Resistance to *Varroa destructor* (Mesostigmata: Varroidae) When Mite-Resistant Queen Honey Bees (Hymenoptera: Apidae) Were Free-Mated with Unselected Drones

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ABSTRACT This study demonstrated (1) that honey bees, *Apis mellifera* L, can express a high level of resistance to *Varroa destructor* Anderson & Trueman when bees were selected for only one resistant trait (suppression of mite reproduction); and (2) that a significant level of mite-resistance was retained when these queens were free-mated with unselected drones. The test compared the growth of mite populations in colonies of bees that each received one of the following queens: (1) resistant—queens selected for suppression of mite reproduction and artificially inseminated in Baton Rouge with drones from similarly selected stocks; (2) resistant × control—resistant queens, as above, produced and free-mated to unselected drones by one of four commercial queen producers; and (3) control—commercial queens chosen by the same four queen producers and free-mated as above. All colonies started the test with ≈0.9 kg of bees that were naturally infested with ≈650 mites. Colonies with resistant × control queens ended the 115-d test period with significantly fewer mites than did colonies with control queens. This suggests that beekeepers can derive immediate benefit from mite-resistant queens that have been free-mated to unselected drones. Moreover, the production and distribution of these free-mated queens from many commercial sources may be an effective way to insert beneficial genes into our commercial population of honey bees without losing the genetic diversity and the useful beekeeping characteristics of this population.

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

Varroa destructor ANDERSON & Trueman, formerly known as *V. jacobsoni* Oudemans (Anderson and Trueman 2000), is an external parasite of the Asian hive bee (*Apis cerana* F.) and the honey bee (*Apis mellifera* L.). These mites feed on the hemolymph of immature and adult bees. Although the traditional Asian host, *A. cerana*, is not severely affected by the parasite, infestation with *V. destructor* normally causes the death of a colony of honey bees.

Suppression of mite reproduction (SMR) is one of many characteristics of honey bees that has been associated with resistance to *V. destructor* (Büchler and Drescher 1990, Boecking et al. 1993, Büchler 1994, Spivak 1996, Thakur et al. 1997, Spivak and Reuter 1998). We focused our breeding work on suppression of mite reproduction because it had been reported in different places around the world as a trait that is associated with colonies of bees that survived an infestation of varroa (Ruttner and Marx 1984, Ritter 1990, Rosenkranz and Engels 1994, Eguaras et al. 1995). Moreover, suppression of mite reproduction was present in our breeding population in 1995; it correlated with changes in our mite populations (Harbo and Hoopingarner 1997), and it was heritable (Harbo and Harris 1999).

Suppression of mite reproduction is a genetic trait of bees that causes mites to become nonreproductive. We define a reproductive mite as one that produces at least one viable daughter (a female that reaches adulthood) after a foundress female has entered a brood cell, where it normally would reproduce. Mites that fail to produce at least one viable daughter (they may or may not lay eggs) are termed nonreproductive. These nonreproductive mites are found in nearly every colony. However, the frequency of nonreproducing mites in European honey bees is typically below 40% (Camazine 1986, Martin 1994, Rosenkranz and Engels 1994). With selective breeding, we have enhanced the expression of this trait such that 100% of the mites in the brood cells are nonreproductive.

Data suggest that there may be two traits that suppress mite reproduction; one with an immediate effect reported by Camazine (1986) and another with a delayed effect that was first described by Fuchs (1994). Both traits were found to be heritable (Harbo and Harris 1999), but the delayed trait was the basis of our selection.

The purpose of this study was to see if mite-resistant queens confer an acceptable level of resistance to their colonies when they are commercially produced and free-mated to unselected drones. If so, the commercial beekeeping system could produce and distribute queen bees that would give beekeepers some immediate relief from varroa mites. Furthermore, this procedure may enable us to insert mite-resistant genes (or any beneficial genes of the honey bee) into a

population without replacing the existing population and without changing the existing beekeeping characteristics of that population. Once in the population at a higher frequency, mite-resistant genes should be favored by natural selection. This should hasten the return of our feral population of bees and eventually eliminate the need to control *V. destructor* with chemicals.

This study tested the following hypotheses, stated below as the alternate hypotheses that we found to be correct: (1) Brood produced in colonies with resistant queens mated to unselected drones (resistant × control) has a greater percentage of nonreproductive mites than colonies with control queens. (2) Colonies with resistant × control queens have fewer mites per 100 cells of brood than colonies with control queens. (3) Colonies with resistant × control queens have a lower population of mites than colonies with control queens.

Materials and Methods

General Design. The experiment had multiple factors within a complete randomized block design conducted in Baton Rouge, LA, in 1999 and repeated with some modification in 2000.

The experiment compared the growth of mite populations in colonies of bees that each received one of the following three queen types (treatments); resistant (R × R)—queens from colonies selected for suppression of mite reproduction and artificially inseminated with drones from colonies that had also been selected for the trait; resistant × control (R × C)—mite-resistant queens, as above, but free-mated with unselected drones; and control (C × C)—queens not selected for resistance to mites and free-mated to unselected drones. We produced and inseminated the R × R treatment in Baton Rouge. The free-mated queens (R × C and C × C) were reared by four different queen producers, mated at their location (Texas, Louisiana, Michigan, or Ohio), and then sent to Baton Rouge for testing. The four cooperating bee breeders produced the R × C queens by rearing daughters from mite-resistant queens that we had sent to them and produced the C × C queens from a breeder queen of their choice.

1999 Experiment. Our experimental design in 1999 did not include the R × R treatment. It included all eight of the possible treatment × producer combinations (four commercial producers, each with two queen types), but these combinations were not equally represented at the two apiary locations. The fully balanced factorial design would have included all 16 possible treatment combinations (two apiaries × two queen types × four producers). Our test in 1999 had 10 of these possible combinations among a total of 33 colonies. We established test colonies on 21 June and 14 July.

2000 Experiment. The 2000 test was more balanced than the 1999 test. In addition to having four cooperators and two treatments (R × C and C × C) as in the 1999 test, the 2000 test had more test colonies (54) and a third location. We also included 13 colonies with R × R queens (four or five at each location). Test colonies were set up on 23 May 31 May and 6 June 2000.

Colony Setup. We established ≈22 colonies of bees at each location with uniform populations of bees and mites. To achieve uniformity, we first collected a large population (>30 kg) of mite-infested bees into a cage that had a volume of ≈340 liters (Harbo 1986, Harbo and Hoopingarner 1997). The bees were taken from colonies in the Baton Rouge area. Bees from the large cage were put into smaller cages to establish the initial bee and mite populations for all the test colonies at a location (≈900 g of bees). The number of mites was calculated from four samples of ≈150 g of bees that were collected from the large cage during the recaging process (Harbo and Harris 1999). Based on data from these samples, we calculated that each colony in 1999 started with ≈740 (first location) or ≈865 mites (second location). Colonies in 2000 (three locations), started with ≈732, ≈487, or ≈717 mites per colony.

Each colony began with a caged population of bees and mites (described above), a test queen (also caged), and five combs (each 20 by 43 cm) in a standard hive that could hold 10 combs. We immediately opened the cages to allow free movement of the worker bees within the hives, but hive entrances remained screened until the following night. Queens were released and began to lay eggs the following day. Combs were added as needed during the experimental period.

Colony Evaluation. We evaluated colonies for mite reproduction and mite population growth during and at the end of the test period that extended for 78 d in 1999 and 115 d in 2000.

Total Mite Population. At the end of the test period, we weighed the bees in each colony (colony entrances were screened at night so that all the bees were weighed), sampled adult bees for mites per 100 g of bees, measured the amount of capped brood, and estimated the mite population in the brood (using the mites per 200 cells of brood described below). From these data, we calculated the total mite population in each colony (Harbo and Harris 1999). Only adult, female mites were counted.

Suppression of Mite Reproduction. We define reproducing mites as those that produce at least one viable daughter (a daughter that could possibly reach adulthood). Nonreproducing mites are mites that enter the cell to reproduce but (1) produce no progeny, (2) produce males only, (3) produce progeny too late to mature, or (4) die in the cell before they can reproduce. Mite reproduction was measured at the end of the test period in 1999. In 2000, measurements were made 18 and 45 d after queen release.

To estimate the percentage of the mites that produced no viable progeny (percentage nonreproduction), we evaluated mites in worker brood, in cells that had progressed 8 to 11 d of their 12-d duration (the duration of the capped stage of the worker bee). Cells of this age were determined by the coloration of the bee pupa in the cell (purple-eyed pupae and older).

Table 1. Mite development in bee colonies containing mite-resistant or susceptible queens

Variable	Type of queen			*F* test = R × C vs C × C only		
	Resistant[a] (R × R)	Res. × Cntl[b] (R × C)	Control[c] (C × C)	*F*	df	*P*
1999 Experiment						
Suppression of mite reproduction on day 78		49 ± 20%	37 ± 18%	1.3	1, 5.2	0.31
Total mite population on day 78		672 ± 321	1128 ± 340	7.5	1, 4.3	0.05
Mites per 100 cells on day 78		11 ± 7	22 ± 11	5.9	1, 4.4	0.07
2000 Experiment						
Suppression of mite reproduction on day 18	64 ± 34%[e]	45 ± 20%	32 ± 18%	2.8	1, 12	0.12
Suppression of mite reproduction on day 45	100 ± 0%	58 ± 22%	40 ± 22%	4.6	1, 12	0.05
Total mite population on day 115	19 ± 20	424 ± 179	834 ± 656	5.3	1, 8.1	0.05
Mites per 100 cells on day 115	0.2 ± 0.25	4.4 ± 1.6	14.0 ± 11.1	12.9	1, 7.1	0.009
Number of cells of capped brood on day 115	2834 ± 1,432[f]	5945 ± 1,437	4393 ± 2,137	13.0	1, 12	0.004
Total adult bee weight on day 115	0.9 ± 0.4 kg[g]	1.9 ± 0.3 kg	1.6 ± 0.6 kg	8.8	1, 11.8	0.01

Data are means ± SD. SD values were calculated as colony to colony variability, pooled across location.

[a] n = 11–13.

[b] n = 16 in 1999 and 23–28 in 2000.

[c] n = 17 in 1999 and 23–26 in 2000.

[d] The mite population refers to the total number of adult female mites in the brood and on adult bees. Each colony started with about 800 mites in 1999 and 650 in 2000.

[e,f,g] The 2000 experiment compared the resistant (R × R) with the control (C × C) group to describe two areas of interest: immediate suppression of mite reproduction (*e*) and the growth of bee populations (*f* and *g*). The respective *F*, df, and *P* for these three characteristics are 15.4; 1, 9; 0.004 (*e*); 12.6; 1, 9.0; 0.006 (*f*); and 15.8; 1, 9; 0.003 (*g*). The remaining R × R values are included to serve as points of reference for the main comparisons (R × C versus C × C).

Mite progeny at this stage must be past the egg stage if there is any hope for them to become adults before the bee emerges from the cell (Harbo and Harris 1999). We found 20 mite-infested cells in each colony, classified each foundress mite as either reproductive or nonreproductive, and then calculated percentage nonreproduction for each colony. Cells with multiple foundress mites were not counted. It was not difficult to collect data at the beginning of the experiment because all colonies had hundreds of mites. However, at the end of the experiment, it was often difficult to find as many as three mites per 1,000 capped cells in some of the most resistant colonies. Therefore, we did not evaluate suppression of mite reproduction on d 115 in 2000.

Mites Per 100 Cells of Brood. We examined 200 cells of capped brood to derive this estimate. All ages of capped brood were examined by opening a row of 50 cells on each side of two combs of capped brood and counting all the live, foundress mites in the 200 cells.

Statistical Analysis. Both experiments had multiple factors within a complete randomized block design. A mixed models approach was used for the analyses of variables within each test (Proc Mixed, SAS Institute 1997). The Kenward-Roger method of estimating degrees of freedom was used in each analysis of variance (ANOVA) (SAS Institute 1997).

The 1999 experiment had three major factors in the model: (a) location of test apiary, (b) type of test queen in a colony, and (c) producer of the test queen. Not all queen producers were represented at the two apiary locations, so to account for this unbalanced design, the model for each dependent variable had four fixed effects (location, type of queen, location x type of queen, and queen producer nested within location) and one random effect (queen producer × type of queen nested within location).

Although similar to the 1999 test, the 2000 experiment was more balanced and had more observations. The factors in the model were as follows: (a) location of test apiary, (b) type of test queen in a colony, (c) producer of the test queen, (d) the interaction of queen type × producer, and (e) the interaction of location × queen type × producer. We included an R × R group in the 2000 experiment. This group was compared with the control group with regard to three variables (immediate suppression of mite reproduction, amount of brood at the end of the test, and bee weight at the end of the test). The analyses used the mixed models procedure (SAS Institute 1997).

Some of the variables resulted in unequal variances (determined by Bartlett's test for homogeneity of variance). In those cases, we forced the mixed model procedure (SAS Institute 1997) to run analyses for unequal variances. Variables evaluated with unequal variances are those with the decimal in the denominator df. The others have df in whole numbers (Table 1).

Results and Discussion

The major finding from this test was that colonies with queens selected for the suppression of mite reproduction trait possessed a significant level of resistance to mites when they were free-mated to drones at commercial beekeeping locations. Therefore, commercially produced queens (mite-resistant queens that are allowed to mate freely) should provide bee-

keepers with some immediate relief from parasitic mites. The following three measures of resistance support this conclusion and correspond to the three hypotheses in the introduction:

(1). The expression of the mite-resistant trait (the percentage of nonreproducing mites) was higher in the R × C group than in the C × C group. The difference was not statistically significant in 1999, but it was in 2000 (Table 1). However, the direction and magnitude of the differences were similar in both years (12% in 1999 and 18% in 2000; Table 1).

(2). Mites per hundred cells of brood were lower in the colonies with R x C queens than in colonies with C × C queens ($P = 0.07$ in 1999 and 0.009 in 2000, Table 1).

(3). In both 1999 and 2000, final mite populations were significantly lower in colonies with R × C queens than in colonies with C × C queens. In colonies with R × C queens, the average mite population declined by 16% during the 1999 experimental period and by 34% during the 2000 experiment. In contrast, mite populations increased in the colonies with C × C queens (70% in 1999 and 97% in 2000) (Table 1).

This was the first time that we detected suppression of mite reproduction in the first reproductive cycle (Table 1). In previous tests, our colonies with resistant or control queens had expressed identical levels of mite reproduction in the first brood cycle (Harris and Harbo 2000). Although we knew that an immediate effect did exist (Camazine 1986) and that it was heritable (Harbo and Harris 1999), our selection had been for mite reproduction that was suppressed only after a queen with resistant genes had been producing brood in a colony for ≈6 wk. We call the delayed effect SMRd and the immediate effect SMRi. We do not know if SMRi is associated with SMRd.

With respect to the growth of bee populations as measured by the number of brood cells and the weight of the adult bees at the end of the test, the R × C group was significantly better than the C × C group (Table 1). This may have been caused by the higher mite load in the C × C group or it may be that the resistant queens provided the commercial queen producers with genes that combined well with their existing stock. The important point is that bee populations showed an acceptable level of growth in colonies with R × C queens.

In contrast, the colonies with R × R queens had a slower growth of their bee populations. The number of brood cells and the weight of the adult bees was significantly less than in colonies with C × C queens (Table 1, statistics in footnotes *f* and *g*). Many colonies with R × R queens developed a very irregular brood pattern as the summer progressed, and this probably caused their relatively low population of bees at the end of the 2000 test. The queens were slightly inbred, but when choosing queens for the test, we chose only queens that had a solid brood pattern in what was then their first month of egg laying, so their poor brood pattern was not a sex allele problem. Also, it was probably not a queen problem because similar resistant queens produced very good populations of brood and bees when they were free mated to produce the R × C group (Table 1). Therefore, the problem with the R × R group was probably related to their mating: inbreeding, a detrimental side effect of the resistant trait when it is present at a high level, or a problem with the inseminations (e.g., diluting and mixing of semen, an insufficient quantity of semen, or queen storage before insemination).

This study demonstrated that selection of honey bees for a single resistant trait (suppression of mite reproduction) can effectively reduce and nearly eliminate mite populations in a bee colony. It may not be desirable to rely on a single mechanism of resistance, but resistance to mites does not necessarily require a combination of genetic effects or genetic effects combined with cultural or chemical treatments.

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