Expression of *Varroa* Sensitive Hygiene (VSH) in Commercial VSH Honey Bees (Hymenoptera: Apidae)

ROBERT G. DANKA,[1] JEFFREY W. HARRIS, AND JOSÉ D. VILLA

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

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ABSTRACT We tested six commercial sources of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), whose breeding incorporated the trait of *Varroa* sensitive hygiene (VSH). VSH confers resistance to the parasitic mite *Varroa destructor* Anderson & Trueman by enhancing the ability of the bees to hygienically remove mite-infested brood. VSH production queens (i.e., queens commercially available for use in beekeepers' production colonies) from the six sources were established in colonies which later were measured for VSH. Their responses were compared with those of colonies with three other types of queens, as follows: VSH queens from the selected closed population maintained by USDA–ARS for research and as a source of breeding germplasm, queens from the cooperating commercial distributor of this germplasm, and queens of a commercial, mite-susceptible source. The reduction of mite infestation in brood combs exposed to test colonies for 1 wk differed significantly between groups. On average, colonies with VSH production queens reduced infestation by 44%. This group average was intermediate between the greater removal by pure ARS VSH (76%) and the cooperators' breeding colonies (64%), and the lesser removal by susceptible colonies (7%). VSH production colonies from the different sources had variable expression of hygiene against mites, with average reduced infestations ranging from 22 to 74%. In addition, infertility was high among mites that remained in infested cells in VSH breeder colonies from ARS and the commercial distributor but was lower and more variable in VSH production colonies and susceptible colonies. Commercial VSH production colonies supply mite resistance that generally seems to be useful for beekeeping. Resistance probably could be improved if more VSH drones sources were supplied when VSH production queens are being mated.

KEY WORDS *Apis mellifera*, *Varroa destructor*, mite resistance, breeding

Parasitism by *Varroa destructor* Anderson & Trueman often is considered to be the most significant problem for honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), and beekeeping worldwide. Recent examples of studies in which the mites were implicated as the primary agent associated with high mortality of colonies include those by Genersch et al. (2010), Guzman-Novoa et al. (2010), and Shäfer et al. (2010). Beekeepers typically manage *V. destructor* with acaricides, but this approach has attendant and increasing disadvantages that include the development of mites that are resistant to the treatments, chemical residues in bees and hive products, and chemical synergisms that stress bees (reviewed by Rosenkranz et al. 2010).

A more suitable and sustainable solution to the *Varroa* problem is expected to be founded on bees that have genetically based mite resistance. One advance in this area has been the selection of bees that actively express the trait of *Varroa* sensitive hygiene (VSH). VSH improves the bees' ability to detect and remove brood infested with *V. destructor* (Harbo and Harris 2005). The trait confers economically useful levels of resistance to colonies by suppressing mite population growth (Harbo and Hoopingarner 1997) and diminishing the need for acaricide treatments (Delaplane et al. 2005, Ibrahim et al. 2007, Ward et al. 2008).

Although pure VSH queens yield colonies with the greatest resistance to *V. destructor*, daughters of VSH queens that outcross by mating with non-VSH (and presumably mite-susceptible) drones usually provide a useful level of resistance. Mite populations in hybrid colonies (VSH queens mated to commercial drones) remained low during tests lasting a few months (Harbo and Harris 2001). Similarly, low proportions of VSH outcrossed colonies reached economic treatment thresholds during critical periods of the annual cycle in stationary honey production apiaries (Ward et al. 2008) and under commercial migratory pollination management (unpublished data).

The VSH trait has been made available to the U.S. beekeeping industry since 2001 through a cooperative agreement between USDA–ARS and Glenn Apiaries (Fallbrook, CA). Glenn Apiaries maintains a breeding population of VSH colonies through instrumental in-

[1] Corresponding author, e-mail: bob.danka@ars.usda.gov.

semination and adds new genetic material yearly from further selections provided by our laboratory. The operation also conducts breeding to combine the VSH trait into multiple stocks that are desirable for beekeeping (Glenn 2010). Instrumentally inseminated breeder queens are then sold to producers of honey bee queens to either incorporate into their own breeding populations or to use as mothers of production queens for sale to beekeepers. The transfer of VSH technology from its source in a research program to beekeepers thus includes two stages of breeding, one stage at Glenn Apiaries and one stage by the creators of production queens. How much these intermediate breeding efforts affect the expression of the trait in VSH production queens is unclear, largely because the difficulty of measuring the trait prevents breeders, queen producers, and beekeepers from assessing it in their bees. Creating production queens also involves outcrossing the queens to multiple drones in which the VSH trait may or may not exist. There was no breeding involved in a prior test of outcrossed VSH queens from the ARS research program (Harbo and Harris 2001) and presumably little breeding in a preliminary test of VSH production queens conducted soon after the germplasm was initially released to the public (Harbo and Harris 2003).

We evaluated the degree of expression of VSH in production queens from commercial sources that represent a variety of efforts to deliver the trait for beekeeping use. Information about how well this mite resistance technology is being transferred could help beekeepers make decisions about how to use the trait, and possibly improve the delivery of the trait by USDA–ARS and bee breeders.

Materials and Methods

Queens were obtained from six commercial sources of bees with the VSH trait. These sources represent several means of incorporating the trait into queens for end use. Some simply use VSH breeder queens supplied by Glenn Apiaries to produce daughters mated to unrelated drones. Others engage in more complex breeding programs in which VSH is one trait being introgressed. The commercial VSH queens were compared with VSH breeder queens from Glenn Apiaries, VSH queens from our ARS research program, and queens from mite-susceptible stock that has low expression of VSH (controls). The control queens were purchased from a commercial U.S. source whose bees in previous tests showed relatively little hygienic removal of mite-infested brood (e.g., Danka et al. 2010). We tested an average of 5.6 (range, 2–10) colonies from each source. Thirty-eight colonies were tested in 2009 (four sources of VSH production queens [n = 3–7 colonies each], VSH from Glenn Apiaries [n = 5], ARS VSH [n = 6], and the control [n = 6]) and 12 colonies were tested in 2010 (two sources of VSH production queens [n = 2–3], ARS VSH [n = 4], and the control [n = 3]). Test queens were added to colonies housed in 1½ story hives in early May of each year. The colonies had estimated mite densities that ranged from 0.0 to 2.6 *V. destructor* per 100 bees when queens were introduced.

VSH activity was evaluated in each colony 8–10 wk after the test was set up, when all bees were from the new resident queen. To test each colony, mite infestation first was measured in a brood comb from a random mite-donor colony (mite-donor colonies were not in the test). We measured infestation in 150 newly sealed brood cells containing larvae, prepupae or white-eyed pupae. Initial infestation was 13.6 ± 4.6 (SD) % in the 50 combs of brood used to test colonies. The comb then was inserted into the broodnest of a test colony and allowed to remain for one week. The comb was retrieved and the final infestation was measured in 170 ± 24 (mean ± SD) cells containing purple-eyed and tan-bodied pupae; these were bees of the same age cohort as those initially measured (Jay 1962). The change in the infestation of brood was calculated as ([final infestation − initial infestation]/initial infestation) × 100. Changes presumably are related to hygienic removal of infested brood. Infestation occasionally (four of 50 observations) was greater in the final measure. We assumed this was due to sampling error and so used the apparent mite gain in analyses rather than adjusting the response to zero. During the final evaluation, mites in singly infested brood cells were examined to determine whether they had offspring; mites without offspring were classified as infertile.

Effects of bee type on two response variables (percentage change in infestation of brood, and percentage of infertility of remaining mites) were evaluated with analysis of variance (ANOVA). Analyses of infertility were weighted according to the numbers of cells with single foundress mites that we observed per colony (12.4 ± 7.6 [mean ± SD]). An initial ANOVA (PROC MIXED, SAS Institute 2010) included all nine sources of queens in a treatment structure that included the four general genetic types of interest (ARS VSH, Glenn Apiaries VSH, VSH production queens, and control) and the six specific sources nested within the VSH production queen type. That analysis showed no differences between the six sources of VSH production queens (Table 1). The next ANOVA included only the four general queen types as the treatment effect. Because the data were collected over 2 yr but all four queen types were not included in both years, year and year × type were included as random effects in the model. This analysis indicated that year was not an important source of variability relative to colony effects (i.e., overall error); for percentage change in infestation of brood, $\sigma^2_{\text{colony}} = 8921.99$, $\sigma^2_{\text{year x type}} = 0.14$, $\sigma^2_{\text{year}} = 0$, and for percentage of infertile mites, $\sigma^2_{\text{colony}} = 2818.70$, $\sigma^2_{\text{year x type}} = 0.04$, $\sigma^2_{\text{year}} = 0$. Means separation following a significant ($P < 0.05$) effect of genetic type was based on lack of overlap of 95% confidence intervals (*Cis*). Pearson's correlation (PROC CORR) was used to assess linear relationships between response variables. Data from commercial sources of production queens are reported anonymously.

Table 1. Expression of two variables associated with *Varroa*-sensitive hygiene when brood infested with *V. destructor* was exposed to bees of several genetic types for 1 wk

Genetic type	% change in infestation of brood			% infertile mites			
	Mean	SE	n_{col}	Mean	SE	n_{col}	n_{cells}
ARS VSH	−76a	8	10	76a	8	8	52
Glenn VSH	−64ab	11	5	55a	9	5	33
VSH production queens	−44b	5	26	17b	3	26	346
control	−7c	8	9	26b	5	5	129
ANOVA results							
Type	$F = 14.49$; df = 3, 46; $P < 0.001$			$F = 20.64$; df = 3, 41; $P < 0.001$			
VSH production queen source							
A	−74	16	2	44	11	2	
B	−49	9	7	12	6	7	
C	−48	13	3	30	9	3	
D	−47	10	5	19	7	5	
E	−36	9	6	16	7	6	
F	−22	13	3	10	9	3	
ANOVA results							
Type	$F = 15.15$; df = 3, 41; $P < 0.001$			$F = 18.52$; df = 3, 26; $P < 0.001$			
Source (type)	$F = 1.46$; df = 5, 41; $P = 0.225$			$F = 1.08$; df = 5, 4; $P = 0.495$			

Table shows results from ANOVA for principal genetic effects. Information in the top of the table is presented as pooled data from the six sources of VSH production queens; information in the bottom of the table involves the individual VSH production queen sources. Within each of the two variables, genetic types whose means do not share a letter have 95% CIs that do not overlap. Mean is the least squares mean; n_{col} is the number of colonies observed for the type; n_{cells} is the total number of cells observed in all colonies of the type.

Results

Genetic effects of queen type affected the change in infestation of brood by *V. destructor* (Table 1). Colonies of ARS VSH and Glenn Apiaries VSH reduced infestation by 76 and 64%, respectively. Infestation was reduced an average of 44% by VSH production colonies (six sources combined) and 7% by control colonies. Hypothesis testing showed that ARS VSH had greater response than VSH production colonies; Glenn VSH responded intermediately between (and did not significantly differ from) these types. Control colonies reduced infestations less than any of the three types of VSH colonies. There was substantial variability in response among the six sources of VSH production queens, with average reductions in infestations ranging from 22 to 74% among these sources. Colony-to-colony variation tended to be greater within the VSH production colonies and control colonies than within ARS VSH and Glenn VSH colonies (Fig. 1).

Genetic trends also are evident when effect size statistics (Nakagawa and Cuthill 2007) are considered. The relevant effect size in this test is the difference in mean reduction of infested brood by any two genetic types; the precision of the estimate of the effect is based on associated 95% CIs. Genetic effects were smallest (and do not differ from zero) between ARS VSH and Glenn VSH, and between Glenn VSH and VSH production queens. Effects were intermediate between ARS VSH and VSH production queens, and largest between each of the two sources of VSH breeding germplasm (ARS and Glenn) and the controls (Table 2).

Bee type also influenced the percentages of *V. destructor* that were infertile after infested brood was exposed to varying levels of hygienic manipulation (Table 1). Mite infertility was significantly greater in ARS VSH (76%) and Glenn VSH (55%) colonies than in control (26%) and VSH production (average 17%) colonies. Infertility of mites among the six sources of VSH production queens ranged from 10 to 44%.

Effect sizes associated with mite infertility were relatively large when ARS VSH was compared with all other genetic types (Table 2). Effects were insignificant between control colonies and VSH production colonies and between control colonies and Glenn VSH colonies.

Mite infertility was significantly correlated with change in mite infestation for the 45 colonies for which both responses were obtained (Pearson's $\rho = -0.448$, $P = 0.002$). This relationship was weaker among the VSH production colonies when they were considered alone (Pearson's $\rho = -0.335$, $P = 0.094$; $n = 26$).

Discussion

When considered as a group, colonies with commercial VSH production queens expressed hygiene

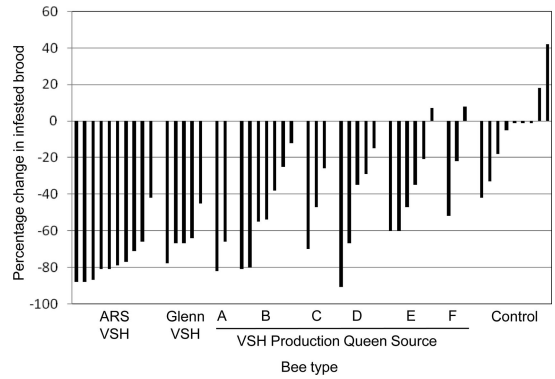

Fig. 1. Change in infestation of *V. destructor* in brood after a 1-wk exposure in individual colonies from each of the nine bee types.

Table 2. Effect size given as estimated differences (with 95% CI) between least squares means for genetic types of interest for the two variables measured

Genetic type	% change in infested brood	% infertile mites
ARS VSH vs. Glenn VSH	12 (−14, 38)	37 (17, 57)
ARS VSH vs. VSH prod. queens	32 (14, 49)	64 (49, 78)
ARS VSH vs. control	69 (47, 91)	55 (37, 74)
Glenn VSH vs. VSH prod. queens	20 (−4, 43)	27 (10, 44)
Glenn VSH vs. control	57 (31, 84)	18 (−2, 38)
VSH prod. queens vs. control	38 (19, 56)	9 (−6, 23)

that is typical of colonies from VSH queens outcrossed to non-VSH drones. Their reduction in brood infestation was intermediate between that of the high expression of pure VSH breeding material (64–76%) and the low expression of nonselected, susceptible bees (7%). The average reduction in infestation was ≈45% in the four VSH production sources that either strictly create outcrossed queens propagated from Glenn Apiaries' breeders (sources B and D with 49 and 47% removal, respectively) or that engage in more complex breeding that introgresses VSH in existing stocks (sources C and E with 48 and 36% removal, respectively). The two extremes of performance among the VSH production sources came from those that were more genetically dissimilar than were the other four sources. One source (A) consisted of daughters of ARS VSH (not Glenn Apiaries) breeder colonies; these colonies removed brood at a rate very similar to ARS VSH bees (74 versus 76%, respectively). The other source (F) consisted of second generation outcross queens (i.e., outcrosses of hybrid VSH queens); these colonies removed an average of only 22% of infested brood. The data show a trend for greater expression of *Varroa* sensitive hygiene in bees that are nearer to ARS VSH germplasm.

It is notable that there is only minor diminishment of VSH activity in the germplasm produced by Glenn Apiaries despite breeding to create multiple stocks having the VSH trait, all of which are derived from a single type of germplasm supplied by USDA–ARS. We tested their breeders designated as "VSH Yellow" and "VSH Dark," and these two types performed similarly.

Populations of *V. destructor* that remain in brood cells following hygienic activity of pure VSH bees typically are highly infertile (Harbo and Harris 2009). Such an association was clear in the ARS VSH but somewhat less in VSH from Glenn Apiaries. For the VSH production queens and control bees, and possibly for Glenn ARS bees, infertility occurred at levels that were inconsistent with their levels of hygienic reduction of mite-infested brood. A similar trend was observed in a prior test when VSH, control and hybrid colonies were compared (Villa et al. 2009). It may be that nongenetic factors that affect the infertility of *V. destructor* (e.g., temperature and relative humidity; Le Conte et al. 1990) have greater relative effects when hygienic activity is low, whereas greater hygienic removal masks these other sources of variation.

A practical interpretation of these data for beekeepers is that the average expression of hygiene in commercial VSH production queens is good. However, there is substantial variability of hygiene expressed both between commercial sources and within colonies from queens supplied by each source. This variability means that beekeepers should be vigilant in monitoring mite levels so that additional mite control activities can be undertaken if needed. Breeders of VSH should recognize that mite resistance in production queens probably could be improved if colonies with VSH drones were supplied when mating VSH production queens. Additionally, simpler techniques to determine the level of VSH in colonies would be a valuable tool to assist with selection and improvement.

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