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Evidence of autogrooming as a mechanism of honey bee resistance to tracheal mite infestation

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SUMMARY

Infestations of tracheal mites (*Acarapis woodi*) were measured in honey bees (*Apis mellifera*) whose autogrooming ability was compromised by having legs or segments of legs amputated. Bees of two stocks, one more resistant (Buckfast) and one more susceptible to tracheal mite infestation, were tested by performing amputations on uninfested, young (0–24 h) adult bees, exposing the treated bees to mites in infested colonies, then retrieving and dissecting the bees to measure parasitism. In both stocks, bees that had mesothoracic legs amputated had greatly increased mite abundances. However, the relative increase in infestation was greater in resistant bees. Mite infestation increased as more (0 *vs.* 1 *vs.* 2) mesothoracic legs were removed. In bees with only one leg removed, mite infestations were greater on the treated side. In subsequent tests with resistant stock bees only, removing the mesotarsi resulted in infestations equalling those found when entire mesothoracic legs were removed, but amputating the four distal mesotarsomeres or the metatarsi resulted in less significant increases. Restraining rather than removing mesothoracic legs also resulted in increased infestation. Young (0–24 h) bees were more affected than older (3–4 day) bees by leg removal, indicating that a factor other than autogrooming accounts for the low susceptibility of older bees to tracheal mites. Together these results are evidence that autogrooming is an important mechanism of protection against tracheal mites, especially in bees known to have genetically-based resistance to the parasite.

INTRODUCTION

Recent research has shown that some lines and stocks of honey bees are comparatively resistant to infestation by parasitic tracheal mites, *Acarapis woodi* (Gary & Page, 1987; Clark *et al.*, 1990; Page & Gary, 1990; Milne *et al.*, 1991; Szabo *et al.*, 1991; Danka *et al.*, 1995; Lin *et al.*, 1996; Guzman *et al.*, 1996). Little has yet been learned, however, about what regulates resistance. In one stock of bees, mite infestation remains low because the migration of mature, mated female mites from older infested bees to young uninfested bees is suppressed (Danka & Villa, 1996). The mechanisms that interfere with this migration are unknown. One relevant behavioural trait may be autogrooming by bees, i.e. individuals cleaning themselves of foreign material. Mesothoracic legs are used to groom the thorax (Snodgrass & Erickson, 1992), and thus might be important in eliminating mites that approach the spiracles to enter the tracheae.

Autogrooming was investigated once previously (Lee, 1963) as a potential factor accounting for the long-recognized low susceptibility of older bees to tracheal mites (e.g. Morganthaler, 1930). Lee sought to determine if old bees were protected by grooming with the mesothoracic legs, by the presence of the 'guard hairs' surrounding the spiracular vestibule, or by the spiracular closing mechanism. He concluded that none of these features significantly decreased the success of mites in entering the spiracles when mites were placed directly on older bees, although leg removal or restraint tended to result in increased mite infestation. Young bees were not evaluated thoroughly, and different bee stocks were not evaluated at all.

Autogrooming often occurs as part of a 'dance' in which allogrooming (i.e. the cleaning of nestmates) is solicited (Haydak, 1945). In a test of the relationship of grooming dance frequency and mite infestation among four lines of bees, the line with most dancing had lowest mite infestation (Pettis & Pankiw, 1994; Pettis & Pankiw, 1998). As a component of such grooming dances, autogrooming may have contributed to lower infestation.

We investigated whether restricting the ability of bees to autogroom, by removing legs or segments of legs, influenced the probability of bees becoming infested by tracheal mites. More importantly, we investigated how these infestation responses varied in stocks of bees that are resistant or susceptible to tracheal mites. This permits insight into the importance of autogrooming as a genetically regulated mechanism of tracheal mite resistance in honey bees.

MATERIALS AND METHODS

Bee stocks

The resistant stock of bees we studied, Buckfast, came from five lines imported into the USA from the UK in 1990. These bees had comparatively low tracheal mite infestation during both field and laboratory tests (Danka *et al.*, 1995; Danka & Villa, 1996). The susceptible stock of bees originated from colonies from Louisiana, USA, that had comparatively high mite infestation in the studies cited above. For each stock, we screened the available colonies during five generations of selection and used bees from six of the most resistant colonies and five of the most susceptible colonies in the experiments described below.

General test procedure

The responses of treated bees (those having legs altered) and control bees (handled similarly but without leg alteration) to tracheal mites were evaluated using a standard procedure: test bees were exposed to mite-infested bees for several days, then retrieved and dissected to determine mite infestations (Gary & Page, 1987). Young (0–24 h) uninfested adult bees were obtained as they emerged from brood combs held in incubators (dark, 35°C, 50–80% RH). Thirty to 80 bees per treatment per colony were coded to colony source with a dot (c. 1-mm diameter) of gloss enamel paint put on abdominal tergites V–VI. Microdissection spring scissors then were used to amputate legs (at the coxal-trochanteral joint) or segments of legs (at the proximal joint of the segments of interest) of treated bees of various stocks and ages; details of treatments follow for each of the six experiments we conducted. Treated bees and control bees were placed into the brood nests of inoculation colonies (having about 40–60% of bees infested with mites) or into cages containing mite-infested bees. In experiments 1 and 2, bees of both stocks were exposed simultaneously to mites. Test bees were retrieved after four days; overall, 54% of 1816 treated bees and 88% of 1063 control bees were recovered. Bees were stored frozen until the thoracic tracheal trunks were dissected and newly infesting adult female mites between the spiracle and first tracheal bifurcation were counted. Experiments were conducted during spring and autumn 1996 at Baton Rouge, Louisiana, USA.

Statistical analyses

Data are reported as mite abundance (the average number of mites per bee among all bees in a sample) for all experiments. In experiments having one-sided treatments, mites per trachea in addition to mite abundances are reported. For experiments 1 and 2, we also analysed mite prevalence (the number of infested bees out of all bees in a sample, and reported here as a percentage). Experiments with both bee stocks consisted of factorial treatment arrangements of bee stock and treatment (fixed effects) in a randomized block design. The fixed effects were assessed by analysis of variance using PROC MIXED of the SAS System (Littell *et al.*, 1996). Mite counts were transformed to $x^{1/2}$ to homogenize variances because counts tended to have variances in proportion to the colony means. Responses for all parameters were analysed as least squares means

FIG. 1. Treatments applied in experiment 2, and results from each side of bees in the three treatment groups. The boxed areas indicate the legs removed from bees of two stocks (R, resistant stock bees; S, susceptible stock bees). Similar amputations were made in experiments 1, 5 and 6. Data are mite abundance ($\bar{x}$ ± s.e. of nontransformed data). Asterisks indicate that means within these bees differed at $P < 0.001$ according to paired t tests.

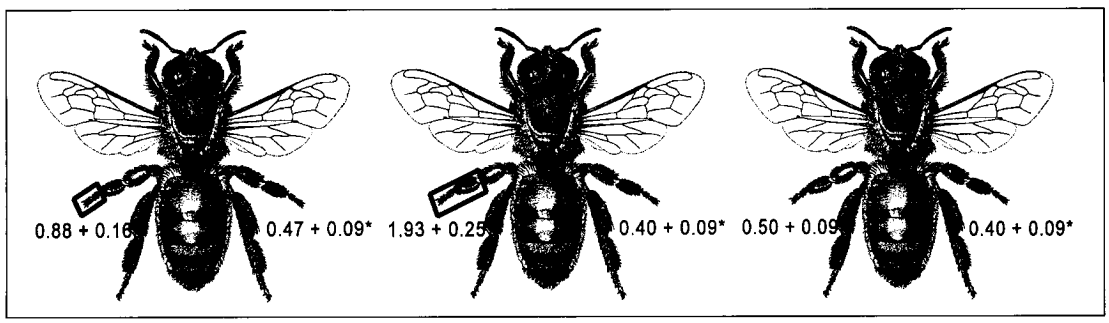

FIG. 2. Treatments applied and results obtained in experiment 3. The boxed areas indicate the leg segments amputated from resistant stock bees (bee on left, the four distal mesotarsomeres; bee in centre, the mesotarsus). Similar amputations were made in experiment 4 (but included the mesothoracic legs (as shown in fig. 1 on right) and the metatarsi). Data are as in figure 1.

(SAS Institute, 1989) because of unbalanced cell sizes. Mean separation was done using least significant differences calculated for pairwise comparisons of means.

Experiment 1

This test measured the overall effect of removing both mesothoracic legs. Treated bees had both mesothoracic legs amputated. Resistant and susceptible stock bees were tested in seven trials. In two of these trials, bees were exposed to mite-infested bees not in inoculation colonies but in cages containing infested bees (250 g and 422 g of bees, respectively, each with 73% prevalence and held at 23°–25°C and 66–75% RH).

Experiment 2

The effect of removing different numbers of mesothoracic legs was investigated. Bees having zero, one or two mesothoracic legs amputated (fig. 1) were compared for mite abundance. Resistant and susceptible stock bees were tested in three trials. For bees that had one leg removed, mite abundances in the tracheae of the treated side were compared to those of the con-

TABLE 1. Tracheal mite abundance in honey bees after bees had legs or leg segments amputated. Mean variance are based on least squares means of transformed data (S = stock; T = treatment; A = age). Mea differen

	Bee stock	Treatment (legs or leg segments removed)	*n*	Abundance ($\bar{x}$ ± s.e.) raw data
Experiment 1	resistant	2 mesothoracic legs	257	2.58 ± 0.11
		control	331	0.74 ± 0.06
	susceptible	2 mesothoracic legs	146	3.15 ± 0.20
		control	309	1.76 ± 0.10
Experiment 2	resistant	2 mesothoracic legs	151	2.54 ± 0.17
		1 mesothoracic leg	149	1.82 ± 0.14
		control	94	0.30 ± 0.08
	susceptible	2 mesothoracic legs	58	3.91 ± 0.38
		1 mesothoracic leg	140	2.98 ± 0.17
		control	116	1.96 ± 0.17
Experiment 3	resistant	1 mesotarsus	40	2.32 ± 0.24
		4 distal mesotarsomeres of 1 leg-	51	1.35 ± 0.21
		control	80	0.90 ± 0.14
Experiment 4	resistant	2 mesotarsi	11	1.55 ± 0.39
		2 metatarsi	6	0.33 ± 0.21
		2 mesothoracic legs	33	1.58 ± 0.26
		control	45	0.16 ± 0.05
Experiment 5	resistant	bees 0–24 h:		
(trial 1)		2 mesothoracic legs	27	1.04 ± 0.22
		control	49	0.12 ± 0.05
		bees 3–4 d:		
		2 mesothoracic legs	23	0.17 ± 0.10
		control	54	0.04 ± 0.03
Experiment 6	resistant	2 mesothoracic legs		
(trial 1)		removed	14	3.50 ± 0.33
		2 mesothoracic legs restrained	12	5.58 ± 0.43
		control	31	1.32 ± 0.23

s are presented for raw data and as least squares means of data transformed to $x^{1/2}$. Results of analysis of ns within an experiment not followed by the same letter differ at $P \leq 0.05$ according to a least significant ce test.

least squares means, transformed data	Results of analysis of variance effect	*F*	*d.f.*	*P*
1.48 ± 0.18a	S	7.47	1, 6	0.033
0.55 ± 0.17c	T	68.29	1, 13	< 0.001
1.61 ± 0.18a	S X T	6.97	1, 13	0.021
1.13 ± 0.17b	max. least significant difference = 0.34			
1.44 ± 0.31ab	S	10.30	1, 2	0.085
1.17 ± 0.31ab	T	27.17	2, 7	< 0.001
0.37 ± 0.32c	S X T	2.84	2, 7	0.123
1.79 ± 0.31a	max. least significant difference = 0.57			
1.59 ± 0.31a				
1.22 ± 0.31b				
1.41 ± 0.11a	T	16.35	2, 168	< 0.001
0.89 ± 0.10b	max. least significant difference = 0.29			
0.65 ± 0.08b				
1.08 ± 0.16a	T	22.37	3, 91	< 0.001
0.33 ± 0.22b	max. least significant difference = 0.54			
1.07 ± 0.09a				
0.16 ± 0.08b				
	T	35.84	1, 149	< 0.001
0.80 ± 0.07a	A	31.26	1, 149	< 0.001
0.12 ± 0.05b	TXA	18.41	1, 149	< 0.001
	max. least significant difference = 0.21			
0.15 ± 0.08b				
0.04 ± 0.05b				
	T	31.19	2, 54	< 0.001
1.84 ± 0.15b	max. least significant difference = 0.33			
2.34 ± 0.17a				
0.92 ± 0.10c				

trol side by a pairwise *t* test; these comparisons were made in bees within each stock.

Experiment 3

We measured infestation resulting when different portions of the mesothoracic legs were removed. Young resistant stock bees had either the mesotarsi or the four distal mesotarsomeres amputated from one side only (fig. 2) in one trial.

Experiment 4

The importance of mesothoracic leg segments or metathoracic leg segments *versus* entire legs was tested. Young resistant stock bees were treated by amputating either mesotarsi only, metatarsi only, or mesothoracic legs from both sides in one trial.

Experiment 5

The effect of bee age on mite infestation when legs are removed was investigated. Young (0–24 h) and old (3–4 day) resistant stock bees were treated by having both mesothoracic legs amputated. Older bees were obtained by holding newly emerged bees in cages supplied with sucrose solution and water for three days in an incubator. Two trials were conducted; these were analysed separately because inoculation conditions differed significantly between trials.

Experiment 6

We assessed the possibility that mite infestation increased in groups of treated bees because of the injury associated with amputating legs, i.e. perhaps migrating mites are attracted to such injury. Young resistant stock bees were treated in one of two ways. In one group, each bee had the mesothoracic legs glued together at the distal tarsomeres with a small drop of fingernail lacquer, while the legs were positioned beneath the thorax. Another treated group had both mesothoracic legs amputated. Two trials were conducted and analysed separately.

RESULTS

Experiment 1

Removing both mesothoracic legs resulted in greatly increased mite abundances in young bees of both resistant and susceptible stocks. Abundances in untreated susceptible bees were intermediate between the low infestations of untreated resistant bees and the high infestations of bees without mesothoracic legs (table 1). This pattern resulted in a significant statistical interaction of bee stock and treatment effects: removing mesothoracic legs resulted in a greater increase in mite abundance in resistant bees than in susceptible bees.

Responses measured as mite prevalence were similar to those for mite abundance. When mesothoracic legs were amputated, the increase in prevalence in resistant bees (from 44 ± 7% to 90 ± 7%) was significantly greater ($F = 15.27$; d.f. = 1,12; $P = 0.002$ for the interaction of stock and treatment effects) than the corresponding increase in susceptible bees (from 77 ± 7% to 92 ± 7%).

Experiment 2

Mite abundance increased in both bee stocks as more mesothoracic legs were removed (table 1). Treated bees (two legs amputated) of both types had statistically similar mite abundances, untreated susceptible bees had intermediate infestation, and untreated resistant bees were least infested.

In treated bees of both stocks that had only one leg removed, mite abundance was greater on the treated side than the untreated side (paired *t* tests, $P < 0.001$ for both bee stocks) (fig. 1).

Responses measured as mite prevalence again were similar to those of mite abundance. A statistical interaction of the effects of stocks and treatment was found ($F = 62.16$; d.f. = 1,7; $P < 0.001$). Prevalences were similar and high (82 ± 10% to 92% ± 10% of bees infested) in resistant and susceptible bees that had one or two legs removed. Untreated resistant bees had low prevalence (27 ± 10%) and untreated susceptible bees had intermediate prevalence (81 ± 10%) that was statistically similar to that of treated resistant bees but less than treated susceptible bees.

Experiment 3

Although mite abundance in resistant bees increased as more segments of the mesotarsus were removed, this increase was significant only when all of the tarsus, not just the four distal tarsomeres, was amputated (table 1; fig. 2).

Experiment 4

Amputating mesotarsi resulted in increased mite abundances that were similar to those found when entire mesothoracic legs were removed (table 1). Amputating metatarsi had relatively little effect on subsequent mite abundances.

Experiment 5

In both trials, mite abundances were greater in young (0–24 h) than in old (3–4 day) bees, and bees with mesothoracic legs removed were more highly infested than control bees (table 1). Responses to treatment were dissimilar in bees of different ages; amputating mesothoracic legs resulted in significantly increased mite infestation (relative to control bees) in young bees but not in old bees. Results shown in table 1 are from trial 1; relative responses were similar in trial 2 but overall infestations were greater, and untreated young bees were infested at levels intermediate between treated young bees and both groups of old bees.

Experiment 6

Mite abundance was greater when mesothoracic legs were restrained than when legs were amputated. Bees treated by either restraining or removing legs had greater infestation than untreated bees. Similar results were obtained in the two trials, but only one trial is reported in table 1. These results do not support the hypothesis that increased mite infestation resulted from injury associated with amputating legs.

DISCUSSION

These experiments provide strong circumstantial evidence that individual honey bees resist tracheal mite infestation by effectively autogrooming and ridding themselves of migrating female mites. Effective autogrooming was not possible when the mesothoracic legs were removed or significantly immobilized. Mite infestation increased as more legs were amputated, and infestation was significantly greater on the side of bees from which one leg or important leg segments were removed. The infestation responses were consistent when measured as mite abundance and as mite prevalence.

Most importantly, impairing autogrooming by removing legs caused greater increases of mite infestation (when treated bees were compared to untreated bees) in resistant stock bees than in susceptible stock bees. This finding suggests that autogrooming is an important factor governing mite resistance in Buckfast bees; as such, it is the first reported specific mechanism of tracheal mite resistance in honey bees. It previously was determined that resistance is founded on the suppression of bee-to-bee migration of female mites (Danka & Villa, 1996). Effective autogrooming of migrating mites appears to keep tracheal mite populations in this stock below levels that cause significant colony mortality (i.e. below mite prevalences of approximately 20% during autumn; Otis & Scott-Dupree, 1992). Resistant bees with only one mesothoracic leg were as well protected from infestation as untreated susceptible bees.

The entire mesotarsus was necessary for effectively removing mites. Bees without this leg segment became as highly infested as bees missing the entire mesothoracic legs. Bees retaining the mesobasitarsus, but lacking the four distal tarsomeres, were able to resist infestation nearly as well as normal bees. Metatarsi were not necessary for effective autogrooming. In subsequent tests we have directly observed (unpublished) the mesothoracic legs being used to groom and remove tracheal mites that were placed on the setae of the thoracic nota or pluera of bees in observation hives and of bees restrained under a dissecting microscope.

Autogrooming, however, may not be the only mechanism of resistance in these bees. Susceptible bees with two legs amputated had slightly greater numerical infestations than resistant bees with two legs amputated. This suggests another factor contributes to resistance in Buckfast bees.

Results from experiment 5 support Lee's (1963) conclusion that removing or restraining the mesothoracic legs does not significantly alter the normally low susceptibility of older bees to infestation. This 'age-related resistance' is apparently founded on another characteristic, perhaps, as Lee posited, decreased attractiveness of older bees to mites. Attractiveness may be mediated by cuticular chemistry; the composition of cuticular hydrocarbons changes rapidly in the first few days of the adult life of honey bees (Francis *et al.*, 1989), and these chemicals have been shown to influence mite movement in *in vitro* tests (Phelan *et al.*, 1991).

The results obtained for the two stocks of bees we studied may or may not be consistent for other bee types that resist mite infestation. The amputation and restraint treatments we describe here and the colony assay should provide a useful system for evaluating tracheal mite resistance in other honey bee types while simultaneously assessing the contribution of autogrooming as a mechanism supporting resistance.

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