An Evaluation of the Associations of Parameters Related to the Fall of *Varroa destructor* (Acari: Varroidae) From Commercial Honey Bee (Hymenoptera: Apidae) Colonies as Tools for Selective Breeding for Mite Resistance

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J. Econ. Entomol. 107(2): 516–522 (2014); DOI: http://dx.doi.org/10.1603/EC13477

ABSTRACT *Varroa destructor* (Anderson and Trueman) trapped on bottom boards were assessed as indirect measurements of colony mite population differences and potential indicators of mite resistance in commercial colonies of Russian and Italian honey bees (*Apis mellifera* L.) by using 35 candidate measurements. Measurements included numbers of damaged and nondamaged younger mites, nymphs, 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. Several measurements differed strongly between the stocks, suggesting that the detailed characteristics of trapped mites may reflect the operation of resistance mechanisms in the Russian honey bees. Regression analyses were used to determine the relationships of these candidate measurements with the number of mites in the colonies. The largest positive regressions differed for the two stocks (Italian honey bees: trapped mites and trapped younger mites; Russian honey bees: trapped younger mites and trapped fresh mites). Also, the regressions for Italian honey bees were substantially stronger. The largest negative regressions with colony mites for both stocks were for the proportion of older mites out of all trapped mites. Although these regressions were statistically significant and consistent with those previously reported, they were weaker than those previously reported. The numbers of mites in the colonies were low, especially in the Russian honey bee colonies, which may have negatively influenced the precision of the regressions.

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

Varroa destructor is generally recognized as the principal biological challenge to beekeeping worldwide. Historically, honey bee (*Apis mellifera*) colonies rarely survived unless *Varroa* infestations were managed with acaricides. Yet, because of issues of chemical contamination of hive products, treatment costs, and development of acaricide-resistant *Varroa*, breeding honey bees resistant to the mites is a more desirable long-term solution to the problem.

There has been substantial recent progress in developing *Varroa*-resistant honey bees, (Büchler et al. 2010, Rinderer et al. 2010). Breeding has yielded four documented *Varroa*-resistant honey bee stocks, which are used in beekeeping. Honey bees bred for the removal of freeze-killed brood (Minnesota Hygienic Bees) have measurable resistance to *Varroa* (Ibrahim and Spivak 2006). Breeding from outcrosses of a North African subspecies in France produced honey bees with strong *Varroa* resistance (Kefuss et al. 2004). Russian honey bees (RHB), found to have comparatively good resistance to *Varroa* (Rinderer et al. 2001a), have been further improved through selective breeding for reduced mite population growth (MPG). Selecting for reduced MPG requires detailed and time-consuming evaluations of the amount of brood and bees in colonies and estimates of the number of mites infesting both brood and bees. The commercial functionality of RHB stock has been well documented (Rinderer et al. 2001a,b; de Guzman et al. 2007; Ward et al. 2008; Danka et al. 2012), and the stock has been released to the beekeeping industry where it is being maintained, improved, and distributed by the Russian Bee Breeders Association (Brachmann 2009). Honey bees that are able to detect and remove brood infested with *Varroa* have been developed with selective breeding. Selecting for this trait, called "*Varroa* sensitive hygiene", requires tedious microscopic examination of sealed brood. However, the functionality of *Varroa* sensitive hygiene has been well documented (Ward et al. 2008, Danka et al. 2012), and breeding material has been delivered to the beekeeping industry and is now widely used (Danka et al. 2013).

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The development of additional or simplified measurements of resistance to *Varroa* would facilitate the breeding of resistant stocks of honey bees. There is potential for resistance based on "grooming," an activity manifested as phoretic *Varroa* falling to the bottom of a hive after being removed by either an infested adult bee itself or by a nestmate (Moretto et al. 1991, Arechavaleta-Velasco and Guzman-Novoa 2001, Mondragón et al. 2005). The standard measurement of grooming is generally based on the proportion of damaged or chewed mites out of mites that fall from colonies. One of the resistant traits in RHB is the increased proportion of chewed mites (Rinderer et al. 2001a). However, not all groomed mites are visibly damaged (Boecking and Ritter 1993). The value of the proportion of damaged mites as a tool for selection is controversial (Boecking and Spivak 1999, Büchler et al. 2010, Rinderer et al. 2010, Guzman-Novoa et al. 2012 reviewed in detail: Rinderer et al. 2013).

Mites that fall from colonies have been used for other studies beyond the determination of the proportion of damaged or chewed mites. Comparisons of numbers of fallen mites before and after acaricide treatment have been used to evaluate the effectiveness of acaracides (Faucon et al. 1995, Delaplane and Hood 1999, Giovenazzo and Dubreuil 2011). Numbers of fallen mites have been used to compare the rates of development of *Varroa* among different sub-species or stocks of honey bees (Moritz and Mautz 1990, de Guzman et al. 1996). Mites in colonies have been estimated from counts of fallen mites for the purpose of determining the most appropriate timing of acaricide treatment (Deleplane and Hood 1999, Strange and Sheppard 2001, Branco et al. 2006). Numbers of fallen mites, when used to estimate the total numbers of mites in colonies, are derived using a model. The model of Martin (1998) indicates that the total number of mites in a colony with brood is 20–40 times the number of the average daily number of fallen mites.

Numerous other studies report both greater and lesser precision (Reviewed by Branco et al. 2006) for a variety of reasons. However, estimating mite populations from numbers of fallen mites is considered adequate for use in integrated pest management (Branco et al. 2006). Aside from assessments of damaged mites, none of these aforementioned studies categorized fallen mites according to age or physiological condition, and none included mite nymphs in their counts.

Recently, Rinderer et al. (2013) studied categories of mites that drop to bottom board traps in both Italian honey bees (IHB) and RHB colonies. They showed that the proportion of "older" mites (regardless of injuries) out of mites that are trapped (O/T) was significantly associated with lower colony mite numbers in both IHB and RHB colonies. Measuring O/T may be an indirect measurement of numerous mechanisms of resistance to *Varroa.* Also, Rinderer et al. (2013) found a strong relationship ($r^2 = 0.62$) between the number of younger trapped mites and total colony mites. Two measurements of the numbers of younger trapped mites, separated by several months during the active growing season in a collection of colonies, may provide a rapid method of comparing MPG among the colonies.

The study of Rinderer et al. (2013) was conducted in one apiary during one season with RHB and IHB not treated with acaricides. It is desirable to determine if the reported relationships of categories of trapped mites to the number of mites in colonies can be found in commercial populations of honey bees that are periodically treated with acaricides. This study was conducted to verify if the measurements of trapped mites have potential value for selective breed for resistance to *Varroa* in commercial populations of RHB and IHB. In addition, the categories of trapped mites were expanded to include the numbers of *Varroa* nymphs.

Materials and Methods

Colonies. Two commercial populations of honey bees were used in this study. Colonies of IHB ($N = 100$) in California that had been treated with a commercial acaricide ≈4 mo earlier were evaluated between 15 and 19 May 2012. These colonies were full-sized colonies that had 12.55 ± 0.34 frames of bees and 4.73 ± 0.14 frames of brood. In addition, colonies of RHB ($N = 100$) in Arkansas were colony divisions derived from colonies that had been treated with a preparation containing thymol ≈6 mo earlier and were evaluated between 19 and 23 June 2012. These colonies were full-sized colonies that had 6.06 ± 0.21 frames of bees and 3.04 ± 0.09 frames of brood. Colonies in both locations were not preselected and were in random apiaries in both beekeeping enterprises. However, the scheduled acaricide treatment for the colonies in California was delayed to allow some development of *Varroa* populations to provide mites for evaluation.

Measurement of *Varroa* Mite Populations. Estimates of the total number of mites in the colonies were derived from counts of mites in 200 worker brood cells (using two brood frames), counts of mites in 50 drone brood cells (using available drone cells), 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). These estimates were made 1 d before mites were trapped from the colonies. These methods are similar to those recommended by Dietemann et al. (2013), except that brood was not frozen because frozen brood is fragile, it is difficult to count all the mites, and exuviae are not discernible.

Measurement of Trapped Mites. Mites were trapped on three consecutive days following the procedures described by Rinderer et al. (2013). Briefly, to collect mites, a cafeteria tray with paper coated with a mixture of vegetable oil and petrolatum was inserted under a screen bottom board. New traps were used each day. On retrieval, papers were folded and placed individually into sealed plastic bags, transported to Baton Rouge, and frozen (−20°C) before examination.

Upon examination, *Varroa* mites were collected from each paper using an insect brush and examined under a dissecting microscope for age (light ochre = younger, darker color = older), injury status (injured

Table 1. Means (±standard error) for 35 candidate measurements of mite fall for IHB and RHB colonies and results of *t*-tests

Measurement	Abbreviation	Italian	Russian	Analysis
Colony mites	C	1501 ± 135[a]	370 ± 36[b]	$t = -10.73$, $P < 0.0001$
Phoretic mites/colony mites	P/C	41.9 ± 2.2%[a]	36.7 ± 2.7%[b]	$t = 2.05$, $P = 0.0005$
Brood mites/colony mites	B/C	47.4 ± 2.4%	55.2 ± 2.9%	$t = 1.69$, $P = 0.092$
Trapped mites	T	41 ± 4[a]	32 ± 3[b]	$t = 2.31$, $P = 0.022$
Trapped mites/colony mites	T/C	3.3 ± 0.2%[b]	11.6 ± 1.2%[a]	$t = 9.14$, $P < 0.0001$
Trapped older mites	O	24 ± 2	25 ± 2	$t = 0.47$, $P = 0.638$
Trapped older mites/trapped mites	O/T	57.8 ± 1.6%[b]	80.4 ± 1.1%[a]	$t = 10.93$, $P < 0.0001$
Trapped older mites/colony mites	O/C	2.0 ± 0.1%[b]	9.3 ± 1.0%[a]	$t = 10.65$, $P < 0.0001$
Trapped older mites/younger mites	O/Y	2.4 ± 0.2[b]	5.9 ± 0.4[a]	$t = 10.12$, $P < 0.0001$
Trapped younger mites	Y	13 ± 2[a]	5 ± 1[b]	$t = -5.64$, $P < 0.0001$
Trapped younger mites/trapped mites	Y/T	28.7 ± 1.2%[a]	15.6 ± 0.9%[b]	$t = -7.70$, $P < 0.0001$
Trapped younger mites/colony mites	Y/C	0.9 ± 0.1%[b]	1.7 ± 0.2%[a]	$t = 3.30$, $P = 0.001$
Total nymphs	N	5.9 ± 0.7[a]	1.5 ± 0.2[b]	$t = -7.31$, $P < 0.0001$
Trapped nymphs/trapped mites	N/T	13.2 ± 0.8%[a]	3.6 ± 0.4%[b]	$t = -9.45$, $P < 0.0001$
Trapped nymphs/colony mites	N/C	0.41 ± 0.03%	0.55 ± 0.10%	$t = -1.31$, $P = 0.192$
Trapped injured mites	I	16 ± 1[a]	9 ± 1[b]	$t = -4.92$, $P < 0.0001$
Trapped injured mites/trapped mites	I/T	37.5 ± 1.1%[a]	27.7 ± 1.2%[b]	$t = -5.90$, $P < 0.0001$
Trapped injured mites/colony mites	I/C	1.3 ± 0.1%[b]	3.2 ± 0.3%[a]	$t = 6.18$, $P < 0.0001$
Trapped fresh mites	F	18 ± 2	20 ± 2	$t = 1.17$, $P = 0.242$
Trapped fresh mites/trapped mites	F/T	40.9 ± 1.1%[b]	62.1 ± 1.3%[a]	$t = 11.95$, $P < 0.0001$
Trapped fresh mites/colony mites	F/C	1.3 ± 0.1%[b]	7.3 ± 0.8%[a]	$t = 10.80$, $P < 0.0001$
Injured fresh mites	IF	1.1 ± 0.1[a]	0.6 ± 0.1[b]	$t = -3.13$, $P = 0.002$
Injured fresh mites/all fresh mites	IF/F	7.2 ± 0.9%[a]	3.1 ± 0.5%[b]	$t = -3.90$, $P = 0.0001$
Injured fresh mites/trapped mites	IF/T	3.1 ± 0.4%[a]	2.1 ± 0.3%[b]	$t = -2.37$, $P = 0.019$
Injured fresh mites/colony mites	IF/C	0.1 ± 0.0%	0.2 ± 0.1%	$t = 1.43$, $P = 0.149$
Trapped injured older mites	IO	10 ± 1[a]	7 ± 1[b]	$t = -2.88$, $P = 0.004$
Injured older mites/older mites	IO/O	41.9 ± 1.7%[a]	27.0 ± 1.2%[b]	$t = -7.05$, $P < 0.0001$
Injured older mites/injured mites	IO/I	63.2 ± 2.1%[b]	80.1 ± 1.7%[a]	$t = 6.20$, $P < 0.0001$
Injured older mites/trapped mites	IO/T	24.4 ± 1.2%	21.6 ± 1.0%	$t = -1.80$, $P = 0.073$
Injured older mites/colony mites	IO/C	0.8 ± 0.1%[b]	2.5 ± 0.3%[a]	$t = 7.10$, $P < 0.0001$
Trapped injured younger mites	IY	4 ± 1[a]	2 ± 0[b]	$t = -4.64$, $P < 0.0001$
Injured younger mites/younger mites	IY/Y	26.4 ± 1.9%[a]	24.9 ± 2.8%[b]	$t = -1.02$, $P = 0.310$
Injured younger mites/injured mites	IY/I	21.3 ± 1.8%[a]	14.2 ± 1.4%[b]	$t = -3.27$, $P = 0.001$
Injured younger mites/trapped mites	IY/T	7.7 ± 0.6%[a]	4.1 ± 0.5%[b]	$t = -4.58$, $P < 0.0001$
Injured younger mites/colony mites	IY/C	0.3 ± 0.0%	0.4 ± 0.1%	$t = 0.61$, $P = 0.539$

[a] *t*-test with higher values.
[b] *t*-test with lower values.

or not injured), and recency of death (fresh or dry). *Varroa* nymphs were counted but not assessed for damage. Mites were considered injured when parts of the gnathosoma (mouthparts), legs, and ventral and dorsal 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. These characteristics remained apparent in the previously frozen mites. 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 from the 3 d of trapping were pooled for each colony. 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), O/T, O/C, trapped younger adult mites (Y), O/Y, Y/T, Y/C, trapped nymphs (N), N/T, N/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 a *t*-test to compare stocks. To determine which mite category for each stock was best related to higher or 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 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, N/T, N/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 2009). Before analyses, data were examined for anomalies and any outliers (> mean ± 3 SD) were deleted.

Results

Several differences were observed between *Varroa*-resistant RHB and *Varroa*-susceptible IHB. Overall, RHB colonies averaged 75% fewer mites than IHB colonies ($P < 0.001$; Table 1). Several measurements of trapped mites reflected this difference; T/C, O/T, O/C, O/Y, Y/C, I/C, F/T, F/C, IO/I, and IO/C all had significantly higher values for RHB colonies (Table 2). IHB colonies had significantly more numbers of younger mites among the total trapped mites along

Table 2. Results of regression analyses to relate 23 candidate measurements of mite fall with mite populations in IHB and RHB colonies

Measurement	Italian	Russian
Trapped mites (T)	$r = +0.796$	$r = +0.509$
	$r^2 = 0.634$	$r^2 = 0.259$
	$P < 0.0001$	$P < 0.0001$
Trapped older mites (O)	$r = +0.683$	$r = +0.532$
	$r^2 = 0.466$	$r^2 = 0.283$
	$P < 0.0001$	$P < 0.0001$
Trapped older mites/trapped mites (O/T)	$r = -0.292$	$r = -0.310$
	$r^2 = 0.085$	$r^2 = 0.096$
	$P = 0.004$	$P = 0.002$
Trapped older mites/younger mites (O/Y)	$r = -0.253$	$r = -0.276$
	$r^2 = 0.064$	$r^2 = 0.076$
	$P = 0.015$	$P = 0.009$
Trapped younger mites (Y)	$r = +0.792$	$r = +0.550$
	$r^2 = 0.628$	$r^2 = 0.302$
	$P < 0.0001$	$P < 0.0001$
Trapped younger mites/trapped mites (Y/T)	$r = +0.239$	$r = +0.373$
	$r^2 = 0.057$	$r^2 = 0.139$
	$P = 0.0197$	$P = 0.0002$
Trapped nymphs (N)	$r = +0.678$	$r = +0.242$
	$r^2 = 0.460$	$r^2 = 0.059$
	$P < 0.0001$	$P = 0.017$
Trapped nymphs/trapped mites (N/T)	$r = +0.253$	$r = +0.148$
	$r^2 = 0.064$	$r^2 = 0.022$
	$P = 0.013$	$P = 0.154$
Trapped injured mites (I)	$r = +0.665$	$r = +0.455$
	$r^2 = 0.442$	$r^2 = 0.207$
	$P < 0.0001$	$P < 0.0001$
Trapped injured mites/trapped mites (I/T)	$r = -0.209$	$r = +0.014$
	$r^2 = 0.044$	$r^2 = 0.0002$
	$P = 0.042$	$P = 0.896$
Trapped fresh mites (F)	$r = +0.791$	$r = +0.540$
	$r^2 = 0.625$	$r^2 = 0.292$
	$P < 0.0001$	$P < 0.0001$
Trapped fresh mites/trapped mites (F/T)	$r = +0.032$	$r = +0.000$
	$r^2 = 0.001$	$r^2 = 0.000$
	$P = 0.747$	$P = 0.954$
Injured fresh mites (IF)	$r = +0.465$	$r = +0.335$
	$r^2 = 0.216$	$r^2 = 0.112$
	$P < 0.0001$	$P = 0.001$
Injured fresh mites/fresh mites (IF/F)	$r = +0.161$	$r = +0.249$
	$r^2 = 0.026$	$r^2 = 0.062$
	$P = 0.123$	$P = 0.014$
Injured fresh mites/trapped mites (IF/T)	$r = +0.118$	$r = +0.232$
	$r^2 = 0.014$	$r^2 = 0.054$
	$P = 0.263$	$P = 0.023$
Trapped injured older mites (IO)	$r = +0.511$	$r = +0.490$
	$r^2 = 0.261$	$r^2 = 0.240$
	$P < 0.0001$	$P < 0.0001$
Injured older mites/older mites (IO/O)	$r = -0.126$	$r = +0.000$
	$r^2 = 0.016$	$r^2 = 0.000$
	$P = 0.223$	$P = 0.953$
Injured older mites/injured mites (IO/I)	$r = -0.240$	$r = -0.285$
	$r^2 = 0.058$	$r^2 = 0.081$
	$P = 0.019$	$P = 0.005$
Injured older mites/trapped mites (IO/T)	$r = -0.241$	$r = -0.048$
	$r^2 = 0.058$	$r^2 = 0.002$
	$P = 0.019$	$P = 0.641$
Trapped injured younger mites (IY)	$r = +0.618$	$r = +0.482$
	$r^2 = 0.381$	$r^2 = 0.233$
	$P < 0.0001$	$P < 0.0001$
Injured younger mites/younger mites (IY/Y)	$r = +0.010$	$r = +0.067$
	$r^2 = 0.0001$	$r^2 = 0.005$
	$P = 0.944$	$P = 0.530$
Injured younger mites/injured mites (IY/I)	$r = +0.179$	$r = +0.263$
	$r^2 = 0.032$	$r^2 = 0.069$
	$P = 0.080$	$P = 0.010$
Injured younger mites/trapped mites (IY/T)	$r = +0.120$	$r = +0.236$
	$r^2 = 0.015$	$r^2 = 0.056$
	$P = 0.243$	$P = 0.020$

with also having significantly fewer older mites among the total trapped mites. Out of the 23 measurements submitted for regression analysis, 13 were significant for positive relationships with colony mites (C) for both stocks (Table 2; Fig. 1). Of them, Y, T, and IY ranked the highest. For both stocks, all absolute measures of trapped mites grew in numbers along with colony mites. In addition, for both stocks, ratios of Y/T, N/T, IF/F, IF/T, F/T, and IY/T had positive regressions with C. For both stocks, three measurements had significantly negative regressions with colony mites (Table 2; Fig. 1). Two involved older mites, O/T and O/Y, disregarding injuries. The third was IO/I (Table 2; Fig. 1).

Discussion

Certainly, the environmental histories of the stocks were different, and direct comparisons between stocks are not experimentally valid. However, when considered as parallel natural history studies, comparisons between the stocks yield insights concerning the usefulness of measurements of trapped mites for selective breeding for resistance to *Varroa* in commercial honey bee populations. Also, data from this study are considered in the context of data from Rinderer et al. (2013), which were derived from an experimentally valid comparison of the stocks for the same parameters.

The central difference between RHB and IHB in this study is that RHB colonies had 75% fewer mites infesting them. This occurred despite the RHB having been treated for *Varroa* with a "softer" chemical and at a longer time period before sampling. The magnitude of the difference was greater than the magnitude of fewer mites (56%) reported by Rinderer et al. (2013), but many of the differences between stocks in mite-drop parameters were similar in the two studies. T/C, O/T, O/Y, O/C, Y/C, I/C, F/C, IO/C, IO/I, and IY/C are all significantly higher for RHB, which is consistent with the report of Rinderer et al. (2013). 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 RHB colonies. However, independent estimates of colony mites are time-consuming; therefore, ratios involving colony mites are not candidates for simplified selection methods. Measurements of fresh mites (IF/F, IF/T, and IF/C) were inconsistent with those of Rinderer et al. (2013), suggesting that they are subject to random variation, are not indicative of a consistent difference between the stocks, and are unlikely to reflect differences in resistance to *Varroa.*

Three measurements (O/T, O/Y, and IO/I) were significantly higher in RHB (Table 1) and had significant negative regressions with C for both RHB and IHB (Table 2; Fig. 1). These measurements were identified by Rinderer et al. (2013), who suggested that they may be good candidates for development as tools for selective breeding for *Varroa* resistance. In this study, these measurements, although statistically significant, have weaker regressions with C, which indicates that they may not be as useful for selective

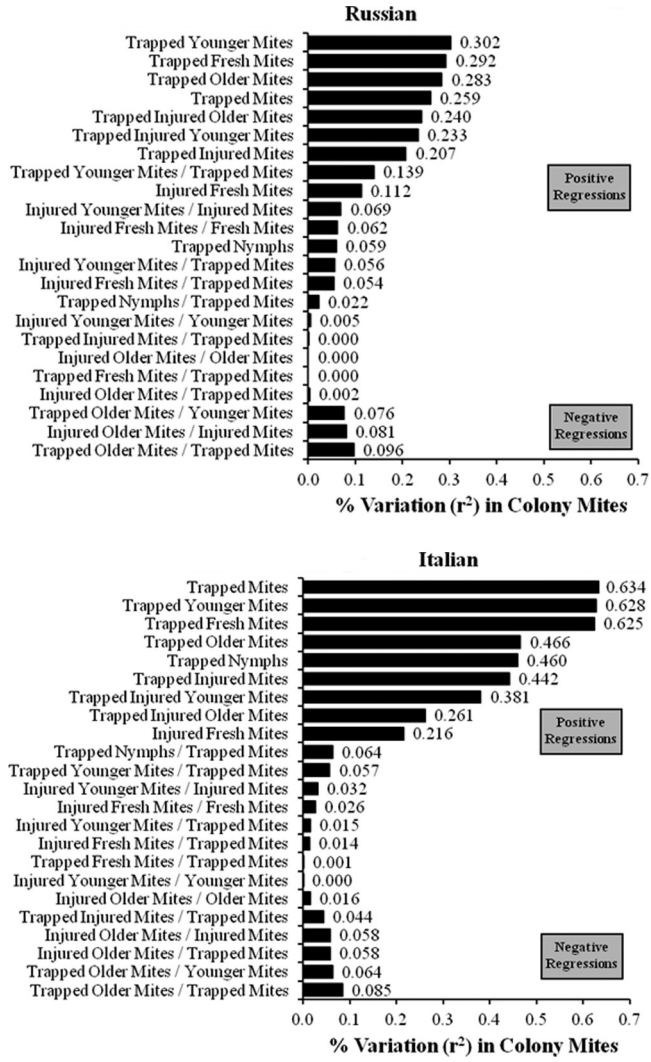

Fig. 1. The percentage variation (r^2) of total mites in a colony that was associated with each of the 23 candidate measurements of trapped mites for Italian and Russian honey bees.

breeding with colonies treated with acaracides on a commercial schedule. An important difference between the two studies is the number of mites (C) in the colonies. IHB colonies in this study averaged 1,501 ± 135 mites, whereas in the prior study, they averaged 3,964 ± 639 mites. Likewise, the RHB colonies averaged 370 ± 36 mites in this study and 1,714 ± 298 mites in the prior study. The lower numbers of mites in colonies may have negatively influenced the precision of the regressions. Total colony mites are derived from estimates of numbers of bees and brood and infestations of samples of bees and brood. A higher sampling error when infestations are low may have resulted in weaker regressions of the measurements with C. Improved methods to measure C may resolve this question. Also, measurements from colonies with fewer fallen mites can be expected to have greater random variation. Perhaps the measurements are only useful in populations that are more highly infested than those studied here.

Two estimates of C, separated by a few months during the growing season, can be used to estimate MPG (Rinderer et al. 2010). Several measurements were positively related to increased numbers of colony mites (C) (Table 2), and the strongest relationships (Y and N) may be useful for estimating C and generating estimates of MPG. Y had the strongest regression with C in both the study of Rinderer et al. (2013) and for both IHB and RHB in this study (Table 2; Fig. 1). N, first measured in this study, also had a strong regression with C for IHB but a weaker one for RHB (Table 2; Fig. 1). Hence, Y remains the best candidate among the trapped mites to use as an indicator of C and as an easier way to estimate a colony's mite population growth for selection purposes.

The proportion of injured mites (I/T), a measurement often used to indicate successful grooming (Rinderer et al. 2013), was significantly higher for IHB (37.5 ± 1.1%) than for RHB (27.7 ± 1.2%). This contrasts with the report of Rinderer et al. (2001a), who found higher rates of injury for RHB and lower rates for IHB. Perhaps the measurement is an unreliable indicator of resistance to *Varroa.* Selection for reduced MPG in RHB has produced substantial improvement in resistance to *Varroa* (de Guzman et al. 2007). It may be selection using the criterion of reduced MPG in part resulted in increased grooming that did not result in visible damage. Certainly, RHB have comparatively fewer mites infesting their colonies and have a higher proportion of trapped older mites than IHB. Some characteristics of RHB colonies result in a greater rate of loss of adult mites. Nonetheless, I/T does not appear to be a promising measurement of mite resistance for the RHB and IHB populations studied here or by Rinderer et al. (2013).

Overall, the use of measurements of fallen mites as tools for selection of increased resistance to *Varroa* requires further investigation. For colonies not treated with acaricides (Rinderer et al. 2013), numbers of young fallen mites are a good indicator of total mites in the colonies. Also, the ratios of fallen older mites to total fallen mites and those of fallen older mites to fallen younger mites are associated with reduced total colony mites, suggesting that they are indicators of resistance to *Varroa.* However, for the colonies in this study that were treated with acaracides, although these relationships were also found, they were insufficiently strong to warrant their use to select honey bees for resistance to *Varroa.* However, stronger evidence of this resistance through characteristics of fallen mites may be apparent when procedures regarding adequate mite populations or appropriate thresholds for mite drop to evaluate and compare resistance are developed.

Acknowledgments

We thank J. A. Stelzer, J. Wales, S. O'Brien, D. Dodge, and R. Watts for their technical assistance, and Coy Honey Farms and Whooten's Golden Queens for providing access to honey bee colonies.

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Received 31 October 2013; accepted 1 February 2014.