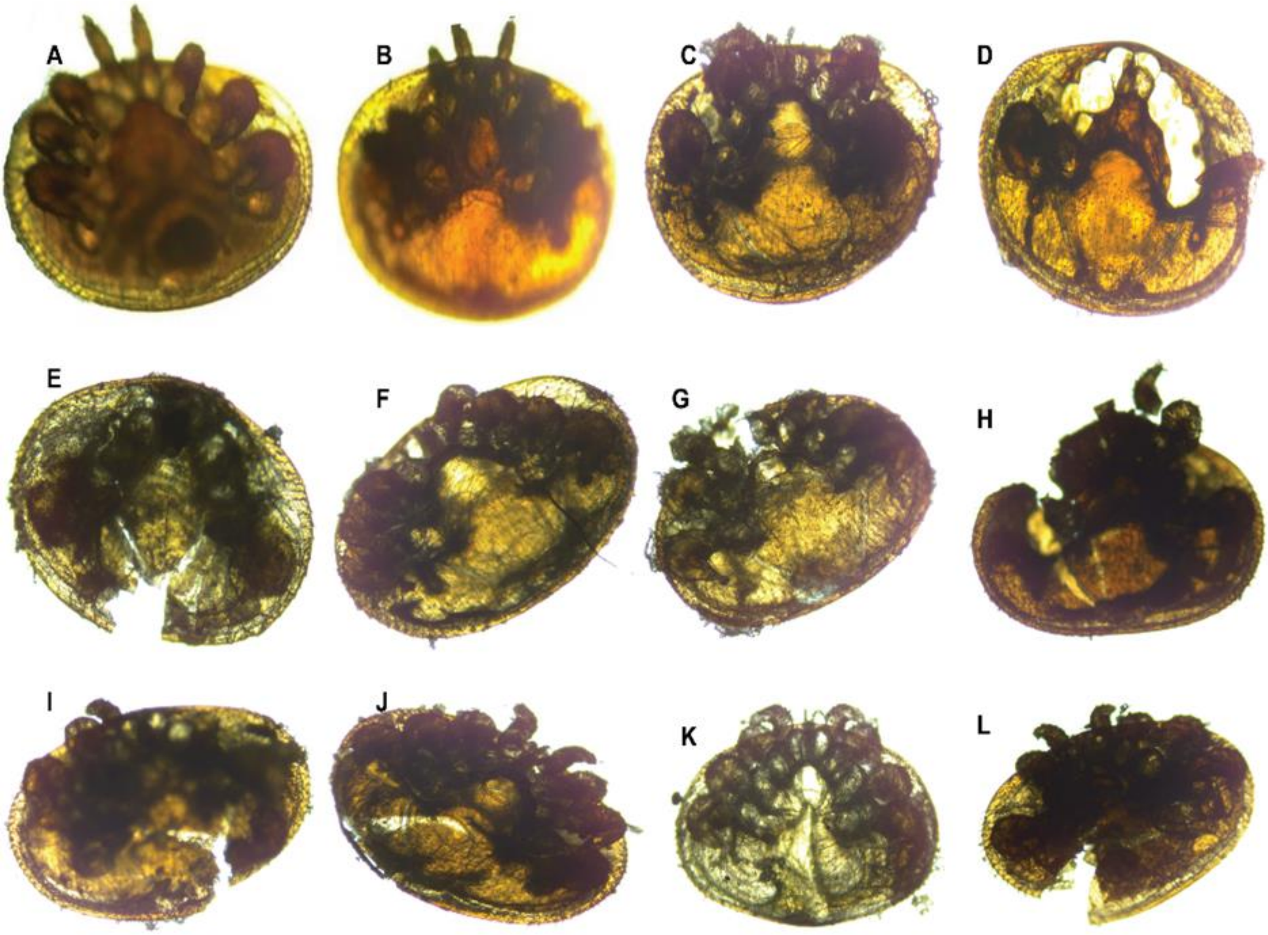

Figure 5. Morphological views of *V. destructor* mite under examination using Primo Star (Zeiss) microscope (40x Mg) during the evaluation of *A.m. bandasii* grooming behavior. The figure illustrates ventral and dorsal view of undamaged mite (A-B) and damage categories inflicted to legs (C), carcass-empty dorsal + empty ventral shield (D), damaged empty dorsal shield + missing legs (E), damaged legs + empty dorsal shield (F), damaged legs + damaged gnathosoma (G), damaged legs + damaged gnathosoma + damaged shield (H), hollow in the dorsal shield + damaged legs (I), damaged dorsal shields (J), hollow in the ventral shield + damaged legs (K) and hollow in the dorsal shield (L).

Discussions

Honeybees exhibit a wide range of natural defensive behaviors against the infestation of pathogens, pests and parasites in order to survive. Particularly, resistant honeybees display various physiological and behavioral defenses to limit the spread of pathogen and parasite infections within a colony (Mondet et al., 2020). However, such natural traits considerably vary based on bees' genetic factors and environmental conditions, as well as prevailing pathogens and parasites (Meixner et al., 2015). Several studies suggested that hygienic and grooming behaviors play key roles as defense mechanisms and enable honeybee populations to survive the effects of brood diseases and parasitic mite (Morfin et al., 2020; Boecking and Spivak, 1999; Khan and Ghramh, 2021; Nganso et al., 2017). In the present study, we assessed the potential contribution of hygienic and grooming behaviors in the central highland honeybee, *A. m. bandasii* in Ethiopia as defense strategies against the infestation of *V. destructor* mite.

Our results reveal a higher level of hygienic behavior (96.7%) expressed in the tested colonies of the *A. m. bandasii* honeybee race. This demonstrates the central highland honeybees have greater ability to detect and remove the infested broods from the comb cells. As explained by Medina-Flores et al. (2014), colonies with a HB of more than 95% within 48 hrs are genetically resistant to different infectious pathogens, and are categorized as strong hygienic colonies. However, the hygienic behaviors expressed in our study varied among experimental colonies. These variations among colonies of the same subfamilies might be influenced by factors such as the age of the worker bees performing the hygienic tasks (Panasiuk et al., 2010), or by their heritable genetic traits in the bee subfamilies (Arathi and Spivak, 2001). In fact, individual worker honeybees within the same subfamilies may also differently respond to different stressors including pest and pathogens (Roberts and Hughes, 2014; Dalmon et al., 2019). In agreement with our finding, a recent investigation by Hunde and Hora (2022) has also shown the performance of *A. m. bandasii* colonies in terms of high level of hygienic behavior (96.42%), good brood rearing, nectar production, and high aggressive behavior. Furthermore, higher percentages of HBs have been reported in different subspecies of Ethiopian local honeybees, including *A. m. scutellata* colonies (95.7%) (Shitaneh et al., 2022) and *A. m. weyi gambella* subspecies (92.16% in 24 hrs) (Aleme et al., 2017).

Compared to reports from other countries, the average hygienic behaviors displayed in Ethiopian honeybee races were higher than those reported in Kenya (81.0%) (Nganso et al., 2017), Egypt (72.5%) (Kamel et al., 2003), Ecuador (80 %) (Masaquiza et al., 2021), and Chile (20-80%) (Araneda et al., 2008). Such differences could be attributed to several factors, including the bees' genetic factors, geographic locations, climatic conditions, and seasonal variations. Although the level of hygienic behavior varies among different bee species, several studies suggested that African honeybees in general, display strong hygienic behavior that suppresses the Varroa mite reproduction cycle and population growth (Muli et al., 2014; Mondet et al., 2015; Mondet et al., 2020; Gebremedhn et al., 2019).

Interestingly, our results demonstrate a negative correlation between the HBs and Varroa mite infestation rate among the tested colonies both at 24 and 48 hrs. This supports the evidence that honeybees with higher HBs can limit the reproduction cycle of the mite, thereby reducing its infestation level in the hive colonies (Kim et al., 2018). Similarly, Muli et al. (2014) reported a strong negative relationship between hygienic behavior and the Varroa mite infestation rate in *A. m. scutellata* colonies in Kenya. This could be linked to specific tasks of worker honeybees in which they exhibit to detect and remove Varroa-infested broods from comb cells, and this specific mechanism of hygienic behavior is termed "Varroa Sensitive hygienic behavior" (VSH) (Spivak and Danka, 2021; Mondet et al., 2020; Harris et al., 2010). Several studies suggest that VSH results in reducing the reproductive potential and population growth of mites, which limits the infestation and spread of mite in colonies (Peng et al., 1987; Spivak and Danka, 2021; Kim et al., 2018). During the activities of VSH, the worker bees' antennal physiology can play a key role in detecting odor coming from Varroa-infested larvae, which then triggers the cleaning of infested broods from the comb cells (Parker et al., 2012; Mondet et al., 2015). Therefore, the local honeybees (*A. m. bandasii*) could have displayed active VSH behaviors to survive against the destructive effects of the Varroa mite by reducing the mite infestation level below the threshold damage level. However, it is worth noting that the timing of brood removal can influence the apparent resistance of honeybees, determining the overall rates of Varroa parasitism in colonies (Spivak and Danka, 2021).

Apart from hygienic behavior, the tested colonies (*A. m. bandasii)* displayed a higher level of grooming behavior in terms of mean daily fallen mites and percentage of damaged mites. The higher percentage of fallen mites and the higher proportion of damaged mites observed in this study reflect the intensive of grooming ability of *A. m. bandasii* to fight against the destructive nature of *V. destructor* mite. The percentage of grooming behavior (80.0±16.3%) recorded in this study was considerably higher when compared to the previous study for *A. m. simensis* colonies in the northern region of Ethiopia, with the GB value of 34.78% and 41.89% during active and dry seasons, respectively (Gebremedhn et al., 2019). In addition, the percentage GB of the resistant, *A. m. scutellata* colonies (21.3%) recorded in Kenya (Nganso et al., 2017) was lower than the present study. This explains a strong grooming behavior of *A. m. bandasii* stocks that could likely inflict damage to the mites' bodies and this might be contributed for colony survival. In agreement with this, Pritchard (2016) explained that colonies inflicting about 60% body damage to the total fallen mites are capable of limiting the mite infestation level and can survive the Varroa infestation without chemical treatment. Consequently, the higher intensity of grooming behavior and injuries that the bees inflict on the body of the mite can significantly influence the mites reproduction cycle and population growth in Varroa-surviving colonies (Dadoun et al., 2020; Russo et al., 2020).

In the present study, about ten body damage categories were examined from the total fallen mites. It appears that all the damages have been inflicted by the grooming activities of worker bees, which is consistent with the previous investigation by Corrêa-Marques et al. (2000). Importantly, a significant overlap of damage categories was observed between our result and the findings of recent studies in Argentina (Russo et al., 2020) and Kenya (Russo et al., 2020; Nganso et al., 2017). Although there were multiple damages examined in our study, the damaged legs (total or partial loss of one or more legs), and damaged dorsal shield were the most frequently recorded damages. This observation suggests that the primary target of worker honeybees is to destruct the legs and external bodies of the fallen adult mites, which then inhibits the mite movement and re-infestation in the hive. Such targeted damages would eventually lead to the death of mites and have potential to reduce Varroa population growth and infestation levels in the hives (Corrêa-Marques et al., 2000). In our study, the successful survival of local honeybee stocks against the Varroa mite infestation might be associated with the ability of worker bees to damage and remove mites from their nest colony. Yet, there were variations in the level of damaged mites across experimental colonies, and this would preclude us from concluding that grooming behavior is an effective mechanism of defense against mites. Because, several driving factors can considerably influence the degree of grooming behaviors among honeybee stocks, even for colonies existing in the same geographic region (Boecking et al., 2000; Masaquiza et al., 2021; Hamiduzzaman et al., 2017). Therefore, considering driving factors would be important during selection breeding programs to enhance resistant bee stocks.

Conclusion

The honeybees exhibit a wide range of resistance mechanisms in order to combat the devastating effects of the *V. destructor* mite. Our investigation into the defensive behaviors of the central highland honeybee, *A. m. bandasii* colonies in Ethiopia reveals the potential expression of hygienic and grooming behavioral traits against the *Varroa destructor* mite. While different local honeybee races may employ diverse defense mechanisms, our study highlights the pivotal roles of hygienic and grooming behaviors in withstanding the effects of the Varroa mite infestations. The strong negative correlation observed between hygienic behavior and the level of Varroa mite infestation indicates the significance of worker bees' hygienic activities in mitigating the reproduction and population growth of the mite within the colonies. Moreover, our finding revealed that *A. m. bandasii* colonies exhibit extensive grooming behaviors, leading to significant damages to the bodies of phoretic mites. This illustrates the critical part in which the combined expression of hygienic and grooming behaviors likely contributes to the survival and reduced vulnerability of local honeybee colonies to the devastating effects of the Varroa mite. These findings provide valuable insights into various natural defense mechanisms that help local honeybee populations in combating the impacts of *V. destructor* mite without the need for beekeepers' intervention. Future research should explore and identify the various defense mechanisms employed by different native honeybee subspecies in Ethiopia to combat against the parasitic Varroa mite. Understanding such natural adaptations and driving factors in local honeybee populations is crucial to design and develop effective selective breeding strategies within the country.

Ethical Statement

There are no ethical issues with the publication of this article.

Funding Information

This research has not received any funding grants from commercial or non-profit organisations.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

A. Gela : Conceptualization, Investigation, Methodology, Writing original draft
A. G: Investigation, Writing, Review & Editing, Supervision
Y. W: Review & Editing, Supervision
A. A: Review & editing, Formal Analysis
Z. A: Investigation, Methodology
A. B: Supervision, Funding acquisition

Acknowledgments

This study was financially supported by Ethiopian Agricultural Research Institute (EIAR), Addis Ababa University (AAU) and Oromia Agricultural Research Institute (IQQO). The authors would like to thank Holeta Bee Research Center (HBRC) for all the logistic and laboratory facility arrangements during the experimental work. Our thanks also go to the bee health team at HBRC for their technical assistance during the laboratory and field work.

References

Abrol, D., & Sharma, D. (2013). Pollinator decline influencing global crop yield and food security. *Integrated Pest Management*, 208.

Aleme, M., Yadessa, E., Tulu, D., Bogale, A., Mengistu, G., & Bezabeh, A. (2017). Performance evaluation of local honey bee races (Apismellifera Wayi Gambella) in Sheka zone. *International Journal of Research in Agricultural Sciences, 4*(6), 282-287.

Allsopp, M., Govan, V., & Davison, S. (1997). Bee health report: Varroa in South Africa. *Bee World,* 78(4), 171-174.

Araneda, X., Pérez, R., Castillo, C., & Medina, L. (2008). Evaluación del comportamiento higiénico de *Apis mellifera* L. en relación al nivel de infestación de *Varroa destructor* Anderson & Trueman. *Idesia (Arica),* 26(2), 59-67.

Arathi, H., & Spivak, M. (2001). Influence of colony genotypic composition on the performance of hygienic behaviour in the honeybee, *Apis mellifera* L. *Animal Behaviour,* 62(1), 57-66.

Aumeier, P. (2001). Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized and Carniolan honey bees. *Apidologie,* 32(1), 81-90.

Begna, D. (2014). Occurrences and distributions of honeybee (*Apis mellifera* Jemenetica) varroa mite (*Varroa destructor*) in Tigray Region, Ethiopia. *Journal of Fisheries and Livestock Production,* 2(3), 10.4172.

Begna, D., Gela, A., Negera, T., & Bezabeh, A. (2016). Identifying the species, effects and seasonal dynamics of honeybee varroa mites: A newly emerging parasite to Ethiopian honeybee. *International Journal of Scientific*

Research in Environmental Science and Toxicology, 1(1), 4.

Boecking, O., Bienefeld, K., & Drescher, W. (2000). Heritability of the Varroa-specific hygienic behaviour in honey bees (Hymenoptera: Apidae). *Journal of Animal Breeding and genetics,* 117(6), 417-424.

Boecking, O., & Spivak, M. (1999). Behavioral defenses of honey bees against Varroa jacobsoni Oud. *Apidologie, 30*(2-3), 141-158.

Büchler, R., Andonov, S., Bienefeld, K., Costa, C., Hatjina, F., Kezic, N., Kryger, P., Spivak, M., Uzunov, A. & Wilde, J. (2013). Standard methods for rearing and selection of *Apis mellifera* queens. *Journal of Apicultural Research,* 52(1), 1-30.

Büchler, R., Berg, S., & Le Conte, Y. (2010). Breeding for resistance to Varroa destructor in Europe. *Apidologie, 41*(3), 393-408.

Castilhos, D., Polesso, A. M., da Silva, A. C. F., dos Santos, A. B., de Carvalho Lopes, A. T., de Oliveira Filho, A. A., Da Cunha, A. F. S., Puker, A., Matioli, A. L., & Campos, A. A. S. (2023). Varroa destructor infestation levels in Africanized honey bee colonies in Brazil from 1977 when first detected to 2020. *Apidologie,* 54(1), 5.

Chemurot, M., Akol, A. M., Masembe, C., De Smet, L., Descamps, T., & de Graaf, D. C. (2016). Factors influencing the prevalence and infestation levels of *Varroa destructor* in honeybee colonies in two highland agro-ecological zones of Uganda. *Experimental and Applied Acarology,* 68(4), 497-508.

Corrêa-Marques, M. H., Issa, M., & Jong, D. D. (2000). Classification and quantification of damaged Varroa jacobsoni found in the debris of honey bee colonies as criteria for selection? *American Bee Journal,* 140(10), 820-824.

Dadoun, N., Nait-Mouloud, M., Mohammedi, A., & Sadeddine Zennouche, O. (2020). Differences in grooming behavior between susceptible and resistant honey bee colonies after 13 years of natural selection. *Apidologie,* 51(5), 793-801.

Dalmon, A., Peruzzi, M., Le Conte, Y., Alaux, C. & Pioz, M. (2019). Temperature-driven changes in viral loads in the honey bee Apis mellifera*. Journal of invertebrate pathology*, 160, 87-94.

Delaplane, K. S., Van Der Steen, J., & Guzman-Novoa, E. (2013). Standard methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicultural Research,* 52(1), 1-12.

Dietemann, V., Nazzi, F., Martin, S. J., Anderson, D. L., Locke, B., Delaplane, K. S., Wauquiez, Q., Tannahill, C., Frey, E., & Ziegelmann, B. (2013). Standard methods for varroa research. *Journal of Apicultural Research,* 52(1), 1-54.

Dietemann, V., Pirk, C. W. W., & Crewe, R. (2009). Is there a need for conservation of honeybees in Africa? *Apidologie,* 40(3), 285-295.

Fazier, M., Muli, E., Conklin, T., Schmehl, D., Torto, B., Frazier, J., Tumlinson, J., Evans, J. D., & Raina, S. (2010). A scientific note on *Varroa destructor* found in East Africa; threat or opportunity? *Apidologie,* 41(4), 463-465.

Fichtl, R., & Adi, A. (1994). *Honeybee flora of Ethiopia*: Margraf Verlag.

Flores, J. M., Gámiz, V., Jiménez-Marín, Á., Flores-Cortés, A., Gil-Lebrero, S., Garrido, J. J., & Hernando, M. D. (2021). Impact of Varroa destructor and associated pathologies on the colony collapse disorder affecting honey bees. *Research in Veterinary Science,* 135, 85-95.

Gebremedhn, H., Amssalu, B., Smet, L. D., & de Graaf, D. C. (2019). Factors restraining the population growth of Varroa destructor in Ethiopian honey bees (*Apis mellifera* simensis). *PLoS One,* 14(9), e0223236.

Gela, A., Atickem, A., Bezabeh, A., Woldehawariat, Y., & Gebresilassie, A. (2023). Insights into varroa mite (*Varroa destructor*) infestation levels in local honeybee (*Apis mellifera*) colonies of Ethiopia. *Journal of Applied Entomology*, 147(9), 798-808.

Gratzer, K., Wakjira, K., Fiedler, S. & Brodschneider, R. 2021. Challenges and perspectives for beekeeping in Ethiopia. A review. *Agronomy for Sustainable Development*, 41, 1-15.

Grindrod, I., & Martin, S. J. (2021). Parallel evolution of Varroa resistance in honey bees: a common mechanism across continents? *Proceedings of the Royal Society B,* 288(1956), 20211375.

Grindrod, I., & Martin, S. J. (2023). Varroa resistance in Apis cerana: a review. *Apidologie,* 54(2), 14.

Hamiduzzaman, M. M., Emsen, B., Hunt, G. J., Subramanyam, S., Williams, C. E., Tsuruda, J. M., & Guzman-Novoa, E. (2017). Differential gene expression associated with honey bee grooming behavior in response to Varroa mites. *Behavior genetics,* 47(3), 335-344.

Harris, J. W., Danka, R. G., & Villa, J. D. (2010). Honey bees (Hymenoptera: Apidae) with the trait of Varroa sensitive hygiene remove brood with all reproductive stages of Varroa mites (Mesostigmata: Varroidae). *Annals of the Entomological Society of America,* 103(2), 146-152.

Hristov, P., Shumkova, R., Palova, N., & Neov, B. (2020). Factors associated with honey bee colony losses: A mini-review. *Veterinary Sciences,* 7(4), 166.

Hunde, T., & Hora, Z. (2022). Determining the performance of *Apis Mellifera bandasii* populations under different agro-ecologies of Central Ethiopia. *Advances in Agriculture,* 2022.

Kablau, A., Berg, S., Härtel, S., & Scheiner, R. (2020). Hyperthermia treatment can kill immature and adult *Varroa destructor* mites without reducing drone fertility. *Apidologie,* 51(3), 307-315.

Kamel, S. M., Strange, J. P., & Sheppard, W. S. (2003). A scientific note on hygienic behavior in *Apis mellifera lamarckii* and *A. m. carnica* in Egypt. *Apidologie,* 34(2), 189-190.

Kebede, D. (2006). Testing colonies of India honey bees *Apis cerana* for hygienic behavior. (Master's thesis), Department of Apiculture of Agricultural Science, Bangalore.

Khan, K. A., & Ghramh, H. A. (2021). An investigation of the efficacy of hygienic behavior of various honey bee (Apis mellifera) races toward *Varroa destructor* (Acari: Varroidae) mite infestation. *Journal of King Saud University-Science,* 33(3), 101393.

Kim, S. H., Mondet, F., Hervé, M., & Mercer, A. (2018). Honey bees performing varroa sensitive hygiene remove the most mite-compromised bees from highly infested patches of brood. *Apidologie*, 49, 335-345.

Le Conte, Y., Ellis, M., & Ritter, W. (2010). Varroa mites and honey bee health: can Varroa explain part of the colony losses? *Apidologie,* 41(3), 353-363.

Locke, B. (2016). Natural Varroa mite-surviving *Apis mellifera* honeybee populations. *Apidologie,* 47, 467-482.

Malfroy, S. (2015). *A model for industry planning and preparedness for an incursion of Varroa mite* (Project

Number: MT12049). Horticulture Innovation Australia Limited.

Masaquiza, D., Vargas, J., Ortíz, N., Salazar, R., Curbelo, L., Pérez, A., & Arenal, A. (2021). Hygienic behavior of *Apis mellifera* and its relationship with *Varroa destructor* infestation and honey production in the Central Highlands of Ecuador. *Insects,* 12(11), 966.

Medina-Flores, C. A., Guzman-Novoa, E., Aréchiga Flores, C. F., Gutiérrez Bañuelos, H., & Aguilera Soto, J. I. (2014). Honey production and *Varroa destructor* infestation of Africanized honey bee (Apis mellifera) colonies with high and low hygienic behavior. *Revista mexicana de ciencias pecuarias,* 5(2), 157-170.

Meixner, M. D., Kryger, P., & Costa, C. (2015). Effects of genotype, environment, and their interactions on honey bee health in Europe. *Current Opinion in Insect Science,* 10, 177-184.

Mekonnon, B., Lakew, B., & Dessalegn, T. (2015). Morphological diversity and association of traits in Ethiopian food barley (*Hordeum vulgare* L.) landraces in relation to regions of origin and altitudes. *Journal of Plant Breeding and Crop Science*, 7(2), 44-54.

Mohammed, N. A. (2002). *Geographical races of the Honeybees (Apis mellifera L.) of the Northern Regions of Ethiopia.* Rhodes University.

Mondet, F., Alaux, C., Severac, D., Rohmer, M., Mercer, A. R., & Le Conte, Y. (2015). Antennae hold a key to Varroa-sensitive hygiene behaviour in honey bees. *Scientific reports,* 5(1), 1-12.

Mondet, F., Beaurepaire, A., McAfee, A., Locke, B., Alaux, C., Blanchard, S., Danka, B., & Le Conte, Y. (2020). Honey bee survival mechanisms against the parasite *Varroa destructor*: a systematic review of phenotypic and genomic research efforts. *International journal for parasitology,* 50(6-7), 433-447.

Morfin, N., Espinosa-Montaño, L. G., & Guzman-Novoa, E. (2020). A direct assay to assess self-grooming behavior in honey bees (*Apis mellifera* L.). *Apidologie,* 51, 892-897.

Muli, E., Patch, H., Frazier, M., Frazier, J., Torto, B., Baumgarten, T., Kilonzo, J., Mumoki, F., Masiga, D., & Tumlinson, J. (2014). Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (*Apis mellifera*) populations in East Africa. *PLoS One,* 9(4), e94459.

Nazzi, F., & Le Conte, Y. (2016). Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. *Annual Review of Entomology,* 61(1), 417-432.

Nganso, B. T., Fombong, A. T., Yusuf, A. A., Pirk, C. W., Stuhl, C., & Torto, B. (2017). Hygienic and grooming behaviors in African and European honeybees-New damage categories in Varroa destructor. *PLoS One,* 12(6), e0179329.

Noël, A., Le Conte, Y., & Mondet, F. (2020). *Varroa destructor*: how does it harm *Apis mellifera* honey bees and what can be done about it? *Emerging Topics in Life Sciences,* 4(1), 45-57.

O'Shea-Wheller, T. A., Rinkevich, F. D., Danka, R. G., Simone-Finstrom, M., Tokarz, P. G., & Healy, K. B. (2022). A derived honey bee stock confers resistance to Varroa destructor and associated viral transmission. *Scientific reports,* 12(1), 4852.

Panasiuk, B., Skowronek, W., Bienkowska, M., Gerula, D., & Wegrzynowicz, P. (2010). Age of worker bees performing hygienic behaviour in a honeybee colony. *Journal of Apicultural Science,* 54(2).

Parker, R., Guarna, M. M., Melathopoulos, A. P., Moon, K.-M., White, R., Huxter, E., Pernal, S. F., & Foster, L. J. (2012). Correlation of proteome-wide changes with social immunity behaviors provides insight into resistance to the parasitic mite, *Varroa destructor*, in the honey bee (*Apis mellifera*). *Genome biology,* 13, 1-15.

Peng, Y.-S., Fang, Y., Xu, S., & Ge, L. (1987). The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology,* 49(1), 54-60.

Pettis, J., & Shimanuki, H. (1999). A hive modification to reduce varroa populations. *American Bee Journal,* 139(6), 471-473.

Pirk, C. W., Strauss, U., Yusuf, A. A., Demares, F., & Human, H. (2016). Honeybee health in Africa—a review. *Apidologie,* 47(3), 276-300.

Plettner, E., Eliash, N., Singh, N. K., Pinnelli, G. R., & Soroker, V. (2017). The chemical ecology of host-parasite interaction as a target of *Varroa destructor* control agents. *Apidologie,* 48(1), 78-92.

Pritchard, D. J. (2016). Grooming by honey bees as a component of varroa resistant behavior. *Journal of Apicultural Research,* 55(1), 38-48.

Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., . . . Ellis, J. D. (2019). *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences,* 116(5), 1792-1801.

Rath, W. (1999). Co-adaptation of *Apis cerana* Fabr. and *Varroa jacobsoni* Oud. *Apidologie,* 30(2-3), 97-110.

Roberts, K. E. & Hughes, W. O. (2014). Immuno-senescence and resistance to parasite infection in the honey bee, Apis mellifera*. Journal of Invertebrate Pathology*, 121, 1-6.

Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology,* 103, S96-S119.

Russo, R. M., Liendo, M. C., Landi, L., Pietronave, H., Merke, J., Fain, H., Muntaabski, I., Palacio, M. A., Rodríguez, G. A., & Lanzavecchia, S. B. (2020). Grooming behavior in naturally Varroa-resistant *Apis mellifera* colonies from north-central Argentina. *Frontiers in Ecology and Evolution,* 8, 590281.

Shegaw, T., Arke, A., Belay, N., & Habte Giorgis, D. (2022). Diagnostic survey on varroa mite (*Varroa distractor*) prevalence in South-Western Ethiopia. *Cogent Food & Agriculture, 8*(1), 2143610.

Shitaneh, E., Arega, H., Getent, M., & Bezabeh, A. (2022). Performance evaluation of local honey bee race (*Apis melifera* secutellata) in the Metekel Zone of North Western Ethiopia. *Veterinary Medicine and Science*, 8(6), 2696-2702.

Spivak, M., & Danka, R. G. (2021). Perspectives on hygienic behavior in *Apis mellifera* and other social insects. *Apidologie,* 52, 1-16.

Spivak, M., & Reuter, G. S. (2001). Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. *Apidologie,* 32(6), 555-565.

Strauss, U., Pirk, C. W., Crewe, R. M., Human, H. & Dietemann, V. (2015). Impact of Varroa destructor on honeybee (Apis mellifera scutellata) colony development in South Africa. *Experimental and Applied Acarology*, 65, 89-106.

Tibatá, V. M., Sanchez, A., Palmer-Young, E., Junca, H., Solarte, V. M., Madella, S., Ariza, F., Figueroa, J., & Corona, M. (2021). Africanized honey bees in Colombia exhibit high prevalence but low level of infestation of Varroa mites and low prevalence of pathogenic viruses. *PLoS One,* 16(5), e0244906.

Traynor, K. S., Mondet, F., de Miranda, J. R., Techer, M., Kowallik, V., Oddie, M. A., Chantawannakul, P., & McAfee, A. (2020). *Varroa destructor*: A complex parasite, crippling honey bees worldwide. *Trends in Parasitology,* 36(7), 592-606.

Yang, H., Shi, J., Liao, C., Yan, W., & Wu, X. (2021). *Varroa destructor* mite infestations in capped brood cells of honeybee workers affect emergence development and adult foraging ability. *Current zoology,* 67(5), 569-571.