

ORIGINAL ARTICLE



Autogrooming and bee age influence migration of tracheal mites to Russian and susceptible worker honey bees (*Apis mellifera* L)

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Summary

The role of autogrooming in controlling tracheal mites in Russian and susceptible colonies was evaluated by gluing together the midlegs of workers and exposing them to mite infestation. In one experiment, young workers (less than 10 h old) from both strains having midlegs glued together at the tarsi (G) or left unglued as controls (C) were introduced into colonies with 50 to 75% of workers infested and retrieved after five or six days. G Russian and susceptible workers had very high mite abundances (8.2 and 7.9 female mites/worker). However, C workers had significantly lower infestations, and strains differed significantly (Russian = 1.5, Susceptible = 3.0 female mites/worker). In a second experiment, C and G workers of both strains and from age classes produced every 24 h (1, 2, 3, or 4 d-old), were simultaneously introduced into an infested colony. Infestation of bees declined significantly in older bees. Differences due to gluing of midlegs and strain became less marked with increasing age of bees. The majority of mites infested the tracheae of workers less than 48 hours old. The autogrooming efficacy of Russian workers during this critical period of 48 hours of high potential for mite migration appears to explain the resistance of their colonies to tracheal mites.

Keywords: *Acarapis woodi*, *Apis mellifera*, grooming, parasitic mites, tracheal mites, honey bees, resistance, susceptibility

Introduction

Honey bee colonies with genetic resistance can prevent problems with tracheal mites (*Acarapis woodi*) because they are able to maintain mite populations below harmful levels (see Danka, 2000 for examples). Despite the reliability and low cost of this genetic solution, commercially available bees in the United States are variable in their susceptibility to these mites (Danka & Villa, 2000; unpublished observations). Unfortunately, other concerns, particularly the more urgent need to address resistance to *Varroa destructor*, are preventing breeding programs from concentrating on achieving useful levels of genetic resistance to tracheal mites. Bees imported to the United States from southeastern Russia due to their resistance to varroa mites (Rinderer *et al.*, 2001) have shown high resistance to tracheal mites in field tests (Guzman *et al.*, 2001a, 2001b, 2002) and are currently available to beekeepers (Rinderer *et al.*, 2005).

In the last ten years, several potential mechanisms of bee resistance to tracheal mites have been evaluated, but only autogrooming has been shown to explain the resistance of young

(<24 h old) workers. Given the reduced movement of female mites into the tracheae of older workers (Morgenthaler, 1930; Lee, 1963; Gary *et al.*, 1989) and further evidence of mediation of mite attraction through cuticular chemicals (Phelan *et al.*, 1989), an early hypothesis was that reduced attraction of young resistant bees could explain their resistance. While there may be minor differences in cuticular extracts from young resistant and young susceptible workers (Danka & Villa, unpublished observations), they do not adequately explain the resistance of individual colonies (Van Engelsdorp & Otis, 2001). Likewise, neither the presence of hairs near the spiracular opening (Lee, 1963; Danka & Villa, 1999) nor morphology of spiracles (Hatjina *et al.*, 2004) have been shown to act as barriers to tracheal mite migration to a level that explains resistance. In contrast, the presence of intact midlegs (and presumably their use in autogrooming) greatly decreases the number of migrating female mites that infest young workers (Danka & Villa, 1998). More importantly, there are significant differences between resistant and susceptible bees in their propensity to autogroom in response to the presence of tracheal mites (Pettis & Pankiw, 1998; Danka & Villa, 2003).

Autogrooming by workers involves midleg movements anteriorly over dorsal areas of the thorax and is usually accompanied by a grooming dance (Haydak, 1945; Land & Seeley, 2004). This grooming dance was described prior to detection of tracheal mites in the U.S. and occurs even in colonies with no tracheal mites (Pettis & Pankiw, 1998). However, colonies with high infestations show a higher frequency of grooming dances (Pettis & Pankiw, 1998) and workers observed autogrooming are much more likely to have migrating female mites on their thorax (Danka & Villa, 2005). Additionally, young resistant bees are more likely to engage in the behavior than susceptible bees when challenged with individual mites (Danka & Villa, 2003).

The exact mechanism of resistance to tracheal mites in Russian bees has not been established. Guzman *et al.* (2002) hypothesized that autogrooming, allogrooming or removal of invading non-Russian infested workers could be possible mechanisms. Given that autogrooming had been shown to be important in other resistant bees (Pettis & Pankiw, 1998; Danka & Villa, 1998), I evaluated its role in conferring resistance to Russian bees. In addition, I investigated the combined effects of grooming and worker age on infestation by tracheal mites in Russian and in susceptible bees.

Materials and Methods

In two experiments, I followed the procedures developed by Gary & Page (1987) to measure migration of female tracheal mites into workers, with some modifications described by Danka & Villa (1996). Additionally, I glued workers' midlegs to stop autogrooming (Danka & Villa, 1998) and caged workers for different time periods before exposing them simultaneously to tracheal mites (Gary *et al.*, 1989).

Resistant Russian (R) and susceptible U.S. (S) colonies were used as sources of workers. Selected colonies had shown two-to four-fold differences in mite abundance (female mites / worker) of young workers introduced into highly infested colonies. The resistant colonies were derived from Russian queen lines 01C-893 (Yellow/Yellow) and 04A-136 (White/Green) (Rinderer *et al.*, 2005). Susceptible colonies came from crosses of two U.S. colonies which had shown high susceptibility in screening tests.

Workers from each source colony were marked with enamel paint on the abdomen (Testors®, Rockford, IL). Four to six marked workers were narcotized at the same time with CO₂. In each group of narcotized bees, the midlegs of about 2/3 of them were glued together at the tarsi with either epoxy glue or fingernail polish (G), and the remaining control bees (C) received similar quantities of glue on the tarsi of one hindleg. Treated and control workers from each colony were then held in an incubator (34–35°C, 50–80% RH) in separate cages provided with water and honey until the time for introduction into an inoculation colony. After inoculation periods (3–6 days), bees were retrieved for dissection. Bees with midlegs previously glued but having separated, were not included in analyses.

Experiment 1: Glued midlegs in Russian and susceptible workers less than 10 h old

On each of four days in October 2004, G ($n \leq 20$) and C ($n \leq 10$) workers from each of two R and two S colonies were treated as

described above. Each afternoon, workers that had emerged since the morning were marked and treated and then added in the early evening to one of two inoculation colonies with high tracheal mite infestation (50–75% of workers infested). Workers were retrieved five or six days after introduction, frozen and then dissected. Mite abundance was analyzed in a random block design with each of the days of introduction into an inoculation colony as a random effect. Strain and treatment were considered fixed effects.

Experiment 2: Glued midlegs in 1, 2, 3 and 4 d-old Russian and susceptible workers

Workers emerged over the previous 24 h from each of two R and two S colonies were marked and treated (G, $n=30$; C, $n=20$ per colony) on each of four days in March 2005. Workers from each colony and day were caged in separate hoarding cages and maintained in an incubator. On the fourth day, bees of all four age classes from all four colonies were simultaneously introduced into an inoculation colony with 78% of its workers infested and from which all emerging brood was removed. Workers were retrieved 3 days later. Mite abundance was transformed ($\log_e x + 0.1$) and then analysed as for experiment 1, except that there was no random effect due to inoculation colony and the added effect of bee age was used as a covariate.

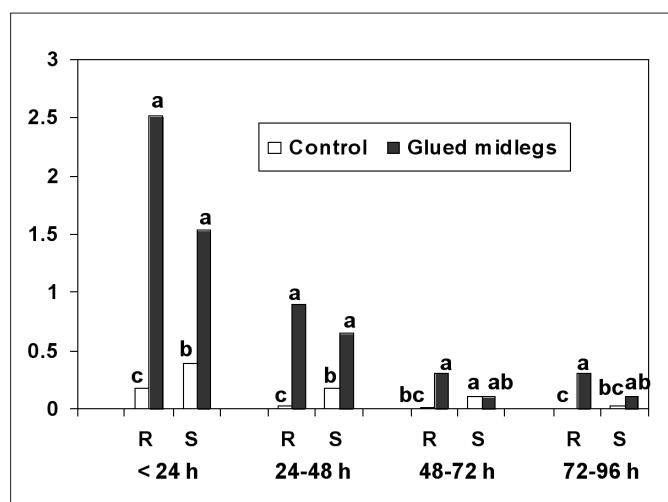


Fig. 1. Mite abundance in Russian (R) and susceptible (S) workers that were reared to four age classes at time of introduction into an infested colony with free midlegs (control) or midlegs glued at the tarsi. Data were transformed [$\log_e (x + 0.1)$] for analysis of covariance incorporating age as a covariate. Separate analyses were later conducted on the transformed variable for each age group to compare least squares means within an age group. Significant differences ($P < 0.05$) of means within age group (transformed to the original variable) are indicated by different letters.

Results

In both experiments, there were strong effects of treatment, but more importantly a significant interaction between treatment and strain (Table 1). In very young bees (10 h old), impairing autogrooming by gluing the midlegs together at the tarsi greatly increased the migration of tracheal mites into bees of both Russian and susceptible origin (Table 2). In control bees, significantly fewer mites migrated into the tracheae of Russian workers than into the tracheae of susceptible bees (Table 2).

As workers aged, the potential for tracheal mite infestation in both treated and control bees greatly decreased, but gluing of midlegs still had strong effects (Fig. 1). The effect of age as a covariate was highly significant, as were the overall effects of treatment over all ages (Table 1). The interaction between strain and treatment seen in bees less than 10h old in the first experiment was evident in bees up to 48 h old, but diminished as they aged and overall infestation decreased (Fig. 1).

Discussion

These results demonstrate that grooming with the midlegs to remove female tracheal mites is the primary mechanism of resistance in Russian bees. This same mechanism was shown to be important in the tracheal mite resistance of Buckfast bees (Danka & Villa 1998, 2003). Higher frequencies of grooming dances (which usually involve autogrooming with the midlegs) were also seen in resistant colonies derived primarily from Italian stocks (Pettis & Pankiw 1998). The same mechanism of resistance has been shown to be present in colonies of three very different origins, suggesting that it is perhaps a universal mechanism by which resistant stocks reduce infestation by tracheal mites.

Bees from susceptible strains are able to partly prevent migration of mites into their tracheae, but not to the point of diminishing infestation to levels consistently below economic thresholds in field colonies. In a previous study, resistant Buckfast bees reacted earlier, and with more intense grooming, to female

Table 1. Results of analyses of variance of effects on mite abundance (female mites/bee). For experiment 2, the variable was transformed to $\log_e(x + 0.1)$.

Experiment	Variance source	df	F	P>F
Experiment 1: Bees < 10-h-old	Treatment	1, 3	35.18	0.012
	Strain	1, 231	2.50	0.115
	Strain × Treatment	1, 228	5.78	0.017
Experiment 2: Bees 1, 2, 3, 4 d-old	Age (covariate)	1, 2	27.58	0.037
	Treatment	1, 9	79.41	<0.0001
	Strain	1, 9	0.16	0.698
	Strain × Treatment	1, 9	12.62	0.006

Table 2. Mite abundances (female mites/worker; least squares means \pm s.e. [n]) in Russian and susceptible bees with free midlegs (control) or midlegs glued at the tarsi. Workers were less than 10-h old at the time they were introduced into highly infested colonies. Means followed by different letters are significantly different ($P < 0.05$).

	Russian	Susceptible
Control Bees	1.46 \pm 0.82 (87) c	2.96 \pm 0.82 (85) b
Bees with Glued Midlegs	8.19 \pm 0.90 (39) a	7.88 \pm 0.92 (24) a

mites placed on the thorax, but the mite removal efficacy of Buckfast bees which initiated grooming was not better than that of susceptible workers which had also reacted (Danka & Villa 2003). Given that grooming behaviour is already present to a degree in young susceptible workers, selection for improved autogrooming in any population of honey bees should be fairly rapid and easy to achieve.

Several factors influence the migration of female tracheal mites into workers. Clearly, autogrooming is an essential active defensive mechanism in young workers, and it differs only in degree between resistant and susceptible bees. Another factor, independent of autogrooming with the midlegs, appears to decrease the infestation potential of all workers as they age. This could be either from changes in attraction to mites or an undiscovered active behavioural defense by workers. As bees age, the proportion of branched hydrocarbons decreases, and the proportion of unsaturated hydrocarbons increases (Francis *et al.*, 1989), and mites tested in locomotory bioassays preferred cuticular extracts of 1 d-old bees over those of 5 d-old bees (Phelan *et al.*, 1991). Alternatively, other unknown defensive reactions, in combination with decreases in attractive chemicals, might become more effective and decrease mite migration into older bees.

Problems with tracheal mite parasitism can be prevented with the use of Russian bees or other resistant strains. Autogrooming with the midlegs is the primary mechanism that allows resistant bees to rid themselves of mites during the migration from older to younger workers. Given that some level of self-grooming is already found in susceptible bees, selection for reduced mite transfer is likely to be based upon this mechanism and should be an efficient way of producing resistance from susceptible strains. There may be differences between strains in allogrooming or in attractiveness to mites (Pettis & Pankiw 1998, Van Engelsdorp & Otis 2001, Villa & Danka 2005), providing more candidates for further selection. However, selecting for other mechanisms of resistance to tracheal mites may not be necessary given the strong effects of autogrooming.

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