Associations of Parameters Related to the Fall of *Varroa destructor* (Mesostigmata: Varroidae) in Russian and Italian Honey Bee (Hymenoptera: Apidae) Colonies

THOMAS E. RINDERER,[1] LILIA I. DE GUZMAN, AND AMANDA M. FRAKE

USDA–ARS, Honey Bee Breeding, Genetics and Physiology Laboratory,
1157 Ben Hur Road, Baton Rouge, LA 70820-5502

J. Econ. Entomol. 106(2): 566–575 (2013); DOI: http://dx.doi.org/10.1603/EC12427

ABSTRACT *Varroa destructor* (Anderson and Truman) trapped on bottom boards were assessed as indirect measurements of colony mite populations and mite fall in colonies of Russian and Italian honey bees using 29 candidate measurements. Measurements included damaged and nondamaged younger mites, damaged and nondamaged older mites, fresh mites and all mites, each as a proportion of total mites in the colonies and as a proportion of all trapped mites or all trapped fresh mites. Regression analyses were used to determine the relationships of these candidate measurements to the number of mites in the colonies. The largest positive regressions were found for trapped younger mites (Y) and trapped fresh mites (F). Measurments of Y and F across time could be used to estimate mite population growth for the purposes of selective breeding. The largest negative regressions with colony mites were observed for: trapped older mites/trapped mites (O/T), trapped older mites/trapped younger mites (O/Y), and trapped injured older mites/injured mites (IO/I). O/T and O/Y are significantly higher for Russian honey bee colonies suggesting that they are related to at least some of the mechanisms used by Russian honey bee to resist *Varroa* population growth. O/T and O/Y have strong negative relationships with colony mites for both Russian honey bee and Italian colonies suggesting that both strains possibly could be selected for reduced colony mites using O/T or O/Y.

KEY WORDS *Varroa destructor*, Russian honey bee, mite fall, grooming behavior, *Varroa* resistance

Breeding honey bees (*Apis mellifera* L.) that are resistant to *Varroa destructor* Anderson and Trueman has used two approaches. Selection based on colonies having lower population growths of infesting *V. destructor* has produced honey bees that are used with minimal acaricide input - Russian honey bees (Rinderer et al. 2001, 2003; de Guzman et al. 2007), and honey bees bred in France (Kefuss et al. 2004, Büchler et al. 2010). The mechanisms underpinning the resistance of Russian honey bee have been mostly identified (Rinderer et al. 2001, de Guzman et al. 2007) but measuring them is time consuming and they have not been used in the selection of the stock. Selecting for a single resistance trait has been used in two programs. Selecting for general hygiene has produced Minnesota Hygienic honey bees that have a moderate resistance to *V. destructor* (Boecking and Spivak 1999, Spivak and Reuter 2001, Ibrahim et al. 2007). Selecting for *Varroa* sensitive hygiene (VSH) has produced VSH honey bees that have strong resistance to *V. destructor* (Harbo and Harris 2005). Selection for additional resistance traits would provide opportunities to produce stocks with an expanded basis for resistance.

Honey bees are thought to have two prominent mechanisms of resistance; hygienic behavior and grooming behavior (Boecking and Spivak 1999). However, while extensive research with hygiene has resulted in the successful development of *Varroa* resistant strains this has not been the case for grooming behavior. One breeding program selecting for increased grooming in Europe used the proportion of damaged mites as its selection criterion but was discontinued owing to a low correlation ($r = 0.27$) between the proportion of damaged mites and colony mite populations. Additionally, the proportion of damaged mites had a low heritability (<0.15), and data collection required laborious sample collection and processing (Büchler 2000, Ehrhardt et al. 2007, Büchler et al. 2010).

Nonetheless, grooming has been extensively studied in *A. mellifera* because these honey bees do remove *Varroa* mites using grooming (Boecking and Ritter 1993, Thakur et al. 1997, Aumeier 2001, Guzman–Novoa et al. 2012) and grooming is thought to be an important resistance mechanism for *A. cerana* (Peng et al. 1987). Injuries to mites are the clearest evidence of grooming. Hence, many of the studies (Table 1) have evaluated proportions of damaged mites in trapped mites. Most of the studies have compared the proportion of damaged mites found for

[1] Corresponding author, e-mail: tom.rinderer@ars.usda.gov.

Table 1. Measurements of grooming behavior and mite fall used by several researchers in studies conducted between 1987 and 2011

Authors	Methods involved/duration	Measurement of grooming
Peng et al. 1987	Observation hives, responses to inoculated mites up to 2 h.	Bees' responses, proportion of fallen mites and injured mites.
Ruttner and Hänel 1992	Full size colonies, daily mite fall for 8 mo.	Proportion of injured mites out of mites that fell.
Büchler et al. 1992	Observation hives and colony of *A. dorsata,* responses to inoculated mites within 15, 60, and 300 s.	Frequency of grooming, removal success.
Moosbeckhofer 1992	Full size colonies, mite fall, three observations within 1 mo.	Relationship between the proportion of injured mites and bee and brood infestations. Light vs dark colored mites differentiated.
Boecking and Ritter 1993	Full size colonies, mite fall at 2–3 h interval for 9 h.	Proportion of live, dead, and injured mites out of mites that fell. Pigmented vs less pigmented mites differentiated.
Eguaras et al. 1995	Full size colonies, 15-d mite fall	Daily mite mortality, proportion of damaged mites.
Fries et al. 1996	Full size colonies, daily mite fall for 1 mo, removal of inoculated mites after 15 min, 30 min, 1, 2, 3, 4, 5, and 6 h.	Total mites that fell, and proportion of injured mites out of mites that fell.
	Observation hives, behavior of bees after mite inoculation, mite removal after 1 h.	Behavior of tagged bees, total mites that fell, proportion of injured mites out of fell mites.
Lodesani et al. 1996	Full size colonies, daily mite fall for 9 d.	Proportion of injured mites out of mites that fell. Dark vs light-colored mites differentiated.
Szabo et al. 1995	Cage bioassay, mite removal after 24, 48, 72, and 192 h.	Mite removal, injuries.
Rosenkranz et al. 1997	Full size colonies, mite fall at 12-h interval (2–7 d).	Proportion of live, dead, and injured mites out of mites that fell. Dark and lightly-colored adult mites differentiated.
Thakur et al. 1997	One-frame cage, removal of inoculated mites,	Bees' behavior towards mites.
Bienefeld et al. 1999	Full size colonies, mite fall at 12, 24, 48, 72, and 168 h.	Proportion of injured mites out of 200 subsampled mites. Relationship between the percentage damaged (immature and mature) and adult mites per colony.
Corrêa–Marques et al. 2000	Full size colonies, mite fall at 24 h interval, repeated 14×.	Proportion of live, dead, and injured mites out of mites that fell. Mature and young mites differentiated.
Webster et al. 2000	Full size colonies, weekly mite fall for nine observations.	Total mite fall, proportion of live mites that fell. Callow vs dark mites differentiated.
Aumeier 2001	Petri dish bioassay, behavior of bees towards inoculated mites, mite removal.	Bees' responses and injuries of mites.
Rinderer et al. 2001	Full size colonies, weekly fall every month for 15 mo.	Proportion of injured mites out mites that fell.
Zaitoun et al. 2001	Full size colonies, mite fall every 2 d for 5 mo.	Proportion of injured mites that fell. Pigmented vs less pigmented mites differentiated.
Al-Ghzawi et al. 2001	Full size colonies, mite fall every 2 d for 3 mo.	Proportion of injured mites that fell. Pigmented vs less pigmented mites differentiated.
Arechavaleta–Velasco and Guzman–Novoa 2001	Jumbo-size colonies, mite fall every week for 5 wk.	Relationships between final infestation levels and the no. of fell mites, injured (subsample) mites and no. of mites recovered in the lab bioassay.
	Cage bioassay, removal of inoculated mites every 12 h.	Number of recovered and injured mites.
Corrêa–Marques et al. 2002	Full size colonies, mite fall every 24 h for ≈ 1 mo.	Frequency of injured mites, types of injuries (n = 100 mites/colony). Lightly colored and alive mites were recorded.
Vandame et al. 2002	Single-frame observation hives, bees' behavior within 8 min.	Bees' behavior towards marked mites, and mite removal.
	Full size colonies, mite fall every 2 mo (three observations).	Proportion of injured mites out of 150 subsampled mites.
Mondragón et al. 2005	Full size colonies, monthly 48 h-mite fall (10 observations).	Relationship between the proportion of injured mites out of the subsampled mites and total no. of mites in colonies in the succeeding sampling period.
Currie and Tahmasbi 2008	Cage bioassay, removal of inoculated mites after 2, 4, 6 d.	Mean daily proportion of dead mites that fell (live mites that fell were returned into the cage).
	Full size colonies, mite fall after 3 d.	Mean daily mite mortality.
Andino and Hunt 2011	One-frame bioassay, full size colonies, mite fall after 72 h.	Relationship between the proportion of mites removed (lab bioassay) and the proportion of injured mites out trapped mites (field colonies).
Ardestani et al. 2011	Cage bioassay, mite fall of inoculated mites after 24 h.	Proportion of injured mites.

different species or strains of honey bees (Moosbeckhofer 1992, Ruttner and Hänel 1992, Boecking and Ritter 1993, Eguaras et al. 1995, Fries et al. 1996, Lodesani et al. 1996, Rosenkranz et al. 1997, Bienefeld et al. 1999, Correa–Marques et al. 2000, Webster et al. 2000, Rinderer et al. 2001, Zaitoun et al. 2001, Al-Ghzawi et al. 2001, Arechavaleta–Velasco and Guzman–Novoa 2001, Vandame et al. 2002, Mondragon et al. 2005).

Fewer studies have related the proportion of damaged mites to populations of mites in colonies. Studies of *A. mellilfera carnica* in Germany have found negative correlations between the proportion of damaged mites and colony mites of $r^2 = 0.69$ (Moosbeckhofer 1992), $r^2 = 0.11$ (Hoffmann 1995), and $r^2 = 0.07$ (Ehrhardt et al. 2007). Studies in Mexico, primarily with Africanized honey bees, have found stronger

negative correlations: $r^2 = 0.51$ (Mondragón et al. 2005) and $r^2 = 0.54$ (Arechavaleta–Velasco and Guzman–Novoa 2001). However, Vandame et al. (2002), studying both full sized colonies and colonies in observation hives, concluded that it seems very unlikely that grooming behavior may explain the lowered levels of *V. destructor* in Africanized honey bee colonies. Guzman–Nova et al. (2012), using four paired studies (Russian honey bee and an unselected stock, Africanized honey bee from Mexico and Italian honey bees from Hawaii, an F_1 of stocks selected for lower and higher mite population growth [MPG] and an F_2 of stocks selected for lower and higher MPG) reported nonsignificant correlations between colony mite infestation as measured by mite fall and damaged mites in all the paired studies except for a strong negative correlation ($r^2 = 0.58$) in the study of F_2 low MPG versus F_2 high MPG.

These varied results in the assessment of the ability of grooming to reduce colony mite populations may arise from several sources. Certainly, there may be a difference between European honey bee and Africanized honey bee (Moretto et al. 1991). In addition, it is difficult to assess damage to mites (Bienefeld et al. 1999, Ruttner and Hänel 1992). Apparent damage may result from predatory insects (Szabo and Walker 1995, Davis et al. 2007), temperature and humidity (Currie and Tahmasbi 2008, Tahmasbi 2009), developmental problems (Davis 2009, Ardestani et al. 2011) and storage and handling (Arechavaleta–Velasco and Guzman–Novoa 2001, Vandame et al. 2002). Further, Bienefeld et al. (1999) suggested that young daughter mites be excluded in the assessment of grooming behavior because they have higher rates of damage (Moosbeckhofer 1992, Lodesani et al. 1996, Al-Ghzawi et al. 2001) and are more likely to have "damages by chance." In addition, grooming may cause damage that is not externally apparent (Büchler et al. 1992). Mites removed by grooming may be unharmed but fall from the nest (Boecking and Ritter 1993, Lodesani et al. 1996) and some may be unable to return.

The majority of mites trapped on bottom boards are not apparently injured (Boecking and Ritter 1993, Lodesani et al. 1996). Many of these mites may have fallen as a result of grooming although certainly many also result from house cleaning of naturally dead mites. Cage studies suggest a relationship between trapped mites (damaged and undamaged) and colony mites. In contrast to their results with field colonies, Arechavaleta–Velasco and Guzman–Novoa (2001) did not find a strong relationship between injured mites in cages of bees and infestation levels in parent colonies ($r^2 = 0.06$). However, they did find a significant relationship ($r^2 = 0.22$) when using the number of mites (injured and noninjured) recovered in cages and the infestations in parent colonies. Additionally, Andino and Hunt (2011) found a negative relationship between mites that fell from bees in cage assays and the mite infestations on adult bees in parent colonies ($r^2 = 0.37$). They also found a significant positive relationship ($r^2 = 0.23$) between the mites that fell from bees in cage assays and the percentage of damaged mites the fell from the parent colonies.

This wide array of studies does not comprehensively evaluate a variety of potential relationships of measurements of fallen mites with numbers of mites infesting colonies. In addition, some of these studies have sought to relate measures of grooming to populations of phoretic mites rather than the overall levels of mites in colonies. These uncertainties about measuring grooming behavior and differing estimates of the relationship of grooming to mite populations in colonies may have led to the perception that European *A. mellifera* rarely exhibits grooming behavior that impacts mite population growth. Hence, increased grooming intensity is not well-recognized as either an important mechanism of resistance to *Varroa* mites or as a useful goal in honey bee breeding (Boecking and Spivak 1999, Büchler et al. 2010).

However, strains of European *A. mellifera* differ in their degree of *V. destructor* infestation and how their resistance to this ectoparasite is expressed. There are several attributes of honey bees in addition to hygiene that contribute to the regulation of *Varroa* mite populations (reviewed by Rinderer et al. 2010). An extended phoretic period for *Varroa* mites negatively influences mite growth in Russian honey bees (Rinderer et al. 2001, de Guzman et al. 2007). Russian honey bee colonies consistently supported higher proportions of phoretic mites than Italian colonies in several studies (Rinderer et al. 2001, de Guzman et al. 2007). Prolonged phoresy reduces the mites' reproductive potential and also exposes *Varroa* mites to increased risks worker bee grooming (Rinderer et al. 2001, de Guzman et al. 2007). In addition, Mondragón et al. (2005) found a strong negative relationship ($r^2 = 0.73$) between mite fertility and mite infestation levels. Reduced mite fertility was identified as a Russian honey bee resistance mechanism (de Guzman et al. 2007, 2008). Perhaps some indications of mite fertility can be found among trapped mites.

Comparing measures of trapped mites in known resistant and susceptible strains may lead to a method of comparing levels of suppression of mite populations infesting colonies by indirectly assessing grooming in combination with other resistance traits, which could be effectively used in a breeding program. Accordingly, we investigated the differences between resistant Russian honey bees and susceptible Italian honey bees using an expanded list of candidate measurements related to trapped mites and total mites in colonies. We compared these candidate measurements both between Russian and Italian stocks and through time using regression analysis to determine their relationship to total colony mites.

Materials and Methods

Colony Setup. Thirty-six colonies were established in April by equally dividing a colony (three brood frames, two honey/pollen frames, and five empty frames) into two or four divisions. Divisions were stacked on the parent colony overnight to allow equal

distribution of adult bees among the divisions. The following day, divisions were labeled according to their origin (parent colony), and provided with a bottom board and a hive cover. All divisions were then moved to a holding location and allowed to settle overnight before queens were introduced. From each parent colony, half of the colony divisions (i.e., one or two) randomly received Italian queens (purchased from a queen breeder in California that advertised Italian queens) and the other half received Russian queens (from the Russian honey bee program at our laboratory). This technique provided colonies presumably having similar levels of *Varroa* infestation (2.42 ± 0.56 mites per 100 adult bees) at the beginning of the experiment for both honey bee stocks. Each colony was provided with two bottom boards with opposing entrances (a screen bottom served as the bees' entrance and a solid bottom received the *Varroa* traps). Each colony was sitting on a block at the middle of a tray of soapy water to exclude scavenging insects such as ants from the *Varroa* trap.

Measurement of *Varroa* Mite Populations. Estimates of the total number of mites in the colonies were derived from counts of mites in 200 brood cells (using two brood frames), mites from adult bee washes (≈300–500 bees), and comb by comb estimates of the number of sealed brood and number of adult bees (Rinderer et al. 2001, de Guzman et al. 2007). Colony mite counts were recorded in June, August, and October 2007. Trapped mites were not collected daily and therefore not included in these estimates.

Measurement of Trapped Mites. Trapped mites were assessed for two consecutive weeks (3–4 consecutive days per week) every month from June to October 2007. To collect mites, a cafeteria tray with petrolatum-coated paper on top was inserted between the two bottom boards. Traps were replaced every day. *Varroa* mites were immediately collected from the traps using an insect brush and examined under a dissecting microscope for age (light ochre = younger, darker color = older), injury status (injured or not injured), and recency of death (fresh or dry). Mites were considered injured when parts of the gnathosoma (mouthparts), legs, and ventral shields (sternal, exopodal, lateral, genitoventral, pleural, and anal shields) were missing or damaged. Mites with dented idiosoma were not categorized as injured. Recency of death was indicated by the presence of hemolymph and fresh tissues when mites were poked or teased apart with an insect pin. The proportion of trapped mites in each category (older, younger, injured, and fresh) for each colony was calculated as the total number of categorized mites divided by the total number of colony mites or the total number of mites trapped.

Data Analyses. Data on the number of mites in the colony (C), phoretic mites (P), mites in the brood (B), total trapped mites (T), trapped older adult mites (O), trapped younger adult mites (Y), O/T, O/C, O/Y, Y/T, Y/C, trapped injured mites (I), I/T, I/C, trapped fresh (young and old adults) mites (F), F/T, F/C, injured fresh mites (IF), IF/all fresh mites (F), IF/T, IF/C, injured younger mites (IY), IY/Y, IY/I, IY/T, IY/C, injured older mites (IO), IO/O, IO/I, IO/T, and IO/C were analyzed. Each variable was first subjected to analysis of variance (ANOVA) for repeated measures with honey bee stock and observation time as fixed effects, and colony as the repeated subject. Where a significant interaction between honey bee stock and time of observation was detected, means were separated with a post hoc "slice test" (*t*-tests controlled for error within the ANOVA) (SAS Institute 2008) to determine differences by stock and by time of observation. To determine which mite category was best related to lower colony mites, a simple linear regression was performed for each variable that was not partially a measure of total colony mites (C) with C as the dependent variable. Before analyses, data on actual mite counts (C, T, O, Y, I, F, IF, IO, and IY) and O/Y were transformed with a square-root transformation and data on proportions (T/C, O/T, O/C, Y/T, Y/C, I/T, I/C, F/T, F/C, IF/F, IF/T, IO/O, IO/T, IO/C, IY/Y, IY/T, and IY/C) were transformed with an arcsine square-root transformation to better approximate normality (SAS Institute 2008). Before analyses data were examined for anomalies and any outliers (>mean ± 3 SD) were deleted.

Results

Several differences were observed between *Varroa* resistant Russian honey bees and *Varroa* susceptible Italian honey bees. Overall, Russian honey bee colonies averaged 57% fewer mites than Italian colonies ($P = 0.0003$; Table 2). Several measurements of trapped mites reflected this difference: T/C, O/T, O/C, O/Y, Y/C, I/C, F/C, IF/F, IF/T, IF/C, IO/C, and IY/C all had significantly higher values for Russian honey bee colonies (Table 2). Italian colonies had significantly more younger mites among the total trapped mites along with also having significantly fewer older mites among the total trapped mites.

For every measurement except O, IY/Y, and IY/C, a significant difference was associated with month of observation. However, the direction of the differences varied. O/T, O/C, and O/Y consistently declined through time (Table 2). Y increased through time ($P = 0.0013$; Table 2). Other measurements related to injury (I, I/T, IF, IF/F, IF/T, IF/C, IO, IO/O, IY, IY/I, and IY/T) tended to rise through time (Table 2).

Out of the 21 measurements submitted to regression analysis, 15 were positively related with colony mites (C) (Table 3; Fig 1). Of them, Y ($r = 0.79$; $P < 0.0001$), F ($r = 0.71$; $P < 0.0001$), T ($r = 0.68$; $P < 0.0001$), and IY ($r = 0.68$; $P < 0.0001$) rank the highest. There were positive regressions between IY/T and C ($r = 0.36$; $P = 0.0005$) and IY/I and C ($r = 0.41$; $P = 0.0001$). All absolute measures of trapped mites grew in numbers along with colony mites. In addition, ratios of Y/T, IF/F, IF/T, and F/T, have positive regressions with C.

Two measurements had significant negative regressions with colony mites (Table 3; Fig. 1). Both involved older mites disregarding injuries: O/T ($r = -0.58$) and O/Y ($r = -0.51$) (Table 3; Fig. 1).

Table 2. Means (±SE) for the 29 candidate measurements of mite fall for Italian and Russian honey bee colonies and through time, and results of the analyses of variance (ANOVA)

Measurement	Italian	Russian	June	July	Aug.	Sept.	Oct.	Analysis
Colony mites (C)	3,969 ± 639[a]	1,714 ± 298[b]	1,234 ± 339[c]		2,966 ± 592[b]		4,790 ± 893[a]	S: $F = 14.64$, $P = 0.0003$ T: $F = 14.48$, $P < 0.0001$ S × T: $F = 1.52$, $P = 0.2243$
Phoretic mites/colony mites (P/C)	19.8 ± 2.2%[b]	30.0 ± 4.1%[a]	31.7 ± 5.8%[a]		19.2 ± 2.0%[b]		22.5 ± 2.7%[b]	S: $F = 6.01$, $P = 0.0163$ T: $F = 3.80$, $P = 0.0263$ S × T: $F = 1.96$, $P = 0.1477$
Brood mites/colony mites (B/C)	80.2 ± 2.2%[a]	70.0 ± 4.1%[b]	68.3 ± 5.8%[b]		80.8 ± 2.0%[a]		77.5 ± 2.7%[a]	S: $F = 6.01$, $P = 0.0163$ T: $F = 3.80$, $P = 0.0263$ S × T: $F = 1.96$, $P = 0.1477$
Trapped mites (T)	112 ± 15	96 ± 12	77 ± 14[b]	131 ± 27[ab]	102 ± 23[ab]	80 ± 24[b]	135 ± 18[a]	S: $F = 0.64$, $P = 0.4260$ T: $F = 2.87$, $P = 0.0252$ S × T: $F = 0.36$, $P = 0.8365$
Trapped mites/colony mites (T/C)	4.5 ± 0.6%[b]	10.3 ± 2.2%[a]	12.5 ± 3.1%[a]		4.7 ± 0.8%[b]		4.7 ± 0.6%[b]	S: $F = 13.39$, $P = 0.0004$ T: $F = 8.53$, $P = 0.0004$ S × T: $F = 1.01$, $P = 0.3682$
Trapped older mites (O)	73 ± 10	72 ± 9	58 ± 10	86 ± 16	68 ± 15	57 ± 17	93 ± 16	S: $F = 0.00$, $P = 0.9854$ T: $F = 2.40$, $P = 0.0529$ S × T: $F = 0.47$, $P = 0.7568$
Trapped older mites/younger mites (O/Y)	2.37 ± 0.26[b]	3.10 ± 0.26[a]	4.23 ± 0.57[a]	2.87 ± 0.30[b]	2.78 ± 0.39[b]	2.16 ± 0.38[c]	1.38 ± 0.11[c]	S: $F = 9.79$, $P = 0.0022$ T: $F = 10.43$, $P < 0.0001$ S × T: $F = 1.38$, $P = 0.2441$
Trapped older mites/trapped mites (O/T)	65.9 ± 1.8%[b]	71.9 ± 1.7%[a]	77.2 ± 2.0%[a]	72.0 ± 2.0%[ab]	69.1 ± 2.5%[ab]	66.5 ± 3.8%[b]	57.4 ± 2.3%[c]	S: $F = 5.34$, $P = 0.0223$ T: $F = 5.50$, $P = 0.0004$ S × T: $F = 1.45$, $P = 0.2197$
Trapped older mites/colony mites (O/C)	3.0 ± 0.5%[b]	7.8 ± 1.8%[a]	10.0 ± 2.6%[a]		3.4 ± 0.6%[b]		2.8 ± 0.4%[b]	S: $F = 15.31$, $P = 0.0002$ T: $F = 11.10$, $P < 0.0001$ S × T: $F = 0.90$, $P = 0.4112$
Trapped younger mites (Y)	44 ± 7	30 ± 4	19 ± 4[b]	45 ± 12[b]	34 ± 9[b]	31 ± 9[b]	60 ± 9[a]	S: $F = 2.76$, $P = 0.0990$ T: $F = 4.71$ $P = 0.0013$ S × T: $F = 0.24$, $P = 0.9170$
Trapped younger mites/trapped mites (Y/T)	34.1 ± 1.8%[a]	28.1 ± 1.7%[b]	22.8 ± 2.0%[c]	28.0 ± 2.0%[bc]	30.9 ± 2.5%[bc]	33.5 ± 3.8%[b]	42.6 ± 2.3%[a]	S: $F = 5.34$, $P = 0.0223$ T: $F = 5.50$, $P = 0.0004$ S × T: $F = 1.45$, $P = 0.2197$
Trapped younger mites/colony mites (Y/C)	1.4 ± 0.2%[b]	2.7 ± 0.4%[a]	2.7 ± 0.5%[a]		1.4 ± 0.2%[b]		2.0 ± 0.3%[ab]	S: $F = 7.44$, $P = 0.0078$ T: $F = 3.79$, $P = 0.0266$ S × T: $F = 1.30$, $P = 0.2783$
Trapped injured mites (I)	28 ± 4	28 ± 4	17 ± 3[b]	39 ± 8[a]	19 ± 4[b]	20 ± 5[b]	44 ± 6[a]	S: $F = 0.26$, $P = 0.6119$ T: $F = 6.29$, $P = 0.0001$ S × T: $F = 0.25$, $P = 0.9066$
Trapped injured mites/trapped mites (I/T)	27.1 ± 1.2%	28.0 ± 1.5%	21.9 ± 2.1%[b]	29.2 ± 1.8%[a]	21.6 ± 1.9%[b]	34.2 ± 2.3%[a]	31.1 ± 1.6%[a]	S: $F = 0.07$, $P = 0.7949$ T: $F = 7.77$, $P < 0.0001$ S × T: $F = 1.69$, $P = 0.1566$
Trapped injured mites/colony mites (I/C)	1.1 ± 0.1%[b]	2.1 ± 0.4%[a]	2.3 ± 0.6%[a]		1.0 ± 0.2%[b]		1.5 ± 0.2%[ab]	S: $F = 6.51$, $P = 0.0126$ T: $F = 3.76$, $P = 0.0275$ S × T: $F = 0.02$, $P = 0.9764$
Trapped fresh mites (F)	26 ± 3	25 ± 4	16 ± 3[b]	28 ± 6[ab]	24 ± 6[b]	20 ± 5[b]	41 ± 7[a]	S: $F = 0.18$, $P = 0.6696$ T: $F = 4.24$, $P = 0.0029$ S × T: $F = 0.47$, $P = 0.7573$

Continued on following page

Table 2. Continued

Measurement	Italian	Russian	June	July	Aug.	Sept.	Oct.	Analysis
Trapped fresh mites/trapped mites (F/T)	25.0 ± 1.3%	25.1 ± 1.4%	20.8 ± 2.1%^{c}	23.2 ± 1.7%^{abc}	22.3 ± 2.3%^{bc}	29.7 ± 2.6%^{ab}	29.4 ± 1.6%^{a}	S: $F = 0.07$, $P = 0.7964$ T: $F = 2.64$, $P = 0.0360$ S × T: $F = 1.26$, $P = 0.2902$
Trapped fresh mites/colony mites (F/C)	1.1 ± 0.1%^{b}	1.9 ± 0.3%^{a}	2.1 ± 0.4%^{a}		1.0 ± 0.2%^{b}		1.3 ± 0.2%^{ab}	S: $F = 8.99$, $P = 0.0036$ T: $F = 4.51$, $P = 0.0138$ S × T: $F = 0.40$, $P = 0.6725$
Injured fresh mites (IF)	1.2 ± 0.3	1.7 ± 0.3	0.1 ± 0.1^{c}	1.1 ± 0.3^{b}	1.9 ± 0.6^{ab}	1.6 ± 0.4^{ab}	2.4 ± 0.5^{a}	S: $F = 3.22$, $P = 0.0747$ T: $F = 8.67$, $P < 0.0001$ S × T: $F = 0.81$, $P = 0.5183$
Injured fresh mites/all fresh mites (IF/F)	3.6 ± 0.6%^{b}	5.5 ± 0.2%^{a}	0.3 ± 0.2%^{c}	2.5 ± 0.6%^{b}	6.4 ± 1.4%^{a}	7.2 ± 1.4%^{a}	6.3 ± 1.0%^{a}	S: $F = 5.03$, $P = 0.0266$ T: $F = 11.79$, $P < 0.0001$ S × T: $F = 1.12$, $P = 0.3505$
Injured fresh mites/trapped mites (IF/T)	0.9 ± 0.2%^{b}	1.4 ± 0.2%^{a}	0.1 ± 0.1%^{c}	0.6 ± 0.2%^{b}	1.4 ± 0.3%^{ab}	1.9 ± 0.4%^{a}	1.7 ± 0.2%^{a}	S: $F = 5.69$, $P = 0.0184$ T: $F = 11.33$, $P < 0.0001$ S × T: $F = 1.88$, $P = 0.1166$
Injured fresh mites/colony mites (IF/C)	0.03 ± 0.01%^{b}	0.07 ± 0.02%^{a}	0.01 ± 0.00%^{b}		0.07 ± 0.02%^{a}		0.07 ± 0.01%^{a}	S: $F = 6.42$, $P = 0.0132$ T: $F = 15.15$, $P < 0.0001$ S × T: $F = 0.96$, $P = 0.3883$
Trapped injured older mites (IO)	17 ± 2	20 ± 3	14 ± 3^{b}	25 ± 5^{a}	12 ± 2^{b}	15 ± 4^{b}	27 ± 4^{a}	S: $F = 0.06$, $P = 0.8091$ T: $F = 4.89$, $P = 0.0010$ S × T: $F = 0.41$, $P = 0.7989$
Injured older mites/older mites (IO/O)	29.6 ± 1.6%	30.7 ± 2.1%	22.7 ± 2.5%^{b}	32.4 ± 2.2%^{a}	22.1 ± 2.5%^{b}	39.1 ± 3.6%^{a}	34.4 ± 2.1%^{a}	S: $F = 0.13$, $P = 0.7161$ T: $F = 8.03$, $P < 0.0001$ S × T: $F = 2.47$, $P = 0.0473$
Injured older mites/injured mites (IO/I)	72.1 ± 2.1%	73.8 ± 2.1%	81.0 ± 3.4%^{a}	79.5 ± 2.4%^{a}	68.7 ± 2.5%^{ab}	71.3 ± 4.3%^{a}	63.0 ± 2.7%^{b}	S: $F = 0.12$, $P = 0.7252$ T: $F = 4.98$, $P = 0.0009$ S × T: $F = 0.86$, $P = 0.4925$
Injured older mites/trapped mites (IO/T)	19.0 ± 1.1%	21.2 ± 1.5%	17.3 ± 1.9%^{bc}	23.4 ± 1.8%^{a}	14.2 ± 1.4%^{c}	25.0 ± 2.8%^{a}	19.9 ± 1.7%^{ab}	S: $F = 1.31$, $P = 0.2537$ T: $F = 4.93$, $P = 0.0009$ S × T: $F = 1.44$, $P = 0.2237$
Injured older mites/colony mites (IO/C)	0.77 ± 0.12%^{b}	1.85 ± 0.47%^{a}	2.27 ± 0.66%^{a}		0.69 ± 0.13%^{b}		0.92 ± 0.12%^{b}	S: $F = 9.62$, $P = 0.0026$ T: $F = 6.95$, $P = 0.0016$ S × T: $F = 0.31$, $P = 0.7366$
Trapped injured younger mites (IY)	10 ± 2	8 ± 1	3 ± 1^{c}	11 ± 3^{b}	8 ± 2^{b}	10 ± 3^{b}	15 ± 2^{a}	S: $F = 0.77$, $P = 0.3828$ T: $F = 5.36$, $P = 0.0005$ S × T: $F = 0.32$, $P = 0.8613$
Injured younger mites/younger mites (IY/Y)	25.4 ± 2.5%	25.6 ± 2.0%	20.1 ± 4.3%	21.4 ± 2.5%	26.4 ± 3.6%	33.4 ± 5.3%	26.3 ± 1.7%	S: $F = 0.01$, $P = 0.9198$ T: $F = 1.61$, $P = 0.1748$ S × T: $F = 0.45$, $P = 0.7693$
Injured younger mites/injured mites (IY/I)	27.9 ± 2.1%	26.2 ± 2.1%	19.0 ± 3.4%^{c}	20.5 ± 2.4%^{c}	31.3 ± 2.5%^{ab}	28.7 ± 4.3%^{bc}	37.0 ± 2.7%^{a}	S: $F = 0.12$, $P = 0.7252$ T: $F = 4.98$, $P = 0.0009$ S × T: $F = 0.86$, $P = 0.4925$
Injured younger mites/trapped mites (IY/T)	7.8 ± 0.7%	7.1 ± 0.7%	4.4 ± 0.9%^{c}	5.9 ± 0.7%^{bc}	7.4 ± 0.9%^{b}	8.8 ± 1.5%^{b}	11.1 ± 1.0%^{a}	S: $F = 0.41$, $P = 0.5218$ T: $F = 5.44$, $P = 0.0004$ S × T: $F = 1.07$, $P = 0.3730$
Injured younger mites/colony mites (IY/C)	0.32 ± 0.05%^{b}	0.55 ± 0.08%^{a}	0.40 ± 0.11%		0.33 ± 0.06%		0.54 ± 0.08%	S: $F = 5.58$, $P = 0.0205$ T: $F = 2.46$, $P = 0.0914$ S × T: $F = 1.36$, $P = 0.2628$

S = stock; T = time. Superscript letters indicate significant ($P \leq 0.05$) post-ANOVA differences.

Table 3. Results of the regression analyses to relate 21 candidate measurements of mite fall to mite populations in Italian and Russian honey bee colonies

Measurement	Regression	Italian	Russian	Measurement	Regression	Italian	Russian
Trapped mites (T)	$r = 0.679$	$r = 0.708$	$r = 0.730$	Injured fresh mites (IF)	$r = 0.541$	$r = 0.566$	$r = 0.738$
	$r^2 = 0.462$	$r^2 = 0.507$	$r^2 = 0.533$		$r^2 = 0.292$	$r^2 = 0.321$	$r^2 = 0.545$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Trapped older mites (O)	$r = 0.584$	$r = 0.607$	$r = 0.699$	Injured fresh mites/all fresh mites (IF/F)	$r = 0.298$	$r = 0.365$	$r = 0.350$
	$r^2 = 0.341$	$r^2 = 0.368$	$r^2 = 0.488$		$r^2 = 0.089$	$r^2 = 0.133$	$r^2 = 0.123$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$		$P = 0.0050$	$P = 0.0117$	$P = 0.0268$
Trapped older mites/younger mites (O/Y)	$r = -0.509$	$r = -0.540$	$r = -0.378$	Injured fresh mites/trapped mites (IF/T)	$r = 0.359$	$r = 0.388$	$r = 0.454$
	$r^2 = 0.260$	$r^2 = 0.292$	$r^2 = 0.143$		$r^2 = 0.129$	$r^2 = 0.151$	$r^2 = 0.206$
	$P < 0.0001$	$P < 0.0001$	$P = 0.0212$		$P = 0.0006$	$P = 0.0064$	$P = 0.0033$
Trapped older mites/trapped mites (O/T)	$r = -0.582$	$r = -0.623$	$r = -0.430$	Trapped injured older mites (IO)	$r = 0.548$	$r = 0.584$	$r = 0.619$
	$r^2 = 0.339$	$r^2 = 0.388$	$r^2 = 0.185$		$r^2 = 0.301$	$r^2 = 0.341$	$r^2 = 0.383$
	$P < 0.0001$	$P < 0.0001$	$P = 0.0056$		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Trapped younger mites (Y)	$r = 0.788$	$r = 0.0.798$	$r = 0.781$	Injured older mites/older mites (IO/O)	$r = 0.209$	$r = 0.221$	$r = 0.208$
	$r^2 = 0.621$	$r^2 = 0.637$	$r^2 = 0.610$		$r^2 = 0.044$	$r^2 = 0.049$	$r^2 = 0.043$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$		$P = 0.0510$	$P = 0.1316$	$P = 0.1974$
Trapped younger mites/trapped mites (Y/T)	$r = 0.582$	$r = 0.623$	$r = 0.430$	Injured older mites/injured mites (IO/I)	$r = -0.408$	$r = -0.463$	$r = -0.296$
	$r^2 = 0.339$	$r^2 = 0.388$	$r^2 = 0.185$		$r^2 = 0.166$	$r^2 = 0.215$	$r^2 = 0.088$
	$P < 0.0001$	$P < 0.0001$	$P = 0.0056$		$P = 0.0001$	$P = 0.0010$	$P = 0.0709$
Trapped injured mites (I)	$r = 0.615$	$r = 0.650$	$r = 0.665$	Injured older mites/trapped mites (IO/T)	$r = -0.000$	$r = -0.024$	$r = 0.094$
	$r^2 = 0.378$	$r^2 = 0.422$	$r^2 = 0.442$		$r^2 = 0.000$	$r^2 = 0.001$	$r^2 = 0.009$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$		$P = 0.9802$	$P = 0.8652$	$P = 0.5629$
Trapped injured mites/trapped mites (I/T)	$r = -0.122$	$r = -0.074$	$r = 0.200$	Trapped injured younger mites (IY)	$r = 0.679$	$r = 0.678$	$r = 0.735$
	$r^2 = 0.015$	$r^2 = 0.006$	$r^2 = 0.040$		$r^2 = 0.461$	$r^2 = 0.460$	$r^2 = 0.541$
	$P = 0.2571$	$P = 0.6153$	$P = 0.2153$		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Trapped fresh mites (F)	$r = 0.706$	$r = 0.721$	$r = 0.772$	Injured younger mites/younger mites (IY/Y)	$r = 0.010$	$r = 0.010$	$r = 0.058$
	$r^2 = 0.498$	$r^2 = 0.520$	$r^2 = 0.596$		$r^2 = 0.000$	$r^2 = 0.000$	$r^2 = 0.003$
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$		$P = 0.9284$	$P = 0.9371$	$P = 0.7268$
Trapped fresh mites/trapped mites (F/T)	$r = 0.227$	$r = 0.218$	$r = 0.282$	Injured younger mites/injured mites (IY/I)	$r = 0.408$	$r = 0.463$	$r = 0.296$
	$r^2 = 0.052$	$r^2 = 0.048$	$r^2 = 0.079$		$r^2 = 0.166$	$r^2 = 0.215$	$r^2 = 0.088$
	$P = 0.0324$	$P = 0.1317$	$P = 0.0782$		$P = 0.0001$	$P = 0.0010$	$P = 0.0709$
				Injured younger mites/trapped mites (IY/T)	$r = 0.361$	$r = 0.333$	$r = 0.392$
					$r^2 = 0.130$	$r^2 = 0.111$	$r^2 = 0.154$
					$P = 0.0005$	$P = 0.0195$	$P = 0.0124$

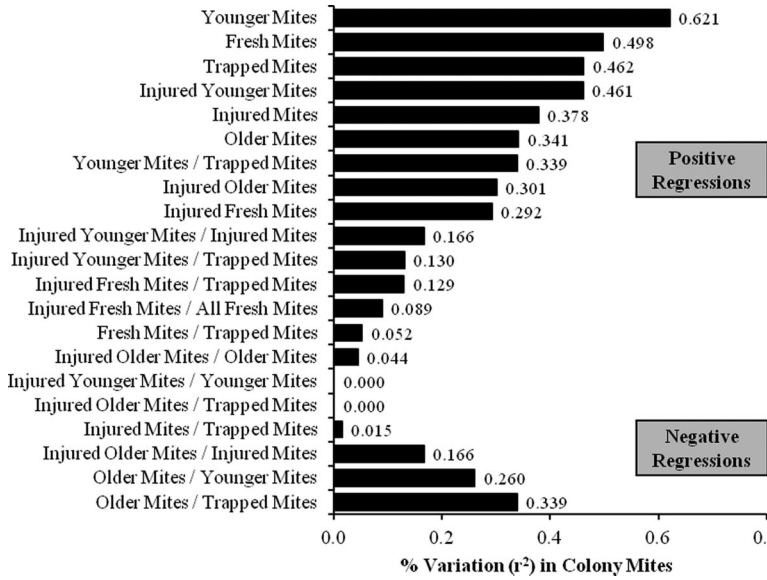

Fig. 1. The percentage variation (r^2) of total mites in a colony that was associated with each of the 21 candidate measurements of trapped mites.

Discussion

The central difference between Russian honey bees and Italian bees in this study is that Russian honey bee colonies had 56% fewer mites infesting them. Additionally, measurements of trapped mites differ between the two stocks with T/C, O/T, O/Y, O/C, Y/C, I/C, F/C, IF/F, IF/T, IF/C, IO/C, and IY/C all significantly higher for Russian honey bees. The majority of these differences related a category of trapped mites to colony mites, suggesting that a higher proportion of colony mites were trapped in Russian honey bee colonies. A proportionally greater number of trapped mites in Russian honey bee colonies may reflect the operation of the resistance mechanisms that led to their comparatively low levels of infestation. However, measurements that require an estimate of C are not good candidates for an improved selection criterion because an estimate of colony mites is very time consuming. In addition, mite population growth (MPG) derived from at least two estimates of C is itself a selection criterion for resistance to *Varroa* (Rinderer et al. 2010). Potentially useful measurements evaluated in this study are I/T, IF/F, IF/T O/T, O/Y, and Y/T.

The proportion of injured mites (I/T) (29%) in this study is similar to that found in many previous studies (Büchler et al. 1992, Aumeier 2001, Currie and Tamashbi 2008). However, in contrast to the report of Rinderer et al. (2001), the proportions of injured mites for Russian and Italian colonies were nearly identical (Table 2). The Italian colonies may have had an elevated I/T in the current study because the I/T of Russian honey bees is very similar to the I/T reported for Russian honey bees by Guzman–Novoa et al. (2012) compared with a lower I/T for the unselected stock they used for comparison. Injury rates generally rose through time (Table 2) suggesting that injuries may be more common with higher overall rates of infestation. However, the regressions of I/T for both stocks and combined data, although negative, were weak and insignificant (Table 3; Fig. 1). These observations are consistent with those of Ehrhardt et al. (2007), Hoffmann (1995), and three of the four regressions reported by Guzman–Nova et al. (2012) who collectively report four similarly low regressions for European honey bee and one for Africanized honey bee. This collection of low regressions contrasts with the strong negative regressions reported for European honey bee (Moosbeckhofer 1992, Guzman–Nova et al. 2012) and African honey bee (Mondragón et al. 2005 and Arechavaleta–Velasco and Guzman-Novoa 2001). These varied results suggest that the relationship of I/T to C must be determined for specific stocks before it can be relied upon as a selection criterion. Our results do not support I/T selection for the Russian honey bee or Italian honey bee stocks in our study.

In addition, our results with proportions of injured fresh mites of all fresh mites (IF/F) and injured fresh mites of all trapped mites (IF/T) do not support their use for selection for resistance. IF/F was higher for Russian honey bees (Table 2; $P = 0.03$) but both IF/F and IF/T had positive regressions with C (Table 3; Fig. 1) making them unsuitable as measurements of potential resistance unless colonies are experimentally manipulated to establish uniform infestation rates.

The measurements that have a positive correlation with C provide some insight concerning the nature of trapped mites. Y, Y/T, IY, IY/I, and IY/T, all have strong positive correlations with C (Table 3; Fig. 1), suggesting that especially Y ($r^2 = 0.62$), but all young mites (injured and noninjured) are more a reflection of mite populations than of reductions in mite popu-

lations resulting from grooming. Bienefeld et al. (1999) made a similar observation causing them to suggest omitting counts of young mites when evaluating proportions of groomed mites. Increased numbers of trapped young mites are associated with hatching brood with 80% of them having a lighter color (Lobb and Martin 1997). More highly infested brood likely results in increased numbers of trapped young mites. Injuries to young mites may result from the removal of dead mites from cells during and after hatching (Bienefeld et al. 1999). Young mites probably are an important part of the strong positive regression of T with C. Numbers of older mites also have a positive regression with C ($r^2 = 0.34$) reflecting natural mite mortality that must rise as the mite population grows. In addition, hygiene may increase as the mite population grows and further increase Y.

As single measures, positive relationships have no value as selection criteria for programs with the goal of reducing colony mites through selective breeding. However, they might be sufficient to determine mite population growth by comparing measurements made across time. In addition, both Y and T contribute to ratios with O that have the strongest negative regressions with C in this study (O/Y, $r^2 = 0.26$; O/T, $r^2 = 0.34$). Of the measurements we studied, these two ratios are the best candidates for employment as one time measurements in a selection program for reduced colony mites. O/T and O/Y are not exclusively measurements of grooming. Various resistance mechanisms could result in an increase in O/T or O/Y. Increased numbers of older mites damaged by grooming, older mites that are removed from the nest by grooming but not damaged and any other physiological or behavioral characteristic of honey bees that shortens the survival of older mites would increase these ratios. Higher proportions of trapped older mites in Russian honey bee colonies may be related to the higher proportion of phoretic mites found in Russian honey bee colonies in this (Table 2) and previous studies (Rinderer et al. 2001, de Guzman et al. 2007). Likewise, any resistance mechanism that reduces fecundity would probably result in lower numbers of T and Y and result in larger ratios of O/T and O/Y.

Considerable research is required before increased values for O/T or O/Y are used as selection criteria for a selection program for improving honey bee resistance to *Varroa* mites. It seems reasonable that O/T and O/Y are related to resistance because they are significantly higher in Russian honey bees (Table 2). However, confirmation studies with the Russian honey bee and Italian honey bees used in this study and with other stocks of bees are necessary to validate the potential of O/T and O/Y including temporal changes and exact measurements rather than estimates of C. In addition, even if O/T and O/Y are confirmed to be measurements of resistance to *Varroa*, the genetic variance for resistance in specific stocks may be limited and selection would yield only marginal results. However, despite these real and possible obstacles, O/T and O/Y may have a place in selection programs for *Varroa* resistance. Certainly they are measurements that can be done quickly without a microscope and hence could be used by commercial beekeepers.

Acknowledgments

We thank J. A. Stelzer, J. Wales, G. Delatte, and R. Watts for their technical help.

References Cited

Andino, G. K., and G. J. Hunt. 2011. A scientific note on a new assay to measure honeybee mite-grooming behavior. Apidologie 42: 481–484.

Al-Ghzawi, A.-M.A., S. T. Zaitoun, and H. K. Shannag. 2001. Damaged varroa mites in the debris of different honey bee subspecies under semiarid Mediterranean conditions. Bull. Soc. Entomol. France 106: 193–198.

Ardestani, M. M., R. Ebadi, and G. Tahmasbi. 2011. Regular dorsal dimples and damaged mites of *Varroa destructor* in some Iranian honey bees (*Apis mellifera*). Exp. Appl. Acarol. 54: 261–268.

Arechavaleta–Velasco, M., and E. Guzman–Novoa. 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. 2001. Bioassay for grooming effectiveness towards *Varroa destructor* mites in Africanized and Carniolan honeybees. Apidologie 32: 81–90.

Bienefeld, K., F. Zautke, D. Pronin, and A. Mazeed. 1999. Recording the proportion of damaged *Varroa jacobsoni* Oud. in the debris of honey bee colonies (*Apis mellifera*). Apidologie 30: 249–256.

Boecking, O., and W. Ritter. 1993. Grooming and removal behaviour of *Apis mellifera intermissa* in Tunisia against *Varroa jacobsoni.* J. Apic. Res. 32: 127–134.

Boecking, O., and M. Spivak. 1999. Behavioral defenses of honey bees against *Varroa jacobsoni* Oud. Apidologie 30: 141–158.

Büchler, R. 2000. Design and success of a German breeding program for *Varroa* tolerance. Am. Bee J. 140: 662–665.

Büchler, R., W. Drescher, and I. Tornier. 1992. Grooming behaviour of *Apis cerana*, *Apis mellifera* and *Apis dorsata* and its effects on the parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae.* Exp. Appl. Acarol. 16: 313–319.

Büchler, R., S. Berg, and Y. Le Conte. 2010. Breeding for resistance to *Varroa destructor* in Europe. Apidologie 41: 393–408.

Corrêa–Marques, M. H., M.R.C. Issa, and D. De Jong. 2000. Classification and quantification of damaged *Varroa jacobsoni* found in the debris of honey bee colonies as criteria for selection? Am. Bee J. 140: 820–824.

Corrêa–Marques, M. H., D. De Jong, P. Rosenkranz, and L.S. Gonçalves. 2002. Varroa-tolerant Italian honey bees introduced from Brazil were not more efficient in defending themselves against the mite Varroa destructor than Carniolan bees in Germany. Genet. Mol. Res. 1: 153–158.

Currie, R. W., and G. H. Tahmasbi. 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.

Davis, A. R., A. Mirakhur, and D. Bikey. 2007. Damage by ants to the honey bee parasitic mite, *Varroa destructor*, pp. 157–158. *In* C.A.C. Gardner, M. A. Harris, R. W. Hellmich, H. T. Horner, J. D. Nason, R. G. Palmer, J. J. Tabke, R. W. Thornburg, and M. P. Widrlechner (eds.), Proceedings of

the 9th International Symposium on Pollination, Ames, IA.

Davis, A. R. 2009. Regular dorsal dimples on *Varroa destructor:* damage symptoms or developmental origin? Apidologie 40: 151–162.

de Guzman, L. I., T. E. Rinderer, and A. M. Frake. 2007. Growth of *Varroa destructor* (Acari: Varroidae) populations in Russian honey bee (Hymenoptera: Apidae) colonies. Ann. Entomol. Soc. Am. 100: 187–195.

de Guzman, L. I., T. E. Rinderer, and A. M. Frake. 2008. Comparative reproduction of Varroa destructor in different types of Russian and Italian honey bee combs. Exp. Appl. Acarol. 44: 227–238.

Eguaras, E., K. Marcangeli, N. Oppedisano, and N. Fernandez. 1995. Mortality and reproduction of *Varroa jacobsoni* in resistant colonies of honey bees (*Apis mellifera*) in Argentina. Bee Sci. 3: 174–178.

Ehrhardt, K., N. Reinsch, R. Büchler, C. Garrido, and K Bienefeld. 2007. Genetic parameters for *Varroa* resistance in the honeybee, pp. 145–146. Abstracts of the 40th Apimondia Int. Apic. Congr., 9–14 2009, Melbourne, Australia.

Fries, I., W. Huazhen, S. Wei, and C. S. Jin. 1996. Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica.* Apidologie 27: 3–11.

Guzman–Novoa, E., B. Emsen, P. Unger, L. G. Espinosa–Montaño, and T. Petukhova. 2012. 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.). J. Invertebr. Pathol. 110: 314–320.

Harbo, J. R., and J. W. Harris. 2005. Suppressed mite reproduction explained by the behaviour of adult bees. J. Apic. Res. 44: 21–23.

Hoffmann, S. 1995. Registration of damaged *Varroa* mites in small colonies for the assessment of grooming behavior. Apidologie 26: 322–323.

Ibrahim, A., G. S. Reuter, and M. Spivak. 2007. Field trial of honey bee colonies bred for mechanisms of resistance against *Varroa destructor.* Apidologie 38: 67–76.

Kefuss, J., J. Vanpoucke, J. D. de Lahitte, and W. Ritter. 2004. *Varroa* tolerance in France of Intermissa bees from Tunisia and their naturally mated descendants: 1993–2004. Am. Bee J. 144: 563–568.

Lobb, N., and S. Martin. 1997. Mortality of *Varroa jacobsoni* Oudemans during or soon after the emergence of worker and drone honeybees *Apis mellifera* L. Apidologie 28: 367–374.

Lodesani, M., M. A. Vecchi, S. Tommasini, and M. Bigliardi. 1996. A study on different kinds of damage to *Varroa jacobsoni* in *Apis mellifera ligustica* colonies. J. Apic. Res. 35: 49–56.

Mondragón, L., M. Spivak, and R. Vandame. 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.

Moosbeckhofer, R. 1992. Observations on the occurrence of damaged *Varroa* mites in natural mite fall of *Apis mellifera carnica* colonies. Apidologie 23: 523–531.

Moretto, G., L. S. Goncalves, and D. De Jong. 1991. Africanized bees are more efficient at removing *Varroa jacobsoni:* preliminary report. Am. Bee J. 131: 434.

Peng, Y. S., Y. Fang, S. Xu, and L. Ge. 1987. The resistance mechanism of the Asian honey bee, *Apis cerana* Fabr. to an ectoparasite, *Varroa jacobsoni* Oudemans. J. Invertebr. Pathol. 49: 54–60.

Rinderer, T. E., L. I. de Guzman, G. T. Delatte, J. A. Stelzer, V. Kuznetsov, L. Beaman, R. Watts, and J. Harris. 2001. Resistance to the parasitic mite *Varroa destructor* in honey bees from Far-eastern Russia. Apidologie 32: 381–394.

Rinderer, T. E., L. I. de Guzman, G. T. Delatte, and C. Harper. 2003. An evaluation of ARS Russian honey bees in combination with other methods for the control of varroa mites. Am. Bee J. 143: 410–413.

Rinderer, T. E., Harris, J. W., G. Hunt, and L. I. de Guzman. 2010. Breeding for resistance to *Varroa destructor* in North America. Apidologie 41: 409–424.

Rosenkranz, P., I. Fries, O. Boecking, and M. Stürmer. 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., and H. Hänel. 1992. Active defense against *Varroa* mites in a Carniolan strain of honeybee (*Apis mellifera carnica* Pollman). Apidologie 23: 173–187.

SAS Institute. 2008. SAS user's guide, version 9.2. SAS Institute, Cary, NC.

Spivak M., and G. S. Reuter. 2001. *Varroa jacobsoni* infestation in untreated honey bee (Hymenoptera:Apidae) colonies selected for hygienic behavior. J. Econ. Entomol. 94: 326–331.

Szabo, T. I., and C.R.T. Walker. 1995. Damages to dead *Varroa jacobsoni* caused by the larvae of *Galleria mellonella.* Am. Bee J. 135: 421–422.

Tamashbi, G. H. 2009. The effect of temperature and humidity on grooming behaviour of honeybee, *Apis mellifera* (Hym.: Apidae) colonies against varroa mite, *Varroa destructor* (Acari: Varroidae). J. Entomol. Soc. Iran 28: 7–23.

Thakur, R. K., K. Bienefeld, and R. Keller. 1997. Varroa defense behavior in *A. mellifera carnica.* Am. Bee J. 137: 143–148.

Vandame, R., S. Morand, M.-E. Colin, and L. P. Belzunces. 2002. Parasitism in the social bee *Apis mellifera:* quantifying costs and benefits of behavioral resistance to *Varroa destructor* mites. Apidologie 33: 433–445.

Webster, T. C., E. M. Thacker, and F. E. Vorisek. 2000. Live *Varroa jacobsoni* (Mesostigmata: Varroidae) trapped from honey bee (Hymenoptera: Apidae) colonies. J. Econ. Entomol. 93: 1596–1601.

Zaitoun, S. T., A.-M.A. Al-Ghzawi, and H. K. Shannag. 2001. Grooming behaviour of *Apis mellifera syriaca* towards *Varroa jacobsoni* in Jordan. J. Appl. Entomol. 125: 85–87.

Received 10 October 2012; accepted 20 December 2012.