

Caste, sex and strain of honey bees (*Apis mellifera*) affect infestation with tracheal mites (*Acarapis woodi*)[★]

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Abstract. Worker honey bees from genetic strains selected for being resistant (R) or susceptible (S) to tracheal mites typically show large differences in infestation in field colonies and in bioassays that involve controlled exposure to infested bees. We used bioassays exposing newly emerged individuals to infested workers to compare the propensity for tracheal mites to infest queens, drones and workers from R and S colonies. In tests with queens, newly emerged R and S queens were either simultaneously confined in infested colonies ($n = 95$ and 87 respectively), or individually caged with groups of 5–20 infested workers ($n = 119$ and 115 respectively). Mite prevalence (percentage of individuals infested) and abundance (foundress mites per individual) after 4–6 days did not differ between R and S queens. In another test, five newly emerged drones and workers from both an R and an S colony, and a queen of one of the two strains, were caged in each of 38 cages with 20 g of workers infested at 60–96% prevalence. Infestations of the R queens ($n = 17$) and S queens ($n = 19$) did not differ significantly, but R workers had half the mite abundance of S workers, while R drones received about a third more migrating mites than S drones. In tests to evaluate possible mechanisms, removal of one mesothoracic leg from R and S workers resulted in 2- to 10-fold increase in mite abundance on the treated side, but excising legs did not affect infestation of the corresponding tracheae in drones. This suggests that differences in infestation between R and S workers, but not drones, are largely determined by their ability to remove mites through autogrooming. If autogrooming is the primary mechanism of colony resistance to tracheal mites, selection for resistance to tracheal mites using infestation of hemizygous drones may be inefficient.

Introduction

The honey bee tracheal mite (*Acarapis woodi*) is an obligate parasite of the honey bee *Apis mellifera* that likely originated in Europe and has spread to many countries of Africa, Asia and the New World (Matheson 1993). Bee colonies with high mite infestation tend to have poor winter survival (Otis and

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Scott-Dupree 1992) due to impaired thermoregulation (e.g. Nasr et al. 1999), and often have reduced honey production (Eischen et al. 1989). Research on genetic resistance to tracheal mites has focused primarily on worker bees because of the effects of high infestation on worker-based colony performance. Generally, genetically resistant colonies restrict infestations in worker bees below an economic threshold while susceptible colonies often become infested at greater, damaging levels (e.g. Danka et al. 1995; Guzman et al. 1998, 2001; Nasr et al. 2001). Autogrooming appears to be the primary mechanism of resistance in workers (Danka and Villa 1998; Pettis and Pankiw 1998; Villa unpublished obs.). Young workers use their mesothoracic legs to remove migrating female mites, but resistant workers are able to remove more mites (Danka and Villa 1998, 2003).

The influence of genetics on the susceptibility of queens and drones to tracheal mite infestation has not been investigated. Large variation in the infestation rates of both queens and drones has been reported, but it is unclear whether this variation is due to genetic resistance. Mite prevalences (% of individuals infested) in groups of queens have been reported to range from 0.7% (Fyg 1983) to 50% (Giordani 1977) in Europe, and from 2.4% (Burgett et al. 1989) to 31% (Pettis et al. 1989) in North America. Mite prevalences of drones in individual colonies range from 3 to 95% (Royce and Rossignol 1991). Young queens are much more prone to infestation than older queens (Anderson 1933; Morgenthaler 1933; Pettis et al. 1989), but a relationship between age and susceptibility has not been established for drones. The infestation of both drones (Royce and Rossignol 1991) and queens (Pettis et al. 1989) tends to increase with the level of infestation in surrounding workers. When simultaneously exposed to infested workers, young drones become infested with more mites than young workers of the same origin (Dawicke et al. 1992).

Clarifying the influence of genetic resistance, caste and sex on the potential for infestation with tracheal mites may provide guidelines for bee breeding and management. If young drones are differentially resistant to tracheal mites as genetically resistant workers are, then selection on hemizygous drones could be expected to produce significantly faster breeding progress than selection on diploid workers or queens (Griffing 1982). If queens that produce resistant workers are themselves resistant to tracheal mites, the commercial production of these queens could reduce the possibility of infestation and possible associated problems in queens.

We used bioassays to clarify the role that caste, sex and strain have on the infestation of bees with tracheal mites. In a series of experiments, young bees were exposed to highly infested workers as sources of migrating female mites. We evaluated possible differences between queens from selected resistant (R) and susceptible (S) strains in their mite infestation potential, whether drones and workers from R and S colonies show similar patterns of infestation, and if the effect of removal of autogrooming legs seen in workers is also found in drones.

Materials and methods

Bees

Queens, workers and drones came from colonies of resistant (R) and susceptible (S) strains that showed divergent responses to tracheal mite infestation in field colonies and in bioassays. In the bioassay, newly emerged workers from different genetic sources are exposed to infested bees in a common environment and then classified according to ensuing infestations of mature female “foundress” mites that have migrated into them (Gary and Page 1987). R colonies were descendants of stock originally imported from Buckfast Abbey, England in 1990. S colonies were derived from local Louisiana colonies. Colonies of each strain with the most extreme infestations in the field and in bioassays with young workers exposed to tracheal mites had been maintained at our laboratory as standards for comparative studies.

For each trial in experiments involving queens, one R and one S colony was used to produce queens. Queens were propagated by standard grafting procedures (Laidlaw and Page 1997). Depending on the experiment, queens were introduced into mite-infested units either while still in queen cells within 24 h of emergence, or when newly emerged (< 24 h) from cells. In experiments with workers and drones, combs with sealed worker and drone brood were removed from colonies, caged individually and newly emerged bees were collected for testing. If queens were included in a trial, the timing of emergence of workers, drones and queens of all colonies was coordinated so that all individuals were less than 24 h from emergence.

R vs. S Queens

Experiment 1 – queens confined in infested colonies

Two R colonies and two S colonies were used to produce queens for four trials. Vertical wooden confinement boards the length and width of a standard comb (45×16×5 cm) with 15 drilled holes (5 cm diam) were fitted with queen excluder material. A queen cell or young queen was placed into a hole. An average of 5 R and 5 S queens were used per board (range 1–15 for both types). A trial consisted of 2–5 boards from one cohort of queens which were placed sequentially within 48 h between the brood combs of a queenless inoculation colony that had a high infestation of tracheal mites (> 70% of workers infested). Queens could thus be classified by strain, trial, and confinement board within trial. Inoculation colonies were kept either in the field or in an air-conditioned room (21–24 °C, 40–70% RH).

After exposure to mite infestation for 4–6 days, both prothoracic tracheal trunks of each queen were dissected and the number of foundress female mites inside the main thoracic tracheae and in the “vestibular” entrance to the tracheae were counted. The mite prevalence (% of bees infested) and mite abundance (number of foundress mites per bee) were calculated for R and S

queens in each confinement board. Analysis of Variance (ANOVA) was used to examine the fixed effects of queen strain; inoculation colony and confinement board within inoculation colony were considered as random effects. We analyzed 95 R queens and 87 S queens.

Experiment 2 – queens caged with small groups of infested workers

Queens were reared from 3 R and 2 S colonies. For each of 8 trials, R and S queens were individually added to groups of workers taken from the honey storage areas of highly infested colonies (> 60% prevalence in workers). Queens were held either with 15–20 workers in wooden “hoarding” cages (10 × 9 × 7 cm; 4 trials) or with 5–10 workers in wooden “Benton” queen-mailing cages (4 trials). Hoarding cages had feeder vials to supply sucrose syrup and water *ad libitum*. Bees in mailing cages had access to moistened powdered sugar (queen candy). Cages were kept in an incubator (35 °C, 40–60% RH) for 4–6 days, and then queens were retrieved and mite infestations were determined. ANOVA was conducted on mite abundance to estimate the fixed effects of strain with trial as a random effect. We analyzed 119 R queens and 115 S queens.

R or S queens with R and S drones and workers simultaneously in hoarding cages

Two R and two S colonies were used to produce queens, drones and workers in two trials. A total of 38 hoarding cages (18 and 20 per trial) were prepared by removing ca. 175 workers (20 g) from honey storage areas of three highly infested colonies (60, 72 and 96% prevalence). Each hoarding cage was given an R or S queen, 5 R and 5 S workers and 5 R and 5 S drones, all < 20 h old. All test bees were marked on the abdomen with Testors® enamel paint to identify the colony origin. Cages were held in an incubator (35 °C, 30–60% RH) for 7 days, and then all living bees were frozen. Queens and all marked drones and workers were dissected to determine mite abundances. For comparisons between R and S workers and drones, ANOVA was used to compare mite abundance among the four combinations of sex and strain, with the effects of trial and cage within trial as random effects. For comparisons between 17 R and 19 S surviving queens, the average mite abundance of drones and workers caged with individual queens was used as a covariate in an analysis of covariance that incorporated the effects of trial and cage as random effects. This separate analysis was necessary because only one free-running queen could be placed in each hoarding cage.

R and S workers and drones with impaired autogrooming ability in infested colonies

We impaired the autogrooming ability of R and S drones and workers in five trials. For each test drone or worker, the mesothoracic leg on a randomly

selected side of the body was amputated at the femur (Danka and Villa 2003). Treated bees were paint marked and placed into an infested colony for 4–5 days, then retrieved and dissected. ANOVA was used to evaluate mite abundance in the treated (amputated) side and in the control side, and the difference in mite abundance between the two tracheae in each bee. For each one of the three variables, the four combinations of strain and sex were considered as fixed effects, and trial was a random effect. We analyzed 42 R workers, 19 R drones, 17 S workers and 17 S drones.

Results

Tracheal mite infestations of R and S queens were low and not significantly different when queens were confined in colonies infested at prevalences higher than 70% ($p = 0.61$ and 0.60 respectively for the variables prevalence and abundance, Table 1). Prevalences within confinement boards ranged from 0 to 43% for R queens and from 0 to 33% for S queens. Infestation also did not differ between R and S queens when individual queens were confined in mailing cages or in hoarding cages with infested workers ($p = 0.92$ and 0.14 respectively for the variables prevalence and abundance, Table 1).

When a R or S queen, and workers and drones of both strains, were simultaneously exposed to infested workers in hoarding cages, several patterns of infestation were evident. R workers acquired significantly fewer mites than S workers (Table 2). In contrast to workers, R drones became more infested than S drones (Table 2). There were no significant differences in infestations of R and S queens ($p = 0.27$). The structure of the experiment did not allow comparisons of the average infestation of queens with those of workers and drones. However, mite abundances in queens were usually (29 of 36 cages) lower than the average of R and S drones and workers in the same cage.

Impairment of autogrooming by removing one of the mesothoracic legs significantly increased the infestation in the trachea on the treated side for both R and S workers (Table 3). In drones, however, removing a leg did not create a significant difference between treated and control sides ($p = 0.34$ and 0.64 for

Table 1. Infestations with tracheal mites in queen honey bees after exposure to infested workers for 4–6 days. Individual queens were either confined simultaneously in holding boards in infested colonies, or caged with 5–20 workers taken from infested colonies. Data are given as least squares means \pm s.e. There were no significant differences ($p > 0.05$) in the prevalence or abundance of foundress mites between R and S queens.

Strain	Queens in confinement board in infested colonies			Queens exposed in cages with 5–20 workers		
	Prevalence (%)	Abundance (mites/bee)	<i>n</i>	Prevalence (%)	Abundance (mites/bee)	<i>n</i>
Resistant	13 \pm 3	0.19 \pm 0.05	95	59 \pm 8	1.52 \pm 0.31	119
Susceptible	10 \pm 4	0.15 \pm 0.06	87	58 \pm 9	1.10 \pm 0.31	115

Table 2. Mite abundances [least squares means \pm s.e. (*n*)] in workers, drones and queens after bees were confined with ca. 20 g of infested workers. Significant differences ($p < 0.05$) among the four means of resistant and susceptible workers and drones are indicated by different letters following the means. Abundances in resistant and susceptible queens did not differ significantly ($p > 0.05$). Infestations in queens could not be compared to those of workers and drones owing to experimental constraints.

Strain	Sex or Caste		
	Worker	Drone	Queen
Resistant	0.43 \pm 0.47 (170) c	1.58 \pm 0.47 (172) a	0.70 \pm 0.25 (17)
Susceptible	0.97 \pm 0.47 (171) b	1.06 \pm 0.47 (150) b	0.17 \pm 0.24 (19)

Table 3. Mite abundance (least squares means \pm s.e., with (*n*) in column head of each group) in each of the two main tracheal trunks of resistant and susceptible workers and drones, and the difference in the infestation between tracheae on the treated (T) side and control (C) side of each individual. Means within a row that are significantly different ($p < 0.05$) are indicated by different letters. Additionally, the difference in infestation between the two sides of each individual were calculated, and differences within the four sex and strain combinations that are significantly different from 0 ($p < 0.05$) are indicated by an *.

	Resistant		Susceptible	
	Workers (42)	Drones (19)	Workers (17)	Drones (17)
Control (C)	0.23 \pm 0.09 c	1.05 \pm 0.34 ab	1.52 \pm 0.31 a	0.71 \pm 0.22 bc
Treated (T)	2.28 \pm 0.28 a	0.63 \pm 0.24 b	3.35 \pm 0.31 a	0.41 \pm 0.17 b
T-C	1.90 \pm 0.37*	-0.48 \pm 0.49	1.98 \pm 0.55*	-0.24 \pm 0.50

R and S drones respectively). The infestation of tracheae on the control side was significantly higher in S workers than in R workers. In the control side of drones, the infestations were similar for R and S drones and were intermediate between those of R and S workers (Table 3).

Discussion

Patterns of infestation varied markedly across castes and sexes of honey bees from R and S strains. As expected from prior research, workers from R colonies consistently became less infested than those from S colonies. However, this differential infestation was not expressed in queens and drones. In three different experiments, statistical tests indicated that R queens were at least as susceptible to infestation as S queens. Furthermore, the numerical values for mean abundance in two of the experiments (and relatively low *p* values associated with the test for differences), suggest that R queens may be more susceptible than S queens under some conditions. For drones, the pattern of infestation was clearly opposite that of workers: R drones became significantly more infested than S drones.

The discrepancy of resistance patterns in drones and queens, when compared to those of workers, raises several issues about mechanisms and breeding of resistance. The finding that R drones had significantly higher infestation than R workers matches the trend reported by Dawicke et al. (1992) in a stock with unknown resistance of workers. The three tests we conducted on queens show that R queens are not any more resistant than S queens, and could potentially be more prone to infestation in a pattern similar to drones. Thus, tracheal mite infestation in drones and queens appears to be regulated differently than in workers. It is possible, for example, that all members of our R colonies had a relatively greater attraction to migrating female mites, perhaps mediated by cuticular compounds (Van Engelsdorp and Otis 2001). R workers – but not R drones – might be able to compensate for this higher attraction by effectively autogrooming and ridding themselves of migrating mites before mites enter the tracheae. Thus, genetic resistance as defined by worker response to tracheal mites does not significantly protect queens and drones from infestation.

It is not known whether tracheal mite infestation impairs performance of queens or drones, but caution by beekeepers would seem warranted. Queens and drones of R strains may be placed at risk by common beekeeping practices. Most notably, when queen cells are placed in mating colonies, queens may become infested if the resident workers are infested. Such a situation may be common, as two surveys in the 1990's found that about a fifth of U.S. commercial queens were infested with tracheal mites (Burgett and Kitprasert 1992; Camazine et al. 1998). Similarly, if drone brood is moved from source colonies to nurse colonies infested with tracheal mites, newly emerged drones could become infested.

With regard to breeding, selection for improved worker autogrooming by propagating hemizygous drones with the lowest mite infestations seems unlikely to be effective given that infestation in R and S drones does not parallel that of workers. Alternatively, the finding that drones do not autogroom successfully may allow the use of drones for selection of other mechanisms of resistance such as relative attractiveness to mites. This selection approach would be most effective if drones and workers of the same genotypes show similar cuticular chemical profiles as they age.

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