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## Genotypic variability and relationships between mite infestation levels, mite damage, grooming intensity, and removal of *Varroa destructor* mites in selected strains of worker honey bees (*Apis mellifera* L.)

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### ABSTRACT

The objective of this study was to demonstrate genotypic variability and analyze the relationships between the infestation levels of the parasitic mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies, the rate of damage of fallen mites, and the intensity with which bees of different genotypes groom themselves to remove mites from their bodies. Sets of paired genotypes that are presumably susceptible and resistant to the varroa mite were compared at the colony level for number of mites falling on sticky papers and for proportion of damaged mites. They were also compared at the individual level for intensity of grooming and mite removal success. Bees from the “resistant” colonies had lower mite population rates (up to 15 fold) and higher percentages of damaged mites (up to 9 fold) than bees from the “susceptible” genotypes. At the individual level, bees from the “resistant” genotypes performed significantly more instances of intense grooming (up to 4 fold), and a significantly higher number of mites were dislodged from the bees’ bodies by intense grooming than by light grooming (up to 7 fold) in all genotypes. The odds of mite removal were high and significant for all “resistant” genotypes when compared with the “susceptible” genotypes. The results of this study strongly suggest that grooming behavior and the intensity with which bees perform it, is an important component in the resistance of some honey bee genotypes to the growth of varroa mite populations. The implications of these results are discussed.

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### 1. Introduction

*Varroa destructor* is a parasitic mite that has become the most important health problem of the western honey bee, *Apis mellifera*, worldwide. This mite has killed millions of colonies, causing the loss of billions of dollars in agricultural crops (Sanford, 2001). There is evidence that the varroa mite is one of the factors associated with the unprecedented loss of honey bee colonies recently experienced in parts of Europe and North America (Stankus, 2008; Guzman-Novoa et al., 2010; Le Conte et al., 2010).

Several synthetic acaricides have been used to control *V. destructor* in honey bee colonies, but mite resistance to the active ingredients in these pesticides has quickly developed and is now widespread worldwide (Milani, 1999). Therefore, it is possible that mite populations have become more difficult to control and are

causing more damage to colonies in recent years. Other ways of controlling these mites are thus necessary. One potential approach to controlling varroa mites would be the development of honey bee strains resistant to them, and one of the natural mechanisms of resistance against these mites seems to be the grooming behavior of worker bees.

Grooming behavior involves the physical removal, and often injury, of parasitic mites from the body of adult bees by individual workers or by their nest-mates. This behavior is one of the main mechanisms of resistance against mites in the Asiatic bee, *Apis cerana*, the original host of *Varroa* spp. (Peng et al., 1987). Grooming behavior is observed in *A. mellifera* as well, but it is expressed at a lower frequency compared to *A. cerana* (Boecking et al., 1993; Fries et al., 1996). In most instances, a bee tries to dislodge a parasite on its own using its legs; this action is called auto-grooming or self-grooming. The bee may also bite and injure the mite (Ruttner and Hänel, 1992; Boecking et al., 1993; Moosbeckhofer, 1997). Alternatively, the bee may solicit the help of nest-mates

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by means of a tremble-dance or grooming invitation dance (Land and Seeley, 2004), which incites nest-mates to clean the bee and remove parasites using their mandibles, an act referred to as allogrooming.

Several studies have shown that grooming behavior confers some degree of resistance to European honey bees against tracheal mites (Pettis and Pankiw, 1998; Danka and Villa, 2003; Villa, 2006), and seems to confer resistance against varroa mites in populations of Africanized bees (descendants of *Apis mellifera scutellata*) (Moretto et al., 1993; Guzman-Novoa et al., 1999, 2002; Arechavaleta-Velasco and Guzman-Novoa, 2001; Mondragon et al., 2005, 2006). However, its effectiveness against varroa mites in European honey bees is still not clear, although variation between European honey bee strains for this behavior has been documented. For example, Rinderer et al. (2001) found that bees from the Primorsky region of Russia seemed to be superior groomers compared to local bees in Louisiana, USA. Grooming behavior has also been associated with higher proportions of mutilated mites falling onto the bottom boards of hives, as well as with lower mite infestation levels in colonies (Moretto et al., 1993; Moosbeckhofer, 1997; Arechavaleta-Velasco and Guzman-Novoa, 2001; Mondragon et al., 2006; Currie and Tahmasbi, 2008; Andino and Hunt, 2011), but these associations are not always evident and the validity of using them to predict the grooming ability of colonies has been questioned because several other factors may cause mite mutilation in addition to the grooming behavior of bees (Rosenkranz et al., 1997; Bienefeld et al., 1999; Boecking and Spivak, 1999).

Several assays to measure grooming behavior in a controlled environment have been used to show differences between genotypes of honey bees for this trait (Hoffman, 1993; Szabo et al., 1996; Arechavaleta-Velasco and Guzman-Novoa, 2001; Currie and Tahmasbi, 2008), but results of these assays had not been directly correlated with the proportion of injured mites in colonies until recently. Andino and Hunt (2011) reported a correlation between the proportion of mites removed by the bees in lab grooming assays and the proportion of damaged mites on sticky boards from the source colonies used in their assays. Their results showed that the bees that removed the highest percentage of mites in their lab assay came from the colonies that had the highest percentage of injured mites.

The relative effectiveness of grooming behavior for mite removal has never been documented at both, colony and individual levels, using the same source colonies. Finding evidence that establishes a relationship between individual behavior and colony behavior is important to demonstrate how individuals with differing propensities to perform a task interact to influence a colony phenotype. Furthermore, a clear relationship between mite loads in colonies, damage to mites, and successful mite removal in individual bees has not been established. Additionally, little is known about the strategies that bees use to successfully remove mites from their bodies. Therefore, the objectives of this study were to measure genotypic variability between different strains of *A. mellifera* for grooming behavior at both colony and individual levels, to establish a relationship between traits associated with grooming behavior at both levels, and to document strategies of mite removal in individual bees.

## 2. Materials and methods

### 2.1. Honey bee genotypes

Experiments were conducted in two locations, at the Center for Beekeeping Development of the Mexican Department of Agriculture (INIFAP-SAGARPA) in Villa Guerrero, Mexico (19 °N, 99 °W) and at the Townsend House, Honey Bee Research Centre, of the University of Guelph, in Guelph, Ontario, Canada (43 °N, 80 °W).

In both locations, genotypes that are presumably resistant and susceptible to the varroa mite were compared.

In Mexico, two honey bee genotypes were used, Africanized and European bees. Africanized bees were derived from swarms captured in the region of study, whereas European bees were derived from Italian bees imported from Hawaii, USA. The genotype of each of the two sources was confirmed by morphometric and mitochondrial DNA analyses (Nielsen et al., 1999). Seven colonies of each genotype were used in the experiments conducted, and none of the colonies had been treated for parasitic mites for at least 1 year.

In Canada, four honey bee genotypes were evaluated: Russian, Local unselected, Local selected for low varroa mite population growth (SL), and Local selected for high varroa mite population growth (SH). Russian bees were originally imported into the USA from the Primorsky region in Eastern Russia by the US Department of Agriculture (Rinderer et al., 2001) in the belief that because these were the first *A. mellifera* colonies exposed to *V. destructor*, they therefore would have developed some degree of resistance to the mite. The strain was later imported into Canada from the USA, and is currently maintained in Ontario by two queen breeders in isolated mating areas. The Russian bees used in our experiments have been selected for honey production and varroa resistance using screened bottom boards and sticky bottom sheets to capture mites. The breeders select the colonies with the lowest mite numbers to rear queens to develop the next generation of bees. The unselected population came from a local beekeeper who does not apply any selection criteria in his queen rearing operation, and the genotypes selected for high and low varroa mite population growth, came from a breeding program currently being conducted at the University of Guelph. Colonies are selected by determining how much mite populations grow between April and August by monitoring mite fall, using sticky bottom boards in hives. The colonies with the highest and lowest rates for mite population growth are selected. To compare Russian and local strains of bees, five colonies of each genotype were used, whereas to compare selected bee genotypes showing high and low growth of varroa mite populations, four and nine colonies of each type were used in years 1 and 2 of selection (SH-1, SL-1, SH-2, SL-2), respectively. None of the colonies in the experiments had been treated against parasitic mites for at least 1 year.

Grooming behavior was evaluated at both the colony and the individual level. This means that each of the genotypes tested was first evaluated with full-size colonies and an indirect quantification of grooming behavior was done for each colony without separating the actions of individual bees. Colony level evaluation therefore would incorporate unique interactions within the colony. For the individual level testing, individual workers of each genotype were evaluated in a controlled environment. Theoretically, this individual level evaluation would more directly measure the genotypic variance among genotypes for the behavior tested, assuming that the common environment would have similar interaction effects on the bee genotypes evaluated.

### 2.2. Grooming behavior at the colony level

Grooming behavior at the colony level was inferred by analyzing the number of fallen mites, as well as the proportion of injured mites, on sticky bottom-board sheets. Sticky bottom-board sheets were made from letter-size, manila file-folders (44 × 30 cm), smeared with vegetable shortening (to trap falling mites) and placed on the hive bottom-boards beneath a hardware cloth (4 mm mesh) to prevent bees from accessing the sticky sheet but allowing mites to fall through. Sticky sheets were replaced every 3 days, the number of mites on the sheets was counted, and mites were collected and preserved in vials containing 96% ethanol. This procedure was performed six times over the course of 18 days for

each colony. The collected mites were counted and examined under a stereoscopic dissecting microscope (40×), and visible damage to their legs and/or idiosomes was recorded. Mites were considered injured by the bees if they were missing legs or parts of legs, or if part of their idiosoma was missing. A percentage of damaged mites was then calculated for each colony and genotype.

### 2.3. Grooming behavior at the individual level

Grooming behavior at the individual level was performed in a laboratory using a modified version of the method described by Aumeier (2000). Mites were collected from heavily infested colonies as described by Arechavaleta-Velasco and Guzman-Novoa (2001) and were transferred to a Petri dish, which was placed in a laboratory room kept at  $28 \pm 2$  °C, and humidified with moist pieces of paper towel placed inside the Petri dish.

Worker bees were collected with a bee vacuum from the brood-nest of the same source colonies of each genotype that were used in the evaluations of grooming behavior at the colony level, and were taken inside the same room as the mites. Individual Petri dishes (9 cm diameter) were prepared in advance of the assays by lining their bottom with a circular piece of white filter paper to provide contrast, thereby facilitating observation of bees and mites. Additionally, Petri dishes were covered with plastic wrap. The plastic wrap was perforated 20–30 times with a nail (50 × 3 mm) in order to allow air to pass through. One bee was introduced into each dish and was then given 2–3 min to become accustomed to the Petri dish. The plastic wrap was then lifted slightly in order to place a single mite on the bee's thorax using a fine brush. A stopwatch was started immediately upon application of the mite, and the bee was observed for 3 min. Grooming instances exhibited by the bee were recorded and the test was discontinued in the event that a bee successfully removed the mite before 3 min had passed. In addition to the above, and only in the experiments conducted in Canada, instances specifically describing grooming intensity by the bees were recorded. "Light grooming" consisted of slow swipes, most often when the bee used just one leg or at most two. "Intense grooming" consisted of more vigorous, wiping and shaking. Most incidences of intense grooming involved the use of more than two legs. Whether or not the grooming was recorded as "light" or "intense" was left to the observer's judgement. However, there was only one observer in each experiment of paired genotypes, and therefore all incidences were judged by the same person. Grooming trials were performed with a total of 700 bees for Africanized and European genotypes (i.e., 350 bees tested from each genotype), 400 for Russian and Local strains, 320 for SL-1 and SH-1 genotypes, and 900 for SL-2 and SH-2 genotypes.

### 2.4. Statistical analyses

A paired comparison of "resistant" and "susceptible" genotypes for mite fall was done using Mann Whitney U tests, because the data were not normally distributed. Additionally, the logistic regression model was used to describe the relationship between the binary response for mite removal and the effect of genotype and colony source on that response; odds of mite removal were computed from this analysis. To find relationships between different components of grooming behavior at the colony and individual levels, data for mite fall, percentage of injured mites, mite removal, and intensity of grooming were subjected to a Spearman Rank Correlation analysis. Data from counts, such as number of injured mites, number of mites removed by individual bees, number of bees that groomed, and number of intense and light grooming episodes, were analyzed with contingency tables, and Chi square values were obtained. All statistical analyses were performed with

the R-Statistical Program (R Development Core Team, Auckland, New Zealand).

## 3. Results

### 3.1. Grooming behavior at the colony level

All four comparisons showed significant differences between genotypes in the number of mites dropped on sticky boards, as well as in the percentage of injured mites. Genotypes that are presumably more resistant to varroa mites (Africanized, Russian, SL-1 and SL-2) had significantly lower mite fall numbers and higher percentages of injured mites than genotypes that are presumably more susceptible to varroa mites (European, Local, SH-1 and SH-2; Table 1).

### 3.2. Grooming behavior at the individual level

Individual Africanized, Russian, SL-1, and SL-2 bees, removed significantly more mites from their bodies than bees from European, Local, SH-1, and SH-2 colonies, respectively (Table 2). The odds of mite removal were high and significant for all presumed resistant genotypes when compared with the presumed susceptible genotypes: Africanized bees were 7.8 times more likely to remove mites than European bees ( $P < 0.0001$ ), whereas bees from Russian, SL-1, and SL-2 genotypes, were 2.1, 2.1, and 9.5 times more likely to remove mites than bees from Local, SH-1, and SH-2 genotypes ( $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.0001$ , respectively). No significant effects of colony source were detected in the analysis ( $P > 0.05$ ).

### 3.3. Efficacy of mite removal at the individual level

Except for Russian bees, genotypes that are presumably more resistant to varroa mites (SL-1 and SL-2) had a significantly higher proportion of bees that groomed. Moreover, all "resistant" genotypes (including Russian bees), groomed at a faster pace than genotypes that are presumably more susceptible to varroa mites (Local, SH-1 and SH-2). Additionally, these two groups of bee genotypes also varied for the number of mites removed by intense grooming compared to light grooming, and all genotypes removed significantly more mites by intense grooming compared to light grooming (Table 3).

### 3.4. Relationship between colony mite infestation, mite damage, and mite removal

Correlations between colony mite infestation, mite damage, and mite removal were not significant for all genotypes, except for the second generation of genotypes selected for low and high mite population growth. The 18 colonies of these two genotypes showed the following significant correlations: mite fall and percentage of injured mites,  $r = -0.76$  ( $P < 0.0001$ ), percentage of injured mites and mites removed,  $r = 0.80$  ( $P < 0.0001$ ), number of bees performing intense grooming and mites removed,  $r = 0.90$  ( $P < 0.0001$ ).

## 4. Discussion

### 4.1. Grooming behavior at the colony level

Our results demonstrate genotypic variability for two traits that may be indirectly associated with grooming behavior at the colony level. Colonies of Africanized and Russian bees as well as those selected for low varroa mite population growth for two genera-

**Table 1**  
Average varroa mite infestation per colony measured as number of mites fallen per day on sticky boards ( $\pm$ SE) and percentage of mites injured collected from colonies of four pairs of honey bee genotypes that vary in their relative resistance and susceptibility to the varroa mite.

Genotype <sup>A</sup>	No. colonies	Mite fall <sup>B</sup>	No. mites	% Mites injured	Chi square <sup>C</sup>
Africanized	7	18.9 $\pm$ 7.3 <sup>a</sup>	663	26.2	29.42 <sup>***</sup>
European	7	45.0 $\pm$ 11.0 <sup>b</sup>	1523	16.3	
Russian	8	9.4 $\pm$ 1.1 <sup>a</sup>	472	30.3	6.65 <sup>**</sup>
Local	8	16.1 $\pm$ 1.8 <sup>b</sup>	857	23.8	
Selected Low-1	4	3.9 $\pm$ 0.3 <sup>a</sup>	45	26.7	5.65 <sup>*</sup>
Selected High-1	4	57.5 $\pm$ 9.7 <sup>b</sup>	690	13.8	
Selected Low-2	9	8.7 $\pm$ 2.5 <sup>a</sup>	79	59.5	99.06 <sup>***</sup>
Selected High-2	9	25.3 $\pm$ 1.8 <sup>b</sup>	228	7.0	

<sup>A</sup> Bee genotypes included Africanized bees from Mexico's high plateau, European bees of Italian origin, imported from Hawaii, USA, Russian bees that have been under selection for mite resistance, local, unselected bees, and selected bees from colonies showing low and high mite population growth in two different years (years 1 and 2).

<sup>B</sup> Different letters between genotypes within the same row indicate significant differences based on Mann Whitney U tests.

<sup>C</sup> Chi square value for proportion of injured mites between two genotypes within the same row.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.0001$ .

**Table 2**  
Varroa mite removal success in the laboratory (measured as percentage of mites removed in  $\leq 3$  min) of individual worker honey bees from colonies of four pairs of genotypes that vary in their relative resistance and susceptibility to the varroa mite.

Genotype <sup>a</sup>	No. bees	% Mite removed	Chi square <sup>b</sup>
Africanized	350	29.4	78.93 <sup>***</sup>
European	350	4.3	
Russian	200	15.0	4.82 <sup>*</sup>
Local	200	8.0	
Selected Low-1	160	28.7	7.20 <sup>**</sup>
Selected High-1	160	16.2	
Selected Low-2	450	34.9	122.33 <sup>***</sup>
Selected High-2	450	5.3	

<sup>a</sup> Bee genotypes included Africanized bees from Mexico's high plateau, European bees of Italian origin, imported from Hawaii, USA, Russian bees that have been under selection for mite resistance, local, unselected bees, and selected bees from colonies showing low and high mite population growth in two different years (years 1 and 2).

<sup>b</sup> Chi square value for proportion of mites removed by individual bees between two genotypes, within the same row.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.0001$ .

tions (SL-1 and SL-2), showed significantly lower rates of mite drop and significantly higher percentages of injured mites than Euro-

pean, Local, and selected colonies for high mite population growth (SH-1 and SH-2). The differences in mite fall and percentage of injured mites between presumably resistant and susceptible genotypes were significant and consistent in the four-paired groups of bees. Additionally, mite fall rates were negatively correlated with percentage of damaged mites, at least for the colonies of two of the genotypes tested (SL-2 and SH-2).

The negative relationship between mite infestation rates and mite damage between genotypes found in this study is supported by earlier research showing a similar relationship between mite infestation levels and the proportion of injured mites found on hive bottom boards (Büchler, 1993; Moosbeckhofer, 1997; Archavaleta-Velasco and Guzman-Novoa, 2001; Mondragon et al., 2005). Other studies have not found this correlation to be clear and significant (Lodensani et al., 2002; Locke and Fries, 2011), or have found that other factors, such as mite reproduction, better explain the variability in mite infestation levels between colonies (Ritter and de Jong, 1984; Rosenkranz and Engels, 1994; Harris and Harbo, 1999; Martin and Medina, 2004; Mondragon et al., 2005; Locke and Fries, 2011). Moreover, the validity of using the proportion of injured mites as a criterion to select bees for mite resistance has been questioned (Rosenkranz et al., 1997; Bienefeld et al., 1999), because a considerable number of injured mites found on hive bottom boards may not have been damaged by the bees. How-

**Table 3**  
Strategy of varroa mite removal in individual workers measured as percentage of bees grooming (%G), grooming at light pace (%GL) or intense pace (%GI), or not grooming (%NG) within 3 min, for three pairs of honey bee genotypes. Numbers in parentheses are numbers of mites removed.

Genotypes <sup>a</sup>	No. bees	%G	%GL	%GI	%NG	Chi-square <sup>b</sup>	Chi-square <sup>c</sup>	Chi-square <sup>d</sup>
Russian	200	69.5 (30)	40.0(6)	29.5 (24)	30.5 (0)	1.13ns	9.54 <sup>**</sup>	22.80 <sup>***</sup>
Local	200	64.5 (16)	48.0(2)	16.5 (14)	35.5 (0)			36.78 <sup>***</sup>
Selected low-1	160	76.2 (46)	23.1(3)	53.1 (43)	23.8 (0)	4.88 <sup>*</sup>	16.64 <sup>***</sup>	21.71 <sup>***</sup>
Selected high-1	160	65.0 (26)	34.4(2)	30.6 (24)	35.0 (0)			28.41 <sup>***</sup>
Selected low-2	450	96.0 (157)	57.1(38)	38.9 (119)	4.0 (0)	48.17 <sup>***</sup>	97.97 <sup>***</sup>	127.43 <sup>***</sup>
Selected high-2	450	81.3 (24)	70.9(7)	10.4 (17)	18.7 (0)			77.17 <sup>***</sup>
Totals	1620	79.7 (299)	52.1(58)	27.6 (241)	20.3 (0)			363.40 <sup>***</sup>

ns = not significant.

<sup>a</sup> Bee genotypes included Russian bees that have been under selection for mite resistance, local, unselected bees, and selected bees from colonies showing low and high mite population growth in two different years (years 1 and 2).

<sup>b</sup> Chi square value for proportion of bees that groomed when a varroa mite was placed on their bodies between two genotypes, within the same row.

<sup>c</sup> Chi square value for proportion of bees that groomed at intense pace when a varroa mite was placed on their bodies between two genotypes, within the same row.

<sup>d</sup> Chi square value for proportion of mites removed by intense grooming compared to light grooming for all bee genotypes.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.0001$ .

ever, in this study, we found consistent significant differences between paired genotypes for all four groups of presumably susceptible and resistant strains of honey bees for both, mite infestation rates, and percentage of injured mites. Furthermore, we found a highly significant and negative correlation between mite fall and proportion of injured mites in one of the paired groups ( $r = -0.76$ ;  $n = 18$ ;  $P < 0.0001$ ). The consistency of our results could have been a consequence of comparing genotypes of bees known for their relative resistance to the varroa mite (i.e., Africanized and Russian bees) or that have been selected for resistance against mites (SL-1 and SL-2), with genotypes of bees known for their relative susceptibility to *V. destructor* (i.e., European or Local, unselected bees) or that have been selected for low resistance to the mite (SH-1 and SH-2). Results could have probably been less consistent if only local, unselected bees had been used in this study.

The percentage of damaged mites found on the bottom boards of hives is a controversial measure of bees' grooming behavior (Boecking and Spivak, 1999). It can be inferred that a higher percentage indicates more grooming, as more mites are injured. This could be true, but mites may also be injured by hygienic bees when they remove infested brood (Rosenkranz et al., 1997), or may be damaged by predators or other factors (Szabo and Walker, 1995; Bienefeld et al., 1999). Nevertheless, there is enough evidence indicating that part of the damage found in fallen mites is indeed caused by the bees' grooming behavior (Büchler et al., 1992; Ruttner and Hänel, 1992; Büchler, 1993; Fries et al., 1996; Szabo et al., 1996; Rosenkranz et al., 1997). What it is still unknown is the relative proportion of damaged mites that result from the bees' active grooming behavior. Moreover, an accurate evaluation of grooming behavior at the colony level is further complicated because it is difficult to account for the proportion of mites groomed off by the bees that fall without any damage, because the bees dislodge them from their bodies using their legs, or because they are removed by rapid body movements performed by infested bees. Despite the above lack of knowledge and the problems associated with mite injury levels in regards to measuring grooming behavior, the findings of previous studies coupled with our results relating mite injury with low infestation rates of colonies, suggest that mite damage may be a more valid indicator of grooming behavior than previously thought, particularly in the light of new evidence linking injured mites with actual grooming behavior in laboratory tests.

To establish a relationship between data on proportion of injured mites and grooming behavior, we used bees from the same colonies that were assessed for mite damage, to test them individually for their ability to remove mites in a controlled setting. If the genotypes showing higher percentages of injured mites also showed higher rates of mite removal at the individual level, then there would be more evidence to support the claim that measuring rates of injured mites is a relatively reliable way of assessing grooming behavior at the colony level (see below).

#### 4.2. Grooming behavior at the individual level

Results showed that the bee genotypes with lower mite infestation levels and higher proportions of injured mites in their colonies were also the ones that removed a higher percentage of mites in individual grooming tests. Bees from Africanized, Russian, SL-1, and SL-2 genotypes, successfully groomed off significantly more mites placed on their bodies (two to seven times more) than bees from European, Local, SH-1, and SH-2 genotypes, respectively. This is the first study showing consistent evidence relating colonies of different honey bee genotypes with high and low levels of varroa mite damage, with rates of successful mite removal of individual bees obtained from the same colonies. These results strongly suggest that the percentage of injured mites is a good indicator of grooming behavior at the colony level. The only other study that

has found a significant correlation between proportions of damaged mites at the colony level and number of mites removed from the bees' bodies in a laboratory assay is the study by Andino and Hunt (2011). The difference between our assay and that of Andino and Hunt (2011) is that they used groups of bees instead of individual workers as in our case, and did not actually see when the bees removed the mites from their bodies.

Other studies have previously demonstrated genotypic variability for grooming behavior against varroa mites at the individual level. For example, Aumeier (2001) and Vandame et al. (2002) deposited mites on the thoraxes of bees of two genotypes and observed them for auto-grooming response. They found variation between the strains tested for percentage of bees that groomed and time to groom, but did not evaluate mite removal rates as in our study.

#### 4.3. Efficacy of mite removal at the individual level

It is clear from the results shown in Table 3, that the proportion of bees that groomed, as well as the intensity of grooming, have a significant effect on the rate of successful mite removal. Again, a higher percentage of bee genotypes most successful at removing mites from their bodies, groomed at a faster pace than did bees of the least successful genotypes. Conversely, a higher percentage of bees of the least successful genotypes groomed at a slower pace. Furthermore, it was found that a significantly higher number of mites were removed by bees performing intense grooming episodes than by bees performing light grooming episodes, regardless of their genotype. Aumeier (2000) also distinguished between "weak" and "intense" grooming, but did not relate this grooming intensity to successful mite removal. This is the first study in which it is clearly demonstrated that intense grooming is significantly more effective at removing mites than light grooming. This result appears to hold true regardless of the strain of honey bee.

#### 4.4. Relationship between colony mite infestation, mite damage, and mite removal

Correlations for these variables were found only for the SL-2 and SH-2 genotypes, but not for the other groups of bee strains. The lack of significant correlation for these traits in three of the paired genotypes compared could be explained in part by the relatively low number of colonies analyzed for the first three pairs of genotypes. In fact, the non-significant correlations in the three comparisons were suggestive of effects that may have been stronger with larger sample sizes. It is also possible that other resistance factors that were not measured may have been restraining mite population growth in "resistant" genotypes. Conversely, the high correlations observed in the second generation of genotypes selected for high and low varroa mite population growth could be at least partially attributed to a further divergence in the expression of grooming behavior between the two groups of colonies as a consequence of selection. This is the first study that reports evidence linking mite infestation at the colony level, mite injury, mite removal, and intensity of grooming behavior. These results suggest that grooming behavior and its associated traits may be more clearly expressed and interrelated after selection, indicating a genetic basis for this behavior.

Overall, results of this study show that bees from colonies with lower mite population rates and higher percentage of damaged mites groomed with more bees (except for the Russian genotype), performed more instances of intense grooming, and intense grooming resulted in dislodging significantly more mites than light grooming in all genotypes tested. Regardless of which bee strain is judged the best groomer, the results of this study clearly show that there is genotypic variability among strains for grooming behavior,

and that several components of this behavior, and their interactions, may result in the successful removal of varroa mites.

#### 4.5. Genetic basis of grooming behavior

Grooming behavior appears to be a quantitative trait that is influenced by genetic effects (Hoffman, 1993; Moretto et al., 1993), but this behavior is also affected by environmental factors such as humidity and temperature (Currie and Tahmasbi, 2008), and may show seasonal variation (Büchler, 1993; Moosbeckhofer, 1997). The degree to which grooming behavior is affected by genetic effects is still not well known, and results of experiments are contradictory. For example, Moretto et al. (1993) calculated an  $h^2$  value of 0.71 for this behavior; however, later studies found low  $h^2$  values for this trait (Harbo and Harris, 1999; Lodensani et al., 2002; Stanimirovic et al., 2010). It is possible that these contradictory results are a consequence of differences in the accuracy with which different assays assess grooming behavior. No assay for measuring grooming behavior is universally agreed upon as accurate. This further adds to the difficulty of learning how effective grooming behavior is at conferring mite resistance to the bees, its genetic basis, and its mode of inheritance. Therefore it is important that future research focuses on standardizing methods to accurately quantify grooming behavior.

The potential selectability of grooming behavior, supported by the results of this study, implies that increased expression of this trait could evolve in feral populations of honey bees or could be achieved through artificial selection. Furthermore, the results of the grooming behavior evaluations conducted in this study, suggest that honey bee populations evolving in response to varroa infestations would also tend towards more intense autogrooming. If the variability in resistance to the varroa mite is partially due to grooming behavior and if this behavior is influenced by additive genetic effects, then selection for grooming behavior should give breeders a means of developing resistant bees and would provide researchers with insight on how this behavior evolved in the genus *Apis*.

The results of this study strongly suggest that grooming behavior is an important component in the resistance of some honey bee genotypes (like the ones tested) to the growth of varroa mite populations, whereas other studies (above cited) have found that other factors, such as the inhibition of mite reproduction, could be more accountable for the observed variability in mite resistance among different populations of bees. It could be that different genotypes of honey bees in different geographical locations adopt different strategies for mite control. However, the question of why different factors seem to have different weights in the resistance of honey bees to varroa mites in different populations and in different locations, is something that remains to be investigated.

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#### References

Andino, G.K., Hunt, G.J., 2011. A scientific note on a new assay to measure honeybee mite-grooming behavior. *Apidologie* 42, 481–484.  
 Arechavaleta-Velasco, M.E., Guzman-Novoa, E., 2001. Relative effect of four characteristics that restrain the population growth of the mite *Varroa destructor* in honey bee (*Apis mellifera*) colonies. *Apidologie* 32, 157–174.

Aumeier, P., 2000. Grooming as a tolerance factor against *Varroa jacobsoni*: a critical assessment on Africanized bees. *Apidologie* 31, 633–634.  
 Aumeier, P., 2001. Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized and Carniolan honey bees. *Apidologie* 32, 81–90.  
 Bienefeld, K., Zautke, F., Pronin, D., Mazed, A., 1999. Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*). *Apidologie* 30, 249–256.  
 Boecking, O., Rath, W., Drescher, W., 1993. Grooming and removal behavior – strategies of *Apis mellifera* and *Apis cerana* bees against *Varroa jacobsoni*. *Amer. Bee J.* 133, 117–119.  
 Boecking, O., Spivak, M., 1999. Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. *Apidologie* 30, 141–158.  
 Büchler, R., 1993. Rate of damaged mites in natural mite fall with regard to seasonal effects and infestation development. *Apidologie* 24, 492–493.  
 Büchler, R., Drescher, W., Tornier, I., 1992. Grooming behaviour of *Apis cerana*, *Apis mellifera* and *Apis dorsata* and its effect on the parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae*. *Exp. Appl. Acarol.* 16, 313–319.  
 Currie, R.W., Tahmasbi, G.H., 2008. The ability of high- and low-grooming lines of honey bees to remove the parasitic mite *Varroa destructor* is affected by environmental conditions. *Can. J. Zool.* 86, 1059–1067.  
 Danka, R.G., Villa, J.D., 2003. Autogrooming by resistant honey bees challenged with individual tracheal mites. *Apidologie* 34, 591–596.  
 Fries, I., Huazhen, W., Wei, S., Jin, C.S., 1996. Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. *Apidologie* 27, 3–11.  
 Guzman-Novoa, E., Vandame, R., Arechavaleta-Velasco, M.E., 1999. Susceptibility of European and Africanized honey bees (*Apis mellifera* L.) to *Varroa jacobsoni* Oud. in Mexico. *Apidologie* 30, 173–182.  
 Guzman-Novoa, E., Vandame, R., Arechavaleta-Velasco, M.E., 2002. Tolerance to *Varroa jacobsoni* Oud. of honey bee (*Apis mellifera* L.) colonies in Mexico. In: Erickson, E., Page, R.E., Hanna, A.A. (Eds.), Proceedings of the 2nd Intern. Conf. Africanized Honey Bees & Bee Mites. Root, Medina, OH, pp. 80–85.  
 Guzman-Novoa, E., Eccles, L., Calvete, Y., McGowan, J., Kelly, P.G., Correa-Benítez, A., 2010. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* 41, 443–450.  
 Harris, J.W., Harbo, J.R., 1999. Low sperm counts and reduced fecundity of mites in colonies of honey bee (Hymenoptera: Apidae) resistant to *Varroa jacobsoni* (Mesostigmata: Varroidae). *J. Apicult. Res.* 92, 83–90.  
 Harbo, J.R., Harris, J.W., 1999. Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). *J. Econ. Entomol.* 92, 261–265.  
 Hoffman, S., 1993. The occurrence of damaged mites in cage tests and under field conditions in hybrids of different Carniolan lines. *Apidologie* 24, 493–495.  
 Land, B.B., Seeley, T.D., 2004. The grooming invitation dance of the honey bee. *Ethol* 110, 1–10.  
 Le Conte, Y., Ellis, M., Ritter, W., 2010. Varroa mites and honey bee health: can Varroa explain part of the colony losses? *Apidologie* 41, 353–363.  
 Locke, B., Fries, I., 2011. Characteristics of honey bee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation. *Apidologie* 42, 533–542.  
 Lodensani, M., Crailsheim, K., Moritz, R.F.A., 2002. Effect of some characters on the population growth of mite *Varroa jacobsoni* in *Apis mellifera* L. colonies and results of a bi-directional selection. *J. Appl. Entomol.* 126, 130–137.  
 Martin, S.J., Medina, L.M., 2004. Africanized honeybees have unique tolerance to *Varroa* mites. *Trends Parasitol.* 20, 112–114.  
 Milani, N., 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides. *Apidologie* 30, 229–234.  
 Mondragon, L., Spivak, M., Vandame, R., 2005. A multifactorial study of the resistance of honeybees *Apis mellifera* to the mite *Varroa destructor* over one year in Mexico. *Apidologie* 36, 345–358.  
 Mondragon, L., Martin, S., Vandame, R., 2006. Mortality of mite offspring: a major component of *Varroa destructor* resistance in a population of Africanized bees. *Apidologie* 37, 67–74.  
 Moosbeckhofer, R., 1997. Observations on reproduction rate of *Varroa jacobsoni* and the occurrence of mutilated mites in *Apis mellifera carnica* colonies. *Apidologie* 28, 193–195.  
 Moretto, G., Gonçalves, L.S., de Jong, D., 1993. Heritability of Africanized and European honey bee defensive behavior against the mite *Varroa jacobsoni*. *Rev. Brasil. Genet.* 16, 71–77.  
 Nielsen, D.I., Ebert, P.R., Hunt, G.J., Guzman-Novoa, E., Kinee, S.A., Page, R.E., 1999. Identification of Africanized honey bees (Hymenoptera: Apidae) incorporating morphometrics and an improved PCR genotyping procedure. *Ann. Entomol. Soc. Amer.* 92, 167–174.  
 Peng, Y., 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. *J. Invertebr. Pathol.* 49, 54–60.  
 Pettis, J.S., Pankiw, T., 1998. Grooming behaviour by *Apis mellifera* L. in the presence of *Acarapis woodi* (Rennie) (Acari: Tarsonemidae). *Apidologie* 29, 241–253.  
 Rinderer, T.E., de Guzman, L.I., Delatte, G.T., Stelzer, J.A., Lancaster, V.A., Kuznetsov, V., Beaman, L., Watts, R., Harris, J.W., 2001. Resistance to the parasitic mite *Varroa destructor* in honey bees from far-eastern Russia. *Apidologie* 32, 381–394.  
 Ritter, W., de Jong, D., 1984. Reproduction of *Varroa jacobsoni* O. in Europe, the Middle East and tropical South America. *Z. Angew. Entomol.* 98, 55–57.  
 Rosenkranz, P., Engels, W., 1994. Infertility of *Varroa jacobsoni* females after invasion into *Apis mellifera* worker brood as tolerance factor against varroaosis. *Apidologie* 25, 402–411.

- Rosenkranz, P., Fries, I., Boecking, O., Stuermer, M., 1997. Damaged Varroa mites in the debris of honey bee (*Apis mellifera* L.) colonies with and without hatching brood. *Apidologie* 28, 427–437.
- Ruttner, F., Hänel, H., 1992. Active defence against Varroa mites in a Carniolan strain of honeybee (*Apis mellifera carnica* Pollmann). *Apidologie* 23, 173–187.
- Sanford, M.T., 2001. Introduction, spread and economic impact of Varroa mites in North America. In: Webster, T.C., Delaplane, K.S. (Eds.), *Mites of the Honey Bee*. Dadant & Sons, Mansfield, OH, pp. 149–162.
- Stanimirovic, Z., Jevrosima, S., Nevenka, A., 2010. Heritability of grooming behaviour in grey honey bees (*Apis mellifera carnica*). *Acta Veterinaria* 60, 313–323.
- Stankus, T., 2008. A review and bibliography of the literature of honey bee Colony Collapse Disorder: a poorly understood epidemic that clearly threatens the successful pollination of billions of dollars of crops in America. *J. Agricult. Food Info.* 9, 115–143.
- Szabo, T.I., Walker, C.R.T., 1995. Damages of dead *Varroa jacobsoni* caused by the larvae of *Galleria mellonella*. *Amer. Bee J.* 135, 421–422.
- Szabo, T.I., Walker, C.R.T., Mueller, A., 1996. Grooming behavior as a *Varroa* resistance characteristic in honey bee colonies. *Amer. Bee J.* 136, 515–517.
- Vandame, R., Morand, S., Colin, M.E., Belzunces, L.P., 2002. Parasitism in the social bee *Apis mellifera*: quantifying costs and benefits of behavioral resistance to *Varroa destructor* mites. *Apidologie* 33, 433–445.
- Villa, J.D., 2006. Autogrooming and bee age influence migration of tracheal mites to Russian and susceptible worker honey bees (*Apis mellifera* L.). *J. Apicult. Res.* 45, 28–31.