Apidologie (1996) 27, 3-11

Original article

Grooming behavior and damaged mites *(Varroa jacobsoni)* in *Apis cerana cerana* and *Apis mellifera ligustica*

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(Received 15 August 1995; accepted 23 November 1995)

Summary — *Varroa* mite mortality and mite damage in colonies of *Apis cerana cerana* Fabr and *Apis mellifera ligustica* Spin, where mites were added to observation hive bees and to full-sized colonies of both bee species, were studied. The results show grooming behavior in *A cerana* but the results also indicate that this behavior may be less effective than previously recorded. In *A mellifera* colonies, phoretic mites were also removed by the bees but less effectively than in *A cerana* colonies. The proportion of experimentally-added live mites in the debris that were visibly damaged in colonies of *A cerana* was 30% (n = 115). From *A mellifera* colonies, 12.5% of the introduced mites had visible injuries caused by the bees (n = 65). The mites recovered from both bee species showed reduced survival rate on bee pupae compared to control mites. Compared to *A mellifera*, *A cerana* is more effective in both removing mites and causing mite damage. However, in *A mellifera* phoretic mites are also removed by the bees, and some of them are injured. Since no reproduction of *Varroa* mites occurs in worker brood in *A cerana*, extremely effective grooming behavior may not be needed to explain the tolerance of *A cerana* to *Varroa* mite infestations. The results presented demonstrate that more research is needed to evaluate the importance of grooming behavior to *Varroa* mite tolerance in both *A cerana* and *A mellifera*.

***Apis mellifera* / *Apis cerana* / grooming behavior / *Varroa jacobsoni* / tolerance**

INTRODUCTION

The parasitic mite *Varroa jacobsoni* attacks both the European honey bee (*Apis mellifera*) and the Asian honey bee (*Apis cerana*). *A cerana* is the original host of the parasite (Koeniger et al, 1981), while the *Varroa* mite has become widely distributed in *A mellifera* comparatively recently. In *A cerana* there is a balanced host/parasite relationship in the sense that the mite does not seriously damage or kill the host. *Varroa*-tolerant populations of *A mellifera* are found in South America in hybrids between Euro-

pean and African bees (Camazine, 1986; Engels et al, 1986; Ritter and De Jong, 1984) and in *A mellifera* populations in Tunisia (Ritter, 1990). However, in most cases *A mellifera* colonies die if the mite population is not controlled.

The mechanisms behind the tolerance of *A cerana* to *Varroa* mite infestations were investigated by Peng et al (1987). They found extensive grooming behavior in *A cerana* that resulted in removal of more than 99% of mites added to colonies in observation hives (n = 270). Only 0.3% of the mites were removed by grooming in colonies of *A mellifera* (n = 270). Of 42 mites examined from the hive bottom of *A cerana*, 73.8% had visible injuries although no mites from *A mellifera* were examined. Büchler et al (1992) also compared grooming in *A cerana* and *A mellifera* and found successful mite removal in 75% of the cases in *A cerana* (n = 36). In *A mellifera*, 48% of the mites were removed by grooming (n = 25). Effective removal of *Varroa* mites has also been reported from colonies of *A cerana japonica* (Takeuchi, 1993).

Damaged mites can be found on the bottom board in all *Varroa*-infested *A mellifera* colonies, and injuries on *Varroa* mites, probably caused by grooming, have been observed in *A mellifera* (Ruttner and Hänel, 1992) in Europe. Moosbeckhofer (1992) found a significant negative correlation between the proportion of damaged mites and the population size of the *Varroa* mite in infested colonies. This indicates that grooming behavior expressed as a proportion of damaged mites may be a useful parameter in selecting for *Varroa* tolerance.

The extent and variation of mite damage in some *A mellifera* populations is well documented (Moosbeckhofer, 1992; Wallner, 1994). There is, however, no information about the level of mite damage from colonies of *A cerana* naturally infested by the *Varroa* mite. This paper reports on observations of mite damage from observation hive experiments and full sized colony experiments using both *A cerana* and *A mellifera* colonies. We also report on mite damage in naturally infested colonies of both bee species.

MATERIAL AND METHODS

The experiments presented were performed near Beijing during August and September 1994. The bees used were a Chinese strain of *A mellifera ligustica* and colonies of *A cerana cerana* from the mountain area 120 km south of Beijing.

All mites used in the presented experiments were phoretic mites collected from one heavily infested *A mellifera* colony. Infested bees were shaken into a box with one side covered by a net, allowing mites to fall through the net. The bees were shaken in the box with the net side up after adding a small amount of wheat flour. Thereafter the box was turned with the net side down and the mites falling from the bees were collected in a container. In the laboratory each mite was transferred and cleaned, if needed, onto a semi-damp piece of cloth and allowed to walk for approximately half an hour. After that the mites were used for inoculation experiments. The mites were inoculated on bees or pupae within an hour of collection.

Observation hive experiments

Two colonies of *A cerana* and one colony of *A mellifera* were used. The addition of mites onto marked bees followed the procedures described by Peng et al (1987) and the observation hives used were the same as used by these authors. Ten to forty mites were added each time to each colony.

Observations were made of the behavior of individual bees and of mites falling from the bees to the bottom of the hive. At the hive bottom there was a white sheet of paper from which the mites could be collected after falling. Each fallen mite was examined under a stereo microscope at 63-fold magnification for visible signs of damage. One hour after inoculating the mites onto each tagged bee, the individual bees were examined for the presence or absence of mites.

The experiment was repeated twice in one *A cerana* colony, inoculating ten mites each time. This colony had one comb and contained approximately 800 bees. In another *A cerana* colony the experiment was conducted once, inoculating 40 mites. This colony had three combs and contained approximately 4 500 bees. In the *A mellifera* observation hive (two combs, approximately 3 500 bees) the experiment was repeated twice, with ten and twenty mites respectively.

Full-sized colonies

Three *A cerana* colonies and three *A mellifera* colonies in Langstroth standard hives were equipped with net bottoms to allow collection of mites under the colonies. The colonies were monitored daily for mite mortality for one month, and all mites from the *A cerana* and some of the mites from the *A mellifera* colonies were examined for mite damage. In the *A cerana* colonies the bees covered four, five and six combs respectively and in the *A mellifera* colonies the corresponding numbers were 10, 12 and 15 combs. All six experimental colonies were put on stands approximately 50 cm from the ground with the legs of the stands in water containers, to avoid ants or other animals gaining access to the hive debris.

At the end of the collection period, two colonies from each bee species were used to investigate mites falling from the colonies after introduction of mites directly upon the bees on top of or in between the frames. Forty eight hours after adding mites, possible residual mites were regarded as an integrated part of the colony and the colony was used for further experiments.

Sixty mites were added into each of two *A cerana* colonies and the experiment was repeated again in one of these hives using 100 mites. In two *A mellifera* colonies 40 mites were added to each hive and the experiment was repeated again in both hives using 100 mites in each hive. Mites were collected from under the colonies 15 and 30 min, 1 , 2 , 3 , 4 , 5 and 6 h after the time of introduction. Each mite which fell during the collection period was examined under a stereo microscope for signs of damage.

A number of fallen mites from each colony where no visible damage could be seen under the microscope were incubated at +34 °C on red-eyed pupae from *A mellifera*. At the same time control mites, collected together with the mites added to the colonies, were incubated in the same way. The survival success of the mites was measured three times at 24 h intervals. The numbers of incubated mites were 26 for *A cerana*, 41 for *A mellifera* and 40 control mites.

RESULTS

Observation hive experiments

In the first *A cerana* colony only six of the 20 mites added were recovered. Five of these mites were found on the bottom of the hive and examined for damage; none had any visible damage. One mite was found on the bee onto which it had been added. In the second *A cerana* colony 11 of 40 mites added were recovered on the bottom board. Two of the recovered mites had visible damage probably caused by the bees. No mite was found on the tagged bee onto which it had been introduced.

In the *A cerana* colonies we observed most of the marked bees (55 out of 60) instantly performing auto-grooming ('self-cleaning') after placing the mite on the bee's body. The remaining bees all performed grooming or appeared disturbed by the presence of the mite but this behavior was observed several minutes later and may be indistinct. After a few minutes some of the bees were involved in allo-grooming ('nest-mate cleaning') as described by Peng et al (1987). However, we also clearly observed, at least on three occasions, a mite leaving a marked bee and moving onto another bee. Separate observations were also made where mites could be seen on bees other than the original mite-receiving bees. It should be noted that it is very difficult to register with certainty the destiny of mites placed on individual bees. They may move to parts of the bee where they cannot be observed, or move onto other bees undetected. With the system used, however, the mites removed from the bees were likely to

be found on the bottom of the hive since the flight activity was low.

In the *A mellifera* colony, mites were added on two occasions making a total of 30 mites. Only 6 of these mites were recovered from the bottom of the hive and examined. None of these had been visibly damaged. Only one mite was found on the bee onto which it was added.

A direct reaction of *A mellifera* to adding the mite onto the bee's body was absent in many cases (17 out of 30). Some cases that could be interpreted as auto-grooming or disturbed behavior were observed (13 out of 30) but not the intense grooming dance performed by many *A cerana* bees. No clear cases of allo-grooming were observed in *A mellifera*.

The results from the observation hives are summarized in table I.

Full-sized colonies

During one month, only four mites were recovered from the bottom of the *A cerana* colonies. From one colony no mites were recovered. From the second colony one mite was recovered, and in the third three mites were found. None of these mites had visible injuries (table II).

In the three *A mellifera* colonies, a total of 258 mites falling naturally from the colonies were collected in 24 hour intervals (table II). Of these mites 26.4% had detectable injuries. Of the 258 collected mites, 132

Table I. Total numbers of mites introduced, recovered on the bottom within 1 h, and damaged in observation hives of *Apis cerana* and *Apis mellifera*.

Species	*Number of introduced mites*	*Number of recovered mites*	*Number of damaged mites*	*Percentage of damaged mites (%)*
Apis cerana	60	16	2	12.5
Apis mellifera	30	6	0	0

Table II. Total numbers of mites introduced, recovered on the bottom within 6 h, and damaged in full-sized colonies of *Apis cerana* and *Apis mellifera*; also given is the number of dead mites investigated from a natural mite population.

Species	*Number of introduced mites*	*Number of recovered mites*	*Number of damaged mites*	*Percentage of damaged mites (%)*
Apis cerana	220	115[a]	34	29.6[a]
Apis mellifera	280	65[b]	8	12.3[b]
Apis cerana	Natural	4	0	0
Apis mellifera	Natural	258	68	26.4

[a,b] Numbers differ significantly ($P < 0.05$).

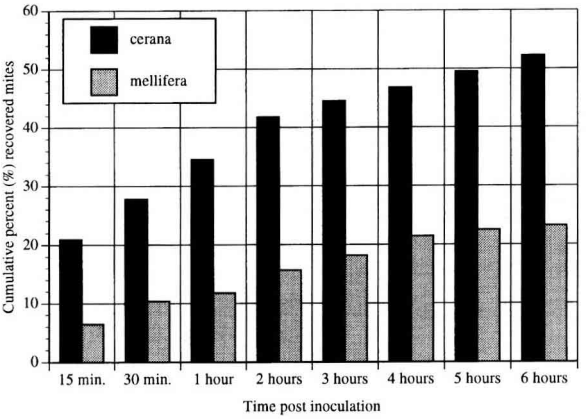

Fig 1. Cumulative percent (%) of all inoculated mites (n = 240 for *A cerana*, n = 280 for *A mellifera*), recovered on the bottom board.

were alive when they were collected. Only 9.1% of the live mites had detectable injuries.

During the 24 h preceding the first experiments with adding mites, the two *A mellifera* colonies used had natural mite mortalities of zero and two mites respectively. When the experiment was repeated, the natural mite mortalities during the 24 h before the experiment were four mites in each of the two colonies used.

Figure 1 presents the cumulative percentage recovery of all mites added during 6 h for the *A cerana* and *A mellifera* colonies. Of the 115 mites recovered within 6 h in the *A cerana* colonies, 34 mites (29.6%) had visible signs of damage caused by the bees. Often one or more legs per mite were missing, but cases where only the pretarsus of one leg was missing were also recorded. The proportion of mites recovered from *A mellifera* colonies within 6 h was significantly lower than from the *A cerana* colonies ($P < 0.001$, $\chi^2 = 45.2$, 1 df). Of the 65 mites recovered, eight had been visibly damaged by the bees (12.3%). Thus, the proportion of damaged mites in the *A mellifera* colonies was significantly lower than in the *A cerana* colonies ($P < 0.05$, $\chi^2 = 6.9$, 1 df). A total of three mites fallen from the *A mellifera* colonies were not considered in the calculations since their appearance (dead, light colored) indicated that they were not mites added to the colonies. The low natural mite mortality in the experimental colonies may slightly influence the calculations but not the conclusions. The data on mites collected from full-sized colonies are summarized in table II.

In figure 2 the survival success of mites fallen from the bees and then incubated on pupae is presented. The mite mortality at 24 h post incubation was already significantly higher than for the control mites incubated one hour after collection from the adult bees, both for *A cerana* ($P < 0.05$, $\chi^2 = 4.72$, 1 df) and *A mellifera* ($P < 0.05$, $\chi^2 = 4.91$, 1 df). At 48 and 72 h post incubation the mortality of the mites from *A cerana* was significantly higher than for those collected from *A mellifera* colonies ($P < 0.05$, $\chi^2 = 5.5$, 1 df and $P < 0.05$, $\chi^2 = 4.4$, 1 df respectively).

DISCUSSION

A substantial proportion of mites are damaged by bees in *A mellifera* colonies. In this investigation, 26.4% of all the naturally fallen mites had injuries while only 9.1% of fallen live mites had injuries. This could indicate either that mites die when they become injured, or that the bees injure already dead or non-vital mites. *A mellifera* do injure

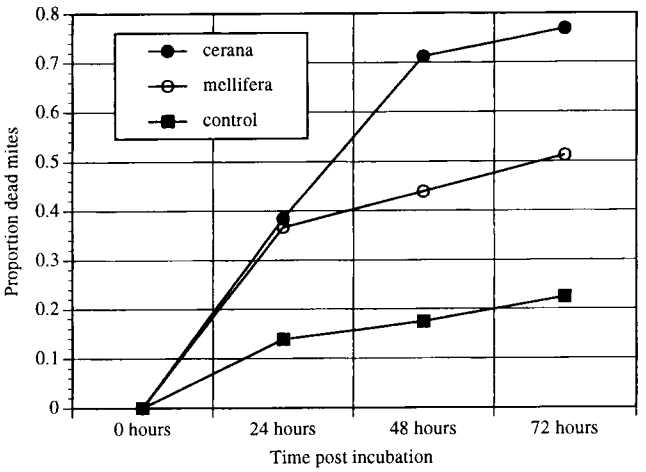

Fig 2. Survival rate of *Varroa* mites fallen from colonies of *A cerana* ($n = 26$) and *A mellifera* ($n = 41$) and incubated on bee pupae compared to incubated control mites ($n = 39$).

already dead *Varroa* mites introduced with hatching brood into colonies (Rosenkranz et al, in preparation).

Although not evident from the observation hive experiments, the presented results demonstrate that *A cerana* is more efficient in both removing and damaging live vigorous *Varroa* mites compared to *A mellifera*. This is congruent with earlier studies where grooming behavior has been considered (Büchler et al, 1992; Peng et al, 1987; Takeuchi, 1993). However, the damage caused to the mite population by the grooming behavior reported here is substantially different from some earlier reports (Peng et al, 1987). The proportion of mites removed from the bees in this investigation is lower in *A cerana* and higher in *A mellifera* than reported by Peng et al (1987). The proportion of damaged mites is also lower in *A cerana* than previously reported. It should be pointed out, though, that the observations of removed mites only refer to mites found on the bottom. In the observation hives removal of mites from the hives was probably limited due to low flight activities. In the full-sized colonies, however, the possibility cannot be excluded that some of the introduced mites were thrown out from the colonies and that this behavior may vary between bee species. Thus, the results presented here should be interpreted with great caution and, rather than demonstrating a specific grooming efficacy in the tested bees, they indicate that more research is needed. It should also be noted that in this experiment most of the mites dropped from the colonies within the first few hours after introduction. This may not reflect natural conditions and it should be emphasized that there is a need to study to what extent mites are damaged in *A cerana* colonies under natural conditions. The few mites collected from the debris in naturally infested *A cerana* colonies in this investigation ($n = 4$) had no visible damage.

For both bee species in this investigation, some mites that fell from the bees, where no visible physical damage could be detected, still seemed to be damaged by the bees. The survival rate of control mites incubated on pupae was significantly higher than that of the mites fallen from *A cerana* or *A mellifera* colonies. This effect could depend in part on dehydration or other experimental effects on the fallen mites but the difference demonstrated between bee species clearly indicate an effect from the bees. Thus, to evaluate the impact of honey bee behavior on the survival of the mites,

it is not sufficient to consider only visibly damaged mites.

There may be several explanations for the described discrepancies to earlier observations. Peng et al (1987), who only worked with observation hives, considered movement of mites from one bee to another as successful mite removal. From the point of view of mite tolerance of the colony, mites need to be removed not only from individual bees but from the colony. Thus, in this investigation we did not consider change of host to be successful removal of mites. The mites we registered as having been removed by the bees were collected from under the bees in all cases (observation hive experiments and colony experiments). In our observation hive experiments we were not able to see when the mites changed their host in many cases.

Another possible explanation for the noted differences to earlier studies may be the source of bees. Peng et al (1987) used *A cerana* from the south of China, while the *A cerana* bees in this investigation came from the Beijing area. The source of mites could also influence the results. We used only mites collected from adult bees, while Peng et al (1987) used both phoretic mites and mites collected from a sealed brood.

The full-sized colony experiments demonstrate that the studied *A cerana* colonies were much more effective in injuring and removing mites from the adult bees than the *A mellifera* colonies. However, in this study this behavior is also present in *A mellifera* to a substantial degree. The source of mites (*A mellifera*) may enhance the grooming effect in *A cerana* since odor is an important cue for detecting mites (Rosenkranz et al, 1993). There is a need to study a much larger number of colonies with respect to grooming behavior before any conclusions should be made concerning its relative importance for *Varroa* mite tolerance in *A cerana* or *A mellifera*. The relative importance of this behavior for *Varroa* mite tolerance is not demonstrated in this or any other investigation and the grooming behavior alone probably cannot explain mite tolerance in *A cerana* (Boecking et al, 1993). In simulations of the population growth of the *Varroa* mite in colonies of *A mellifera*, the relative importance of reproduction in the worker brood is obvious (Fries et al, 1994). These simulation studies indicate that, if there is no reproduction in the worker brood, the need for effective grooming to keep the mite population under control may be reduced. Understanding the relative importance of various factors that contribute to the *Varroa* mite tolerance in *A cerana*, or in other mite-tolerant honey bees, may be important for evaluating available options in *A mellifera*. In the search for a possible solution to the *Varroa* mite problem through breeding in colonies of *A mellifera*, the host–parasite relationship in the original host of the mite needs to be studied further.

ACKNOWLEDGMENT

This study was supported by the Swedish Council for Forestry and Agricultural Research.

Résumé — Étude comparative du comportement de toilettage et du nombre d'acariens lésés *(Varroa jacobsoni)* chez *Apis cerana cerana* et *Apis mellifera ligustica*. On a étudié la mortalité de l'acarien *Varroa jacobsoni* et ses lésions (principalement la perte ou les lésions d'une ou plusieurs pattes) dans des ruches d'observation, auxquelles des varroas avaient été ajoutés, et dans des colonies entières des deux espèces *Apis cerana cerana* et *Apis mellifera ligustica*. Dans les ruches d'observation, des varroas prélevés dans des colonies d'*A mellifera* ont été introduits sur des abeilles marquées, selon le protocole de Peng et al (1987). Trente varroas ont été ajoutés aux colonies d'*A mellifera* et 60 à

celles d'*A cerana*. Les varroas éliminés par les abeilles et tombés sur le plancher de la ruche dans l'heure suivant l'introduction ont été récoltés et leurs lésions étudiées. La présence de varroas sur les abeilles marquées a été recherchée une heure après leur introduction. Dans les colonies entières, les varroas, prélevés dans des colonies de *mellifera*, ont été introduits directement audessus des abeilles sur les barettes supérieures (en tout 280 dans les colonies de *mellifera* et 220 dans celles de *cerana*). Les varroas tombés des abeilles ont été récoltés sous les fonds grillagés des ruches 15 minutes, 30 minutes, 1, 2, 3, 4, 5 et 6 heures après leur introduction. Leurs lésions ont été étudiées. Les résultats montrent l'existence chez *A cerana* d'un comportement de toilettage, mais il n'est pas aussi efficace que précédemment décrit. Dans les ruches d'observation d'*A cerana*, le comportement de toilettage décrit par Peng et al (1987) a été observé, mais il est clair également que certains varroas changent simplement d'hôte lorsque que survient le toilettage. D'après les observations, 27 % seulement des varroas introduits (16 sur 60) ont été éliminés par les abeilles en 1 heure. Sur les varroas éliminés, seuls deux (12,5 %) ont été visiblement lésés par les abeilles. Sur les 30 varroas introduits sur les abeilles *A mellifera*, six ont été éliminés par elles et retrouvés sur le plancher (20 %). Aucun d'entre eux ne présentaient de lésion. Dans les colonies entières d'*A mellifera*, les varroas ont également été éliminés par les abeilles mais de façon bien moins efficace que dans les colonies d'*A cerana*. La proportion de varroas vivants retrouvés dans les débris dans les 6 heures suivant l'introduction et présentant visiblement des lésions a été de 30 % (n = 115) dans les colonies d'*A cerana* et de 12 % (n = 65) dans les colonies *d'A mellifera*. Comparée à *A mellifera*, *A cerana* est plus efficace pour se débarrasser des acariens et leur causer des lésions. Néanmoins, ces deux comportements sont également présents chez *A mellifera*. Puisque *V jacobsoni* ne se reproduit pas dans le couvain d'ouvrières d'*A cerana*, la grande efficacité du comportement de toilettage mentionnée auparavant n'est peut-être pas nécessaire pour expliquer la tolérance d'*A cerana* aux infestations par *V jacobsoni*. Nos résultats montrent que des recherches complémentaires sont nécessaires pour comprendre l'importance du comportement de toilettage dans la tolérance à *Varroa*, aussi bien chez *A cerana* que chez *A mellifera*.

***Apis mellifera / Apis cerana* / comportement toilettage / *Varroa jacobsoni* / sensibilité résistance**

Zusammenfassung — Putzverhalten und beschädigte Milben *(Varroa jacobsoni)* bei *Apis cerana cerana* und *Apis mellifera ligustica*. Mortalität und Verletzungen (meist ein oder mehrere Biene verletzt oder fehlend) von *Varroa* wurden an Völkern von *Apis cerana cerana* und *Apis mellifera ligustica* untersucht. Hierzu wurden die untersuchte Milben zu normalen Bienenvölkern und zu Beobachtungsstöcken zugesetzt. In den mit *A mellifera* und *A cerana* besetzten Beobachtungsstöcken wurden phoretische Milben aus Völkern von *A mellifera* entsprechend den Methoden von Peng *et al* (1987) auf gekennzeichnete Bienenarbeiterinnen aufgebracht. Bei *A mellifera* wurden 30, bei *A cerana* 60 Milben zugesetzt. Die innerhalb der folgenden Stunde von den Bienen entfernten Milben wurden vom Beutenboden aufgesammelt und auf Verletzungen untersucht. Die gekennzeichneten Bienen wurden nach dieser Stunde auf Milbenbefall untersucht. In den normalen Bienenvölkern beider Bienenarten wurden die ebenfalls von *A mellifera* abgesammelten Milben direkt auf die auf den Oberträgern der Waben sitzenden Arbeiterinnen aufgesetzt. Die herunterfallenden Milben wurden 0,25, 0,5, 1, 2, 3, 4 und 6 Stunden nach Einsetzen der Milben unterhalb eines Drahtgitters im

Beutenboden eingesammelt. Alle diese Milben wurden auf Beschädigungen untersucht. Insgesamt wurden 280 Milben in Völker von *A mellifera* und 220 in Völker von *A cerana* eingesetzt. Die Ergebnisse zeigen zwar einen deutlichen Effekt des Putzverhaltens bei *A cerana*, allerdings deuten sie darauf hin, daß dieser wesentlich geringer ist als früher berichtet. Das von Peng *et al* (1987) beschriebene Putzverhaltens konnte zwar ebenfalls beobachtet werden, es war aber deutlich, daß ein Teil der Milben daraufhin lediglich auf eine andere Wirtsbiene wechselte. Nur von 27% der eingesetzten Milben (16 von 60) konnte beobachtet werden, daß sie innerhalb einer Stunde von den Bienen entfernt wurden. Von diesen zeigten nur 2 (12,5%) sichtbare Beschädigungen durch die Bienen. Von den 30 auf Arbeiterinnen von *A mellifera* aufgebrachten Milben wurden 6 (20%) von diesen entfernt und auf dem Beutenboden wiedergefunden. Keine dieser Milben war sichtbar verletzt. Auch in normal großen Völkern wurden aufsitzende Milben von *A mellifera*-Arbeiterinnen entfernt, allerdings weniger effektiv als von *A cerana*-Arbeiterinnen. Bei *A cerana* betrug der Anteil verletzter Milben an den innerhalb von 6 Stunden auf dem Beutenboden wiedergefundenen Milben 30% (N = 115), bei *A mellifera* 12% (N = 65). *A cerana* kann damit wirkungsvoller als *A mellifera* die Milben entfernen und ihnen Verletzungen beibringen. Allerdings ist auch *A mellifera* hierzu in der Lage. Da *Varroa* sich in den Arbeiterinnenbrutzellen von *A cerana* nicht vermehrt, ist die Toleranz von *A cerana* gegenüber einem Befall durch *Varroa* auch ohne ein so hocheffektives Putzverhalten, wie dies früher berichtet worden war, erklärlich. Die vorliegenden Ergebnisse zeigen, daß zur Abschätzung der tatsächlichen Bedeutung des Putzverhaltens für die Toleranz sowohl von *A mellifera* als auch von *A cerana* weitere Untersuchungen erforderlich sind.

***Varroa jacobsoni* / *Apis mellifera* / *Apis cerana* / Putzverhalten / Toleranz**

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