Inheritance of Resistance to *Acarapis woodi* (Acari: Tarsonemidae) in First-Generation Crosses of Honey Bees (Hymenoptera: Apidae)

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ABSTRACT The tendency of honey bees, *Apis mellifera* L, to become infested with tracheal mites, *Acarapis woodi* (Rennie), was measured in six different types of F_1 colonies. The colonies were produced by mating a stock (Buckfast) known to resist mite infestation to each of five commercially available stocks and to a stock known to be susceptible to mites. Young uninfested bees from progeny and parent colonies were simultaneously exposed to mites in infested colonies, then retrieved and dissected to determine resultant mite infestations. Reduced infestations similar to but numerically greater than those of the resistant parent bees occurred in each of the six crosses made with resistant bees regardless of the relative susceptibility of the other parental stock. Reciprocal crosses between resistant and susceptible queens and drones proved equally effective in improving resistance. Therefore, allowing resistant stock queens to mate naturally with unselected drones, or nonresistant queens to mate with drones produced by pure or outcrossed resistant queens, can be used for improving resistance of production queens.

KEY WORDS *Apis mellifera, Acarapis woodi,* genetic resistance, breeding, heritability

SOME GENETIC LINES of honey bees, *Apis mellifera* L, are comparatively resistant to infestation by parasitic tracheal mites, *Acarapis woodi* (Rennie) (Gary and Page 1987, Clark et al. 1990, Page and Gary 1990, Milne et al. 1991, Szabo et al. 1991, Rinderer et al. 1993, Danka et al. 1995, Lin et al. 1996). In colonies of resistant bees, mites are suppressed during the migratory phase of their life cycle, and mite infestations tend to remain relatively low. Because honey bee colonies often are weakened or killed when tracheal mite populations are high (Eischen 1987, Otis and Scott-Dupree 1992), mite suppression based on genetic resistance to the parasite is of interest to beekeepers and bee breeders who are trying to improve bee stocks. Tracheal mite resistance levels currently vary widely among colonies in the U.S. commercial queen breeder population (Danka and Villa 2000), which suggests that enhancement through breeding is possible.

Efforts toward breeding for tracheal mite resistance have been few, perhaps because of the limited information available about how the trait is inherited. Resistance is under genetic control and its expression can be increased by selection (Page and Gary 1990, Nasr and McRory 1998). Field observations by a beekeeper indicated that progeny colonies maintained resistance when queens of a resistant commercial stock, Buckfast honey bees, were mated to drones of a susceptible stock (Calvert 1957). In summary statements about his development of the Buckfast stock, Adam (1968, 1987) stated that his field experience over many years showed that resistance is a hereditary characteristic. More recently, Lin et al. (1996) used short-term bioassays and field evaluations to show that reciprocal hybrids of resistant Buckfast bees and susceptible bees had mite infestations that often were as low as those of the resistant parent or were intermediate between those of the parents. The issue of performance of reciprocal crosses is raised because Adam (1968, 1987) gave conflicting views about the influence of queens versus drones in passing on resistance or susceptibility. This has implications for bee breeding because breeders must know if they can maintain tracheal mite resistance by propagating just queens or drones of resistant stock or if both sexes must be provided to retain resistance in offspring.

Another commercial stock, ARS-Y-C-1, also has been shown to pass tracheal mite resistance to its offspring. Crossing ARS-Y-C-1 bees with a less resistant stock yielded hybrids that were as resistant as the ARS-Y-C-1 parent, which suggested that the trait is dominant (Rinderer et al. 1993).

We studied first-generation progeny of resistant bees to further address breeding issues. The three objectives were as follows: (1) compare the level of tracheal mite resistance in a known resistant stock, several commercially available but unselected stocks, and F_1 progeny of the commercial stocks and the resistant stock; (2) compare the level of tracheal mite resistance in a known resistant stock, a known susceptible stock and the F_1 progeny of a cross between these two stocks; and (3) evaluate possible differences in progeny of reciprocal crosses between the known resistant and susceptible stocks used in objective number 2.

Materials and Methods

Stocks and Matings. The resistant bees came from five lines of Buckfast bees imported into the United States from the United Kingdom in 1990. These bees had comparatively low tracheal mite infestation in field and laboratory tests (Danka et al. 1995, Danka and Villa 1996). The bees we used had been propagated for five generations after importation. The susceptible bees we used came from colonies chosen for their susceptibility to tracheal mite infestation in field and laboratory tests (e.g., see Danka and Villa 1996). All tests were conducted at Baton Rouge, LA, during 1995–1997.

In the first of two tests, breeder queens of five U.S. commercial honey bee stocks that had not been actively selected for resistance to tracheal mites were crossed with drones of the resistant stock. The group of commercial stocks comprised two stocks advertised to be of Italian bee (*A. m. ligustica* Spinola) ancestry, two Caucasian (*A. m. caucasica* Gorbachev) stocks, and one Carniolan (*A. m. carnica* Pollmann) stock. Daughter queens were propagated from one breeder queen of each stock and crossed to drones of nine resistant colonies by allowing bees to naturally mate at a coastal island in Louisiana where no other honey bees were present. Mated queens were used to establish F_1 colonies of which we tested seven and six, respectively, of the two Italian crosses, six of each of the two Caucasian crosses, and five of the Carniolan cross.

In a second test, resistant bees were crossed with bees of the selected susceptible stock. Reciprocal crosses were made by conducting natural matings at different times at the isolated island mating site. Queens from these matings were used to establish F_1 colonies of which we tested five of the cross of resistant queens × susceptible drones and six of the cross of susceptible queens × resistant drones.

Evaluations of Responses to Tracheal Mites. For each cross, bees of various genetic types (i.e., parent colonies and F_1 progeny colonies) were evaluated simultaneously for responses to tracheal mites as explained below. In the tests of the resistant × commercial stock crosses, bees from a colony of the susceptible stock also were included as a reference.

Bees were tested for resistance by using a bioassay similar to that developed by Gary and Page (1987). Young (<24 h old), uninfested adult bees were obtained as they emerged from individually caged brood combs held in incubators (dark, 35°C, 50–80% RH). Each of 30–40 bees per colony was coded to colony source by marking with a 1-mm dot of gloss enamel paint on the posterior abdominal tergites. Marked bees were placed into the brood nest of an inoculation colony that had ≈30–50% of resident bees infested with tracheal mites. Bioassays of each cross usually were replicated using several different inoculation colonies. The two Italian crosses were tested in two and one inoculation colonies, respectively, the two Caucasian crosses in three and two inoculation colonies, the Carniolan cross in two inoculation colonies, and the resistant × susceptible crosses in three inoculation colonies. Marked bees were retrieved from inoculation colonies after 4 d and stored frozen until the prothoracic tracheal trunks were dissected and newly infesting adult female mites between the spiracle and first tracheal bifurcation were counted. Data are reported as mite prevalence, i.e., the percentage of bees that were infested in a sample. For the resistant × susceptible crosses we also measured mite abundance, i.e., the average number of adult female mites per bee in the sample.

Data Analysis. In both tests, replication of bioassays in different inoculation colonies created randomized block designs having inoculation colony as a random effect and genetic type as a fixed effect. In addition, in the first test, data from all five of the resistant × commercial crosses were pooled, and specific commercial parents as well as F_1 progeny colonies were considered as random effects in an overall analysis of the crosses. To further test for possible interactions between genetic type and the specific commercial parents, we conducted an additional analysis in which the effect of commercial parent was designated as fixed. Effects were submitted to analysis of variance (ANOVA) using PROC MIXED of the SAS System (Littell et al. 1996). Table 1 gives the ANOVA structure for both tests. Mite infestation responses were analyzed as least squares means, and means were separated using least significant differences of the least squares means.

Results and Discussion

F_1 progeny of resistant bees routinely showed good resistance to tracheal mites in crosses made with the other parents we studied. The analysis of the pooled crosses of resistant and commercial stocks showed that the F_1 progeny had desirable resistance that was similar to that of the resistant parent (Table 2). Good resistance in the F_1s occurred despite the commercial parents having an average response to tracheal mites that was intermediate between the responses of the resistant parent and the susceptible reference stock. Furthermore, this trend of improved performance held when each of five specific commercial crosses were considered separately in the analysis, because there was no statistical interaction of effects of commercial parents and genetic type. The consistent performance of the F_1 progeny groups suggests that the trait of tracheal mite resistance features good general combining ability rather than specific combining ability, i.e., the trait is manifested in all or most crosses rather than in just some crosses.

Reciprocal crosses of resistant and susceptible stocks yielded resistant F_1 bees regardless of the sex of the resistant parent (Table 2). This result was consistent whether resistance was measured as mite prevalence or mite abundance, although the effect was greater for mite prevalence. Thus, the source of genes for resistance (queens or drones) does not change the inheritance of the trait.

Table 1. ANOVA structure of tests involving tracheal mite resistant honey bees mated to either commercial bee stocks or a stock susceptible to tracheal mites

Stock mated to resistant	Mite infestation response	Fixed effects				Random effects[b]	
		Source	F	df	$P > F$	Source	Variance[b]
Commercial	Mite prevalence	Genetic type[c]	31.63	3	<0.001	Commercial parent	0
						Inoculation colony (commercial parent)	276.5
						Commercial parent × genetic type	0
						Colony (genetic type)[d]	0
						Residual	55.0
Susceptible	Mite prevalence	Genetic type[e]	7.77	3	<0.001	Inoculation colony	348.5
						Genetic type × inoculation colony	0
						Colony (genetic type)	0
						Residual	56.6
	Mite abundance	Genetic type	3.33	3	0.12	Inoculation colony	0.154
						Genetic type × inoculation colony	0.046
						Colony (genetic type)	0
						Residual	0.018

[a] Covariance parameters.
[b] PROC MIXED calculates variance using restricted maximum-likelihood estimation, and sets random effects components with low variance to zero.
[c] For the resistant by commercial stock cross, the effect of genetic type contains each parent, all F_1 colonies and the susceptible reference colony.
[d] The effect of colony (genetic type) is calculated only for groups of F_1 progeny colonies.
[e] For the resistant by susceptible stock cross, genetic type contains each parent and all F_1 colonies.

The overall results from the two types of progeny tests indicate that tracheal mite resistance could be described as an incompletely dominant trait: F_1 colonies of a resistant parent generally had resistance that approached that of the resistant parent. Resistance is likely to be under polygenic control because it is regulated at least in part by a complicated behavioral mechanism (autogrooming by worker bees; Danka and Villa 1998). We speculate that some of the genes involved have major, dominant effects, whereas the remaining genes have additive effects.

Our results further define the inheritance of tracheal mite resistance in Buckfast bees, a phenomenon first reported by Calvert (1957) and Adam (1968). The results also support the conclusion of Lin et al. (1996), whose preliminary data indicated that hybrids tended to have the resistance of the resistant parent rather than having a strictly intermediate phenotype. Thus, bee breeders can expect to improve tracheal mite resistance relatively easily by adding resistant bees into their breeding population. Furthermore, resistant stocks can be propagated effectively without the need to make completely controlled matings of queens and drones. New production queens (i.e., those queens propagated from pure breeding stock that are to be used in field colonies by beekeepers) will produce colonies of resistant F_1 worker bees if the queens are simply reared from resistant stock; the drones with which they mate need not be of resistant stock. Such queens will also produce resistant drones that will be available for future breeding work. Conversely, queens of nonresistant stock can be mated in an area with an abundance of resistant drones (or instrumentally inseminated with semen from resistant drones) to produce resistant production colonies. In this way, bee breeders could continue selecting stock for other traits of interest (e.g., honey production), propagate queens, and incorporate tracheal mite resistance via matings to drones of resistant stock. Overall, these results lend support to the recommendation that a program of testing, selection, and propagation can be

Table 2. Tracheal mite infestations in a parental stock of tracheal mite-resistant honey bees, in another parental stock, and in first generation progeny bees

Stock mated to resistant	Mite infestation variable	Mite infestation (least squares mean ± SEM) in:			
		Resistant parent	Commercial parent	F_1 progeny	Susceptible parent (or reference)
Commercial	Mite prevalence	14 ± 6c	23 ± 6b	17 ± 4c	(40 ± 6a)
Susceptible	Mite prevalence	25 ± 12b	—	$♀_R \times ♂_S$: 31 ± 11b $♀_S \times ♂_R$: 30 ± 11b	51 ± 12a
	Mite abundance	0.38 ± .27	—	$♀_R \times ♂_S$: 0.46 ± 0.26 $♀_S \times ♂_R$: 0.40 ± 0.26	0.93 ± 0.27

Resistant bees were mated to commercial bee stocks and in reciprocal crosses to susceptible bees. Parental bees and progeny bees were simultaneously exposed to mites in infested colonies, and resulting mite infestations were measured as mite prevalence (i.e., the percentage of infested bees in a sample) or mite abundance (i.e., the average number of mite per bee in a sample). Means within rows (i.e., within a mite infestation variable within a stock cross) that are followed by different letters differ at $P \leq 0.05$ according to ANOVA and pairwise least significant difference tests.

used by beekeepers to effectively manage tracheal mite parasitism.

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