

## REVIEW ARTICLE

### Grooming by honey bees as a component of varroa resistant behavior

Dorian J. Pritchard\* 

Oakwood Lodge, Heddon-on-the-Wall, Newcastle-upon-Tyne, NE15 0HZ, UK

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Grooming behavior of honey bees can be considered in two major categories: autogrooming or self-grooming and inter-bee grooming, called allogrooming. Allogrooming can be one-on-one, or social, involving several nestmates acting collaboratively. In addition, some house bees become allogrooming specialists, and for them grooming their nestmates can be a full-time occupation for most of their lives. Early observations on the Eastern honey bee, *Apis cerana*, recorded autogrooming, one-on-one, and social allogrooming, all of which result in dead, visibly mutilated varroa mites falling to the hive floor. Similar behavior has been sought in the Western honey bee. *Apis mellifera*, with variant observations for the different subspecies. Most descriptions relate to *A. m. carnica*, some to *A. m. ligustica*, but with one notable exception, almost none to *A. m. mellifera*. The most impressive findings are from “Africanized” bees, which provide some of the best cases of natural, long-lasting tolerance to varroa mites in *A. mellifera*, although even some of these are controversial. The speed of both autogrooming and allogrooming responses is generally reported to be much slower in *A. mellifera* than in *A. cerana*, and the frequency and degree of damage to mites are also lower. Serious damage, such as severance of limbs and gashes of the idiosoma, shows a heritability ( $h^2$ ) of around .16 in *A. mellifera* overall.

#### Acicalamiento de las abejas como un componente del comportamiento de resistencia a varroa

La conducta de acicalamiento de las abejas puede ser considerada en dos categorías principales: *autogrooming* o auto-aseo y cuidado personal inter-abeja, llamada *allogrooming*. *Allogrooming* puede ser uno-a-uno, o social, incluyendo la participación de varias compañeras de la colonia que actúan en colaboración. Además, algunas abejas de interior se convierten en especialistas en el *allogrooming*, y para ellas el aseo de sus compañeras de colonia puede ser una ocupación a tiempo completo durante la mayor parte de sus vidas. Las primeras observaciones sobre la abeja de la miel oriental, *Apis cerana*, registraron *autogrooming*, uno-a-uno y *allogrooming* social, todo lo cual resultó en ácaros varroa muertos, visiblemente mutilados caídos en el suelo de la colmena. Se ha buscado un comportamiento similar en la abeja occidental de la miel, *Apis mellifera* con observaciones variables en las diferentes subspecies. La mayoría de las descripciones se refieren a *A. m. carnica*, algunas a *A. m. ligustica*, pero casi ninguna a *A. m. mellifera*. Los hallazgos más impresionantes son de abejas “africanizadas”, que proporcionan algunos de los mejores casos de tolerancia natural de más larga duración a los ácaros varroa en *A. mellifera*, aunque incluso algunos de ellos son controvertidos. La velocidad de las respuestas tanto *autogrooming* como *allogrooming* está resultando ser mucho más lenta en *A. mellifera* que en *A. cerana*, así como la frecuencia y el grado de daño a los ácaros que también son más bajos. Daños graves tales como ruptura de las extremidades y heridas del idiosoma, muestran una heredabilidad ( $h^2$ ) de alrededor de 0,16 en *A. mellifera* en general.

**Keywords:** *Apis mellifera*; *Apis cerana*; varroa resistant behavior; varroa tolerance; grooming

#### Introduction

Every aspect of an animal’s phenotype derives from interactions between its genotype and its environment. If resistant behavior in a colony of honey bees against the parasitic *Varroa* spp. (varroa) mite occurs in a stock of bees, it is, therefore, unlikely to be an entirely predictable, simple, genetically based response, but rather to involve perhaps a series of interactions between bees and mites. It can also be expected to vary between species, subspecies, apiary conditions and handling regimes, and such variation makes its investigation difficult. The extent to which a character depends on genotype can be expressed in the mathematical term “heritability” ( $h^2$ ), the value of which indicates its capacity for selection by breeders (see Büchler, Berg, & Le Conte, 2010).

The Eastern honey bee, *Apis cerana cerana*, has long experience of one species of varroa, namely *Varroa jacobsoni* specifically in Java and of its close relative, *Varroa destructor* in much of the rest of its range (Anderson & Trueman, 2000) and has developed a range of ways of avoiding or resisting its attacks. These include apparent physiological limitation of mite growth in worker brood (Harris, 2007; Harris & Harbo, 2000); so-called “hygienic behavior,” and “*Varroa* sensitive hygiene,” involving uncapping of brood cells that contain mites and ejection of the mites (see e.g., Carreck, 2011; Rinderer, Harris, Hunt, & de Guzman, 2010); “entombing” of parasitized drone brood cells beneath propolis, causing the non-emergence of both bee and parasites (Rath, 1999); and aggressive “grooming,” involving removal and destruction

\*Email: [dorian.pritchard@btinternet.com](mailto:dorian.pritchard@btinternet.com)

of adult mites on the external surfaces of adult bees (see below).

The Western honey bee, *Apis mellifera*, exists in many subspecies, which have only recently become parasitized by *V. destructor* (Anderson & Trueman, 2000), and defensive behaviors and other attributes are as yet only poorly developed (see reviews by Büchler et al., 2010; Carreck, 2011). However, the guard bees of some *A. mellifera* strains are said to deny entrance to their hives of bees with mites on their bodies and chase or carry those bees away (Osterlund, 2015; Rummer-Downing, 2010). Some catch free mites and eject them from the nest (Lodesani, Vecchi, Tomasini, & Bigliardi, 1996; Wallner, 1991), and some have developed other means of defense similar to those of *A. cerana*. Several relevant observations are as yet unconfirmed, but one of the most effective recognized means of defense is body grooming, the subject of this review.

### Mite population dynamics

The dynamics of *V. destructor* population growth in *A. mellifera* colonies have been described by Fries, Camazine, and Sneyd (1994), Martin (1998) and Calis, Fries, and Ryrie (1999). The first model was based on a literature review and identified major gaps in our knowledge of the number and viability of mite offspring. The per capita number of reproductive cycles per female mite was set at 1.4, and the rate of invasion of brood cells by mites was determined from the mean length of the phoretic period, but excluded the mite mortality rate.

In the later models, cell invasion rates and mite mortality are based more directly on observational data. Martin's model envisages a 12-fold increase in mites during a 128 day breeding season. Calis et al. describe a mite doubling time of 30 days, leading to inevitable colony death in the fourth year. However, serious deficiencies of both models are that the *A. mellifera* subspecies which provided the parameters for the models are not specified, nor whether they were well adapted to the area where they were studied.

Another major criticism is that neither Calis et al. nor Martin accommodate what Calis et al. referred to as "the negative impact that high mite levels may impose on bee colonies" (Calis et al., 1999). Both models envisage mite populations' increasing way beyond levels actually tolerated by bees. Calis et al. admit this is an important limitation, as "interaction between the mite population and the colony is evident at high mite populations." This note of caution seems to have gone unnoticed, for example, by Ball, Brown, and Wilkins (2010) and Brown, Learner, Marris, Wilford, and Semmence (2013) in their directives to British beekeepers on colony management, as their population growth curves are mathematically perfect, without regard to defensive actions by bee hosts or attrition at high densities, and their directives make no attempt to harness or develop defensive responses by the bees to the advantage of the

colony. Mites removed by grooming are mentioned by Martin, but included in postemergence mortality with no special distinction. Brown et al. (2013) do encourage beekeepers to select for and retain bees that appear to show increased tolerance to varroa and they report on an ongoing project at the University of Sussex, UK (Carreck, 2011) that identifies and selects for "hygienic traits or behaviors" in adult worker bees, but they take no account of grooming in any regard.

### Behavioral interactions of varroa and bees

When a parasitized *A. mellifera* worker bee emerges from her brood cell, the female parent mite and young mature females move out into the nest, but the male(s) and immature females quickly die. Lobb and Martin (1997) estimated that around 50% of fallen dead mites die within sealed brood cells, the rest mainly shortly after emergence. This may explain Wallner's deduction that if 60% of fallen mites show damage inflicted by bees, those bees should be capable of survival without mite treatment (Dews, 2008; Wallner, 1990a, 1990b). For example, of 100 non-emerged mites, around 50 die before emergence. Some of these, say 10, would have damage inflicted on them during cell cleaning and tidying up of the brood nest. The remaining 50 emerge alive and are initially phoretic; if 40 of these die as a consequence of grooming, but 10 live, reproduce and eventually die uninjured, then 50% of all the fallen mites would show damage, but the mite infestation could be maintained by reproduction of the 10 that escaped injury. However, if all those 50 that initially emerged alive are soon lethally injured by alert bees and fall to the floor, then 60% of the fallen mites would show damage and the mite population would have no chance of recovery.

Usually within a day or two of emergence, mature female mites attach to adult bees where they pierce the host's intersegmental abdominal integument and begin to feed on her hemolymph. *V. destructor* favors distinct attachment sites, particularly at the 3rd and 4th ventrolateral abdominal tergites (Bowen-Walker, Martin, & Gunn, 1997). Adult female mites can recognize potential host bees in close proximity and frequently react by jumping rapidly to attach themselves "phoretically" to their new hosts. By contrast, bees are often unaware of mites, even when exposed on the thorax of a neighboring bee. They may even touch the mite with antenna or mouthparts without any further reaction to it. Rath (1999) claims that *A. cerana* workers display little reaction to mites that remain calmly in their phoretic feeding positions in lateral intersternite folds. However, host bees are disturbed and react by self-grooming or the grooming dance when a mite leaves a "phoretically safe position" and moves on that bee's body.

Mites are indirectly guided to safe positions on the bee by her grooming attempts; 87% choosing phoretic sites between the second and third lateral tergites (Rath, 1999). On *A. cerana* drones, this preference is

even more restricted (52 of 54 cases) to the second lateral intertergite on the left side (Rath, 1991). In an *A. cerana* colony, 25% of mites were on drones, 75% on workers. Drones do not practice self-grooming nor participate in social grooming (Rath, 1999). Adult *V. jacobsoni* females have an elliptical concave ventral surface which permits a close fit to the rounded surface of the host's body. Once ensconced in position, the only way a bee can grip the mite is to bite it from the side, with one mandible on the dorsal, the other on the ventral side of the mite (Rath, 1999). In *A. cerana*, drone brood tends to be invaded by 2–5 mites per cell (Rath, 1999).

Virus infections can be transferred by varroa mites, or triggered in the bee's body, and are the primary cause of mortality in severely infested *A. mellifera* colonies (Boecking & Spivak, 1999).

On its original host, *A. cerana*, varroa (both *V. jacobsoni* and *V. destructor*; see Anderson & Trueman, 2000) is not a serious pest, largely because it does not reproduce successfully in *A. cerana* worker brood. Consequently, the total number of mites within an *A. cerana* colony is always low, less than 800 (Boecking & Ritter, 1993). *V. destructor* can, however, reproduce well in *A. mellifera* worker brood, and if the mite population reaches 2000–3000, this is considered sufficient to kill a colony (Carreck, Ball, & Martin, 2010; Martin, 1998).

Peng (1988) and Büchler, Drescher, and Tornier (1992) observed *A. cerana* seizing mites with their mandibles. By contrast, of 25 individually artificially mite-infested *A. mellifera* workers, removal of mites was observed in 12 cases, but there was no evidence this was done with the mandibles. In Božič and Valentinčič's (1995) study with *A. m. carnica*, not a single groomer was seen to seize and remove a mite from another bee. The vigorous movements of *A. cerana* were also not seen in *A. m. carnica* and typically *A. mellifera* groomers worked singly, whereas in *A. cerana*, 2–4 bees sometimes groomed one recipient.

The behavioral and physiological adaptations of *A. cerana* colonies that limit mite population growth are accordingly widely believed to be lacking or only poorly developed in *A. mellifera*, and it is generally advised that if no action is taken by the beekeeper, an *A. mellifera* colony infested with varroa is sure to collapse within 1–3 years, depending on its initial level of infestation (Fries, Imdorf, & Rosenkranz, 2006; Korpela, Aarhus, Fries, & Hausen, 1992; Martin, 1998). However, some populations of the British near-native *A. m. mellifera* bee appear to thrive indefinitely without treatment (see Pritchard, 2012, 2015), and there are accounts of varroa resistant *A. mellifera* in North America, Europe, and other parts of the world (e.g., Fries et al., 2006; Seeley, Tarpay, Griffin, Carcione, & Delaney, 2015).

In "Africanized" *A. mellifera* (i.e., other *A. m.* subspecies hybridized with *A. m. scutellata*), mite populations stabilize at 1000–3000 without killing colonies (Medina, Martin, Espinosa, & Ratnieks, 2002; Vandame, Colin, Morand, & Otero-Colina, 2000). These hybrids are

partially tolerant because their brood is relatively unattractive to mites, anti-varroa hygienic behavior is efficient, there is reduction in the post-capping period (Gonçalves, 2001), and its workers actively groom mites off their bodies. Non-Africanized Brazilian *A. mellifera* colonies also show partial tolerance of varroa infestation, which has been ascribed to low mite fertility in the brood of these bees (Mondragón, Spivak, & Vandame, 2005). Anecdotal reports suggest that the high level of resistance of some British near-native *A. m. mellifera* strains may be due to grooming, but no detailed reports have yet been published (see Dews, 2008; Rummer-Downing, 2010; Pritchard, 2012, 2015).

### Autogrooming

Self-grooming, or "autogrooming," enables bees to remove ectoparasites, dust, and pollen from their own bodies and helps disperse pheromones (Boecking & Spivak, 1999). It is described as involving biting and licking with the mouthparts, as well as movement of the pro- and/or mesothoracic legs (Danka & Villa, 2005). With *A. m. carnica*, it includes cleaning of the antennae and mouth parts with the first legs, cleaning of the first legs with the antenna cleaners, and brushes on the first legs with cleaning spines on the middle legs, cleaning of the middle leg spines with brushes on the hind legs, and finally cleaning of the hind leg brushes between themselves, to put the dust particles or pollen grains onto the corbicula of the hind legs (Božič & Valentinčič, 1995).

Varroa resistant grooming behavior was detailed by Peng, Fang, Xu, and Ge (1987) in *A. cerana*. They described how a worker bee uses her legs and mandibles to remove the mite from her body and mutilate it. Arechavaleta-Velasco and Guzmán-Novoa (2001) deduced that decreases in mite infestation levels observed in *A. mellifera* colonies could likewise be explained by higher proportions of mite injuries inflicted by grooming, an observation confirmed by Moosbeckhofer (1992) and Mondragón et al. (2005). Grooming to remove varroa mites by *A. cerana* typically first involves signs of irritation, followed by self-cleaning behavior. If this fails to displace the mite, the bee may perform a grooming invitation dance by rapidly shaking her abdomen, which attracts the attention of nestmates (Ruttner & Hänel, 1992).

Wallner (1990a, 1990b) was the first to postulate active defense of *A. m. carnica* against varroa, based on his observation of fallen mites with damaged legs and cuticle of the dorsal shield, or idiosoma. This was confirmed and illustrated by Ruttner (1991) and Ruttner and Hänel (1992). According to Arechavaleta-Velasco, Alcalá-Escamilla, Robles-Rios, Tsuruda, and Hunt (2012), an *A. mellifera* worker bee's initial reaction to a mite penetrating her integument is "swiping motions in the direction of the mite with the front pair of legs." If on failing to displace the mite, as with *A. cerana*, she

performs the grooming dance, and this can elicit allogrooming by a single nestmate or sometimes “group cleaning” by several bees (Haydak, 1945; Milum, 1955; Peng et al., 1987).

In Moosbeckhofer’s (1992) study of *A. m. carnica*, the percentage of dark, damaged fallen mites correlated with bee- and brood infestation rates. Peng et al. (1987) recorded 73.8% of the fallen mites as showing damage in *A. cerana* hives, but grooming was (initially) claimed to be less effective in *A. mellifera*, one sample of *A. mellifera* (subspecies not defined) destroying only .3%, while *A. m. ligustica* showed an average mite removal rate of 5.75%. However, Rosenkranz, Fries, Boecking, and Stürmer (1997), working with *A. m. ligustica* and *A. m. carnica*, recorded mite damage rates averaging 45% (44–62%), while Africanized *A. mellifera* damaged 38.5% (Moretto, Gonçalves, & De Jong, 1991). Markthaler (2004) mentions Wallner’s introduction of “Varroa Killer Factor” (vkf) scores, these being the percentage of fallen mites showing damage. He reported that Wallner’s strong *A. m. carnica* hives eventually achieved a vkf of 93%.

Büchler et al. (1992) added live mites to healthy colonies and watched the bees’ response. Autogrooming was found to be delayed in *A. mellifera*, whereas close to 90% of *A. cerana* showed autogrooming with the mandibles within 60 s and, although 48% of the mites were removed by *A. mellifera*, none were seen to be caught with the mandibles. However, Wallner (1991) reported an *A. m. carnica* worker catching a free mite in her mandibles and holding it horizontally, flying away with it.

Aumeier (2001) identified eight aspects of responsive behavior by bees after having a live mite placed on their thorax, and on this basis compared the behavior of highly responsive Africanized bees and susceptible *A. m. carnica*. These included (1) Weak cleaning or shaking; (2) Intense cleaning or shaking, corresponding to the “grooming dance” (Peng et al., 1987; Thakur, Bienefeld, & Keller, 1997); (3) “biting” of the mite; (4) “rolling,” using all pairs of legs, and (5) “attempting to fly.” Grooming success was evaluated by counting the number of mites removed, the number of times a mite remounted the bee, and the number of bees that gave up the struggle. The total number of responses, especially the vigorous aspects, was more than twice as high in Africanized bees, while *A. m. carnica* tended to delay their reaction. However, in both types of bee, about 80% of mites removed remounted their hosts and remarkably, no physical damage was visible on any mites, even after bees had been seen vigorously shaking and even chewing them. The final stages in the lethal grooming response found in some bees, therefore, seem to be only poorly developed in these strains. Interestingly, even mites that appeared to be extremely disturbing to their hosts were instantly ignored after leaving the bee. None of the *A. m. carnica* and only 4 of 115 Africanized bees solicited allogrooming, and this never succeeded in dislodging a mite.

Nevertheless, in a Mexican study of eight genetically diverse sets of colonies, the principal identified basis of resistance was grooming. Arechavaleta-Velasco and Guzmán-Novoa (2001) found that those *A. mellifera* colonies with the lowest rate of mite population growth showed more grooming behavior, more mites falling to the hive floor, higher proportions of fallen chewed mites, and reduced infestation levels of adult bees. Mondragón et al. (2005) also concluded that the proportion of mutilated mites found on the hive floor is one of the best predictors of mite population decline in *A. mellifera* colonies.

Invernizzi, Zefferino, Santos, Sánchez, and Mendoza (2016) attempted to explain the reputational differences with respect to varroa resistance between susceptible *A. m. ligustica* and “Africanized bees,” i.e., hybrids between *A. m. mellifera* and *A. m. scutellata*, and tested representatives of both categories at individual, group, and colony levels. Following Aumeier (2001), mites were first placed on the thorax of individual bees, which were then observed for 2 min. for responses that could be considered as attempts to get rid of them. Overall, the Africanized bees showed a higher number of reactions ( $p = .02$ ), but only 8% of bees bit a mite and only 6.3% dislodged one successfully. In the group comparison, 20 mites were introduced to groups of 30 caged bees and the number of fallen mites recorded after 24 h. An average of 63% of mites was dislodged, but there were no differences between the bee types, and no mites were damaged. In the colony comparisons, there was a significant difference in the percentage of fallen mites with leg injury collected over a period of 10 days: 29% in the Africanized colonies, 18% in the *A. m. ligustica* ( $p = .009$ ). The percentage of mutilated mites in this field test showed marginally significant correlation with bee behavior in the group tests, but only after one colony was excluded ( $p = .05$ ). There was, therefore, little evidence for bees being capable of mutilating mites as an aspect of autogrooming, but the field observations suggest this may occur as a consequence of some other behavior of whole colonies in their defense against varroa mites.

Ruttner and Hänel (1992), working with Wallner’s mite-resistant *A. m. carnica* bees found a significant positive correlation between the numbers of dead and mutilated mites in the course of a season and observed the same range of behaviors described by Peng et al. (1987) and Moretto et al. (1991) for *A. cerana*. However, only 12 of Wallner’s 700 colonies (1.7%) showed appreciable mite resistance. In those, 30–50% of dead mites had damaged legs, especially the anterior pair. Damage to the idiosoma cuticle was relatively rare (1–2%), but the level of damage increased significantly in line with mite mortality. Ruttner and Hänel (1992) concluded that both leg and idiosoma injuries were caused to mites by the workers’ mandibles acting like sharp scissors and that there was no difference in the type of damage caused by *A. mellifera* and *A. cerana*. In Africanized

*A. mellifera* colonies, injuries to the body were also relatively rare, but leg damage was seen in over 75% of all injured mites; 25% had just one leg damaged (Rosenkranz et al., 1997).

Bak and Wilde (2016) compared autogrooming reactions and grooming intensity with regard to *V. destructor* in three honey bee subspecies and a hybrid between *A. m. capensis* and *A. m. carnica*. The other subspecies were *A. m. carnica*, *A. m. caucasica* and *A. m. mellifera* of the Polish Augustowska line. This was done by a modification of the method of Aumeier (2001), in which a live mite was placed on the thorax of one bee of a caged group of three. The behavior of the recipient bee was then compared with that of its fellows for a period of 3 min. Responses were assessed as “no reaction,” “weak,” or “strong,” also as “immediate,” undertaken within 30 s, and “continuous,” lasting longer than 30 s. The number of identified reactions was also recorded (Aumeier, 2001), the total number of mites that fell and the proportion that showed injury. The *A. m. mellifera* bees were outstandingly more reactive to varroa mites than all the others, 98% showing some response to contact with a mite. The average number of reactions shown (5.4) was significantly higher, and the average duration time of the reaction(s) was also significantly longer (64.7 s) than those of the other groups ( $p = .000$ ). In most trials, bees manifested at least 3 defensive reactions, the highest number being shown by *A. m. mellifera* ( $p = .0182$ ). Reaction time was also faster, 83.2% of *A. m. mellifera* workers reacting immediately. Importantly, 90% or more bees were unable to remove their mites and none of them used their mandibles. No body damage was found on fallen mites, and mites were ignored once they had been removed from their host and no allogrooming was observed. Aumeier (2001) and Guzman-Novoa, Emsen, Unger, Espinosa-Montaño, and Petukhova (2012) also found that under laboratory conditions, no mites showed damage, even though mites collected from floorboards in the field frequently did (Fries, Wei, Shi, & Chen, 1996; Guzman-Novoa et al., 2012; Mondragón et al., 2005; Spivak, 1996). Interestingly, in comparison with Africanized *A. m. mellifera* resistant to *V. destructor* and susceptible *A. m. ligustica*, Corrêa-Marques and De Jong (1996) found that frequency of mite body injuries showed no correlation with bees’ autogrooming capacity. This suggests that most injuries to mites are likely to be caused by an activity other than autogrooming per se, e.g., possibly during allogrooming, or hunting of non-phoretic individuals (see below).

### Allogrooming

Both autogrooming and allogrooming depend on the ability of workers to detect mites and remove them successfully. When this takes place, *A. cerana* bees can be seen grabbing and crushing mites in their mandibles (Thakur, Bienefeld, & Keller, 1996; Thakur et al., 1997).

During autogrooming, bees brush the accessible parts of their own bodies. The grooming dance in *A. m. carnica* bees also commences with rapid self-cleaning movements with any pair of legs and wiping of the surface of the abdomen with their hind legs before wagging the body (Božič & Valentinčič, 1995; Milum, 1947). This provokes social grooming in “temporarily specialized” groomer bees, which often then clean the dancer and several other bees repeatedly over several days (Kolmes, 1989).

After the *A. m. carnica* groomer bee has made its first cleaning attempts on a recipient, the latter fully spreads her wings in the “social grooming display,” standing motionless with abdomen lifted, both bees usually being orientated up the comb (Božič & Valentinčič, 1995). After being groomed, the recipient bee was usually seen to shake for ~.33 s, and then fold her wings to the normal backward position. The median duration of grooming behavior directed by a groomer at a dancer was 8 s, that directed at a resting bee, 22 s (Božič & Valentinčič, 1995).

In allogrooming by *A. cerana*, one or several nestmates touch the thorax, propodeum, and abdomen of the other bee with their antennae, front legs, and mandibles, and it appears that distinct areas are routinely searched (Rath, 1999). This may induce the mite to leave the host, or the allogroomers may remove it, at the same time damaging it with their mandibles. In 60–100% of cases, the mite is driven from its host and in 50–60%, the mite is killed (Rath, 1999).

Fries et al. (1996) introduced intact live mites to hives and counted the proportion that ended up physically damaged in the floor debris. It was 30% in *A. cerana*, but only 2.5% in *A. m. ligustica*. In *A. cerana* colonies, within 5 min of introducing the mites, a third of bees carrying mites were subjected to allogrooming by up to 4 nestmates. “Group cleaning” was first observed in *A. cerana* colonies by Peng et al. (1987). Successful mite removal occurred in 75% of observations with *A. cerana* and a third of the mites were caught with the mandibles (Büchler et al., 1992). Allogrooming was rarely observed by Büchler et al. (1992) among *A. mellifera* within 5 min of adding live mites.

House bees are stimulated to begin allogrooming when they perceive another bee performing the grooming dance. On their response, the latter holds her wings perpendicular to her body axis while her nestmate(s) works on those parts inaccessible to autogrooming. Allogroomers generally seem unaware of mites on the other bee’s body before they begin grooming (Boecking & Spivak, 1999), although Wallner (1991) reported an *A. m. carnica* worker seizing a free mite that had escaped from a damaged drone cell 2 cm away from it. Allogrooming is typically age-specific, but some individuals perform repeated acts of grooming and a few even become full-time grooming specialists.

Bouts of grooming by *A. m. carnica* lasted 45s and were directed at the wing bases 44.6% of the time, the

petioles 18%, and sternite regions of the abdomen 2.8%. This removed dust and pollen and realigned body hairs (Božič & Valentinčič, 1995). During 18% of their time, groomer bees cleaned their own mouth parts and antennae.

During allogrooming in *A. cerana*, Peng (1988) and Büchler and coworkers (1992) observed bees seizing varroa mites with their mandibles. Božič and Valentinčič (1995) observed 12 cases of *A. mellifera* removing mites from other workers, but this was always with the legs, not the mandibles.

### Grooming specialists

Many observations support the theory that the honey bee, hive contains a pool of inactive, uncommitted workers that become recruited to the work force only when alerted by specific environmental stimuli. These individuals are available for mobilization to compensate for deficiency in a class of workers, to exploit newly available resources, or to respond to emergencies. Only a small proportion of bees become specialists, but these notably include allogroomers (see Kolmes, 1989; Robinson, 1992).

The normal progression of most workers through the regular series of short-term specialisms is known as “age related polyethism” and is governed by increasing levels of juvenile hormone. By contrast, long-term adoption of specialist tasks is triggered by environmental factors (Robinson, 1992). Key factors in the creation of allogrooming specialists must logically include the presence of varroa mites and performance of the grooming invitation dance by parasitized house bees. Both of these relate directly to mite population density as well as inherited predisposition.

Moore, Angel, Cheeseman, Robinson, and Fahrbach (1995) introduced 200 one-day-old adult bees, each marked on the thorax with colored and numbered plastic disks, into an unrelated colony housed in an observation hive and observed their behavior from day 4 after emergence. One individual, Red 93, began by performing brood care as normal, but by day 7, she was doing almost nothing but groom her nestmates. Her age cohort began foraging at 19 days, but up to day 31, when observation was discontinued, Red 93 remained a grooming specialist. Typically, social grooming occupies 1.5% of a house bee’s time, but with Red 93 it was 84%. Throughout her life, Red 93 did virtually nothing other than groom her nestmates. Creation of a force of specialist allogroomers would be what we might expect in a colony of honeybees with the ongoing threat of overwhelming mite infestation.

Božič and Valentinčič (1995) reported that among *A. m. carnica*, “regular groomers” groomed up to 8 recipients one after the other, with breaks of less than 2 min. for searching for new recipients or responding to the grooming dances of neighbors. The median searching time was 6.5 s, and 50% of the grooming sessions lasted

over 22 s. Between grooming sessions, the groomers occasionally cleaned their own antennae with a single movement of their forelegs. Specialized groomers preferentially cleaned grooming dancers and to a lesser extent, other resting bees. During the search for potential recipients, the groomer bees palpated nestmates (Božič & Valentinčič, 1995)

### Environmental influences on grooming

Büchler (1993) found a strong seasonal effect in the percentage of naturally fallen damaged mites, ranging from 10% in early March to 40% in mid-June. Currie and Tahmasbi (2008) carried out a study to assess how variation in temperature and humidity affects the “costs and benefits” of grooming as a defense against *V. destructor* in high- and low-grooming rate strains of *A. mellifera*. Cages of 100 mite-infested bees were assigned to three temperatures (10, 25, and 34 °C) and three humidity (low, medium, and high) regimes, and bee and mite mortality rates were quantified. Differences in grooming behavior between high- and low-grooming rate lines were most pronounced at 25 °C and slightly greater at low than high humidity. Mite mortality was greater with the high-rate groomers at 25 and 34 °C, but similar to that in the low at 10 °C. The mortality of bees was, however, greater in the high- than the low-rate lines when maintained at 10 °C, indicating a “biological cost” to the colony associated with grooming at low temperatures.

Martin (1998) reported that fallen mites increased by a factor of 6 (Lobb & Martin, 1997), or 7–15 (Martin & Kemp, 1997) when *A. mellifera* brood was emerging, compared to when it was not. The total proportion of the emergent mite population dropping daily during brood emergence was 30% from worker brood, and an estimated 20% from drone. Rosenkranz et al. (1997) monitored the proportion of damaged mites in the floor debris of *A. mellifera* hives when brood was and was not emerging and also when varroa-sensitive hygiene was stimulated by the insertion of newly killed, but otherwise intact brood. They found that dead mites removed from brood cells by the bees were damaged to a similar extent as those removed by grooming, which was maximal when brood was emerging. This suggests that when both means of defense are in operation, the proportion of damaged mites is not a reliable indicator of the extent of grooming taking place.

Using replicate caged samples of 180 g of queenright *A. mellifera* bees and 200 mites, Hoffman (1995) assessed damage rates in fallen mites in relation to the development of the brood nest. When no brood was emerging, the damage rate was 10.2%, but was significantly higher at 16.7%, with emerging brood, when the multiple injury rate of mites was also higher. Overall, 12.8% of the mites showed physical damage. He deduced that 10% of the infestational variation between the original colonies could be explained on this basis. His earlier experiments with 30 g of bees and 100 mites per cage (Hoffman,

1993) revealed no significant differences between *A. m. carnica* hybrid stocks, and the mite damage rates in the caged bees were lower than those with natural mite mortality in the parent hives.

Although influenced by several biological and environmental factors, the proportion of seriously damaged mites in the debris of a colony is nevertheless now generally considered an acceptable indicator of grooming success under “field conditions” (see Andino & Hunt, 2011; Rinderer et al., 2010; Ruttner & Hänel, 1992). Autogrooming can be artificially stimulated by dusting the colony with powdered sugar (Stevanovic, Stanimirovic, Lakic, Djelic, & Radovic, 2012).

### Genetics and physiology of grooming behavior

The effectiveness of grooming behavior has often been inferred from the proportion of mutilated mites falling, but the validity of using this indicator has been questioned (Bienefeld, Zautke, Pronin, & Mazeed, 1999; Boecking & Spivak, 1999; Rosenkranz et al., 1997; Szabo & Walker, 1995). Szabo and Walker pointed out that injuries to mites could have arisen: (a) during their killing by house bees; (b) at ejection of mites that died of other causes; or (c) as a consequence of predation by greater wax moth larvae or ants after their natural death from other causes. However, severed legs and pieces missing from the idiosoma, as distinct from mere “dorsal dimples,” cannot be considered normal aspects of mite development (Davis, 2009; Rinderer et al., 2010). Estimates of the heritability of anti-grooming behavior have been very variable, and environmental influences have also been shown to be significant (see above). Several related criticisms were, however, laid to rest by the insightful analysis of Guzman-Novoa et al. (2012). These authors sought to substantiate genetic variability in grooming behavior and also to analyze the relationships between infestation levels, rate of damage of fallen mites, and grooming intensity.

Guzman-Novoa et al. (2012) took several strains of reportedly mite-resistant bees with up to 15-fold lower mite populations and up to 9-fold higher percentages of damaged mites than supposedly susceptible genotypes. The autogrooming behavior of individual bees was assessed by placing a single mite on the thorax of confined representatives of each colony and observing them for 3 min. Slow swipes with just one or two legs was scored as a “light grooming” response, while “intense grooming” involved more vigorous shaking and wiping, usually with more than two legs. The supposedly mite-resistant bees were found to perform up to 4-fold more intense grooming, and up to 7-fold more mites were dislodged from bees’ bodies than by the light grooming strains.

At a colony level, the resistant bees had significantly lower mite falls, but higher percentages of injured mites than the susceptible. In one paired group, there was a highly significant negative correlation between mite fall

and the proportion of injured mites ( $p < .0001$ ). It was also shown that bees in the colonies most successful at removing mites from their own bodies groomed at a faster pace and that a greater proportion performed anti-varroa grooming. Overall, these results strongly suggest that the percentage of injured mites is a good indicator of grooming behavior at the colony level. Andino and Hunt (2011) made similar deductions with caged groups of bees, as distinct from individual workers.

This study, therefore, establishes a sensible and valid link between mite infestation rates at the colony level, mite injury levels, mite removal efficacy, and anti-varroa grooming intensity. That these associated traits are more clearly expressed and remain interrelated in strains selected for mite resistance shows they have a genetic basis and that there is relevant genetic variability between honeybee strains. The implication is that honeybees can be bred for effective autogrooming and that varroa-infested populations under natural or artificial selection should tend to evolve toward more intense autogrooming activity.

Descendent colonies of high- or low-grooming strains showed the strain-specific level of grooming, confirming that those behaviors are inherited, despite evidence of strong environmental influence (Boecking & Spivak, 1999; Wallner, 1991). According to Villa and Rinderer (2008), the genetic basis for autogrooming is polygenic, with some alleles exerting strong dominance. On the other hand, line-mixed colonies showed a consistently lower performance than the parent lines (Fuchs, Büchler, Hoffmann, & Bienefeld, 1996), implying recessive inheritance, or interstrain communication incompatibilities.

House bees have been shown to groom a disproportionately large number of their full sisters (i.e., those of their own patriline) as distinct from their half-sisters (Frumhoff & Schneider, 1987). The observation that propensity for allogrooming varies between patrilines (Frumhoff & Baker, 1988; Robinson, 1992) reinforces the concept of inherited predisposition for allo- as well as autogrooming.

Colonies selected for grooming behavior typically show significantly lower infestation rates and more damaged mites, compared to unselected colonies (Markthaler, 2004; Wallner, 1990a, 1990a, 1991), but the estimate of heritability in *A. mellifera* ( $h^2 < .15$ , Erhardt, Reinach, Büchler, Gavido, & Bienefeld, 2007) was considered by Büchler (2000) too low to justify a large-scale selection program. Moretto, Gonçalves, and De Jong (1993) had earlier calculated a heritability of .71 in European and Africanized *A. mellifera* strains, but others have generally found much lower values. For example, Harbo and Harris (1999) noted that dents in the idiosoma are found on young mites still within the cell and, therefore, not due to mandibular action by adult bees (see also Davis, 2009). They found neutral heritability ( $h^2 = .00$ ) for all physical damage to mites,

but when dents were ignored and only severed legs and gashed bodies considered, this rose to  $.17 \pm .52$ .

Arechavaleta-Velasco et al. (2012) considered autogrooming in *A. mellifera* colonies to be the key trait for suppressing mite population growth. What they deemed “high grooming” lines averaged 18 s to respond to the presence of a mite placed on their bodies, whereas “low groomers” took 30 s. By analysis of crosses between them, they identified a candidate autogrooming gene on Chromosome 5, which they named “groom-1”.

Recently Tsuruda et al. (2014) followed up their mapping of quantitative trait loci associated with grooming by an investigation of possible *neurexin* gene involvement. Neurexin has been shown to be involved in aspects of autism spectrum disorder in humans, notably of repetitive and agitated movements and hypersensitivity to stimuli. In mice, it is known to affect grooming behavior. Grooming intensity in honeybees was assessed with regard to use of multiple legs or one leg, and fast or slow movements. Tsuruda et al. (2014) found significant differences in expression of the B form of neurexin between intense- and slow-grooming strains of bees and between grooming that lead to mite removal compared to no grooming at all. They also found correlation between response time and neurexin gene expression and a significant difference in neurexin production between bees that took <8 s and those that took >8 s to respond to the presence of a mite placed on its thorax. The only bee species mentioned in this report is *Apis cerana*.

## Conclusions

We have seen that many *A. mellifera* strains are capable of some degree of varroa resistant grooming, as first seen in *A. c. cerana*, although there is a great variability in this trait. Those *A. mellifera* bees generally reported as having the highest degree of resistance are some strains of *A. m. carnica* and some “Africanized” hybrids of *A. mellifera* with *A. m. scutellata*, but there are also anecdotal accounts of, for example, British *A. m. mellifera* with such high degrees of grooming-related resistance that they thrive without treatment. The recent *A. mellifera* subspecies comparison by Bąk and Wilde (2016) revealed that *A. m. mellifera* of the Augustowska line were outstandingly the most reactive to the presence of a mite placed on their bodies, 98% of bees reacting to shed the mite, reacting faster and for longer, and with a greater variety of responses than the other subspecies, although no mites were actually damaged in the laboratory experiments. A nagging worry in relation to the claimed aggressive defense of Africanized bees against *V. destructor* is that although their non-African parent line is sometimes stated to be *A. m. mellifera* (e.g., Invernizzi et al., 2015), that issue is usually overlooked. It could be that much of the resistant behavior of the Africanized bees is owed to their *mellifera*, as well as, or rather than, their *A. m. scutellata* ancestry.

Invernizzi et al. (2016) paid no regard to a possible role for the *A. m. mellifera* component of their Africanized bees, while Mondragón et al. (2005) ascribed the partial tolerance of non-Africanized *A. mellifera* to low mite fertility. The three best known accounts of *V. destructor* population dynamics are all deficient in making no allowance for defensive responses by the bees. Nor do they accommodate normal variations in the developing broodnest, including those associated with swarming and the presence or absence of emerging brood.

Several studies of autogrooming in *A. mellifera* stress that both colony and individual bee responses are relatively less intense compared to those of *A. cerana*, and the bees either use only their legs to displace mites or if, such as *A. cerana*, they also use their mandibles, they tend to leave the mite undamaged and capable of remounting the bee. The full lethal grooming response of *A. cerana* does occur in some *A. mellifera* strains, but this seems to be rare.

When *A. cerana* bees fail to dislodge a mite by self-grooming, they perform a grooming invitation dance that attracts the attention of nestmates, stimulating allogrooming from some. This can be provided by temporarily specialized groomer bees or irreversibly specialized allogroomers, which may perform in groups of up to 4 bees per recipient. Allogroomers also search for suitable recipients of their attention among resting bees. The frequency of allogrooming behavior is recorded as being lower in *A. mellifera* and is usually done by groomer bees acting alone. Specialized groomers seek out bees carrying phoretic mites, but usually seem not to notice mites before touching them while searching distinct areas of their neighbors’ bodies. *A. cerana* allogroomers remove mites with their mandibles, and in so doing frequently damage them. The proportion of damaged mites is also typically much larger among *A. cerana* than *A. mellifera*.

There are strong seasonal biases in the proportion of damaged mites on hive floors and this also depends on environmental temperature and humidity. It is highest when brood is emerging, as is the rate of multiple injuries to mites.

In assessing the effectiveness of grooming from the proportion of mutilated fallen mites, care must be taken to ensure those mites were not injured in unconsidered ways. If properly treated, however, this proportion is a valuable indicator of the disease status of the colony. Related research reinforces the assumed links between infestation rates at the colony level, mite injury levels, mite removal efficacy, and anti-varroa grooming intensity. That these features tend to be associated in strains selected for varroa resistance demonstrates that they are inherited and a range of observations and experiments imply that this deduction applies to both auto- and allogrooming. The identification of neurexin as possibly playing a role in grooming behavior suggests a DNA marker that could be used for selection of varroa resistant stock.



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No potential conflict of interest was reported by the author.

**ORCID**

Dorian J. Pritchard  <http://orcid.org/0000-0001-9777-5002>

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