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RESEARCH PAPER

Mating disruption of the honeybee mite *Varroa destructor* under laboratory and field conditions

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Abstract Mating in the mite *Varroa destructor*, an ectoparasite of the honeybee, takes place within the sealed brood cell of its host and is triggered by a female sex pheromone consisting of three fatty acids and their respective ethyl esters. In a laboratory bioassay, we observed interactions among offspring of a *Varroa* female at 11 days after host cell capping in the absence and in the presence of the sex pheromone and clearly demonstrated that male mites were not able to distinguish between receptive daughters and either older or immature and unreceptive females when exposed to the pheromone. In addition, mating attempts in the presence of the pheromone with otherwise receptive females were clearly of shorter duration and males often failed to select a receptive female. In order to evaluate the effect of pheromone exposure on successful copulations and the number of spermatozoa transferred under field conditions, we sprayed one of the pheromone components, oleic acid, on an empty brood comb before host egg-laying and *Varroa* infestation and counted the spermatozoa of daughter mites taken from this comb. We could show that the number of spermatozoa was indeed reduced, and 20 % of females lacked spermatozoa. Our results open up new possibilities and represent a promising step toward biological control of *Varroa* mites in beehives.

Keywords *Varroa destructor* · Mating behavior · Bioassay · Mating disruption

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Introduction

The ectoparasitic mite *Varroa destructor* has become the major pest of the western honeybee *Apis mellifera* and is considered the main reason for the periodically high colony losses (Genersch et al. 2010; Guzmán-Novoa et al. 2010; Le Conte et al. 2010; Rosenkranz et al. 2010). The symptoms of a *Varroa* infestation strongly depend on the infestation rate and the presence of associated viruses. Damages can range from the weakening of individual worker bees by the loss of hemolymph (De Jong et al. 1982; Schneider and Drescher 1987; Schatton-Gadelmayer and Engels 1988) to damages through immunosuppression and secondary infections (Yang and Cox-Foster 2007; de Miranda and Genersch 2010; Genersch 2010; Richards et al. 2011; Francis et al. 2013). Infested colonies usually collapse within 1–3 years without periodical treatments. Recommended pest control strategies often combine biotechnical methods like the removal of drone brood with the use of acaricides. The latter include "soft" chemicals like organic acids or essential oils and "hard" chemicals like organophosphates and synthetic pyrethroids. However, the use of hard miticides is associated with the risk of the pollution of bee products and development of resistance of the mites while soft chemicals often show poor efficacies, are not easy to apply and might even cause damage to bees or brood (reviewed in Rosenkranz et al. 2010). Biological approaches that could overcome these disadvantages do not exist so far.

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A promising avenue for sustainable treatment would be the control of mite reproduction (Dietemann et al. 2012). Successful reproduction by a *Varroa* female requires the finding of a suitable larval host (Dillier et al. 2006), the initiation of oogenesis (Garrido and Rosenkranz 2004), the timing of egg laying (Frey et al. 2013) and subsequent mating of the young daughter mites (Donzé et al. 1996). Only inseminated daughter mites can lay fertilized female eggs during the next reproductive cycle and, therefore, contribute to an increase of the mite population within the host colony.

Recently, the chemical composition of the female *Varroa* sex pheromone has been identified (Ziegelmann et al. 2013a, b), offering now for the first time options to disrupt the mating behavior of mites. The mating process of *V. destructor* takes place exclusively within the sealed host brood cell. Male mites reach adulthood approximately 9 days after cell capping, followed by the oldest daughter, which matures some 20 h later (Rehm and Ritter 1989). Males start mating as soon as the first daughter has molted. One to two daughter mites can normally reach maturity and mate before the host bee completes development and emerges from its cell.

Until now the exact number of sperm cells transferred by a male *Varroa* mite has not been analyzed in detail. Donzé et al. (1996) described that up to 35 spermatozoa are stored within a female's spermatheca. A clear preference by mate mites for freshly molted daughters, as observed by Donzé et al. (1996), was confirmed in our previous studies by use of a laboratory bioassay (Fahle and Rosenkranz 2005; Ziegelmann et al. 2013a), in which female deutochrysalis, young adult daughters and mother mites were offered to males. This preference can be explained by the fact that there is only a limited time window available for the mating process until the host bee hatches. If a male mite wasted time with immature or already mated females, it would most likely reduce the mating success of the adult daughter mites. Consequently, by mating with the youngest adult daughter until the next daughter reaches maturity, a male can ensure that all adult daughters receive a sufficient amount of spermatozoa whilst also maximising his reproductive success.

The complete mating behavior of the male is triggered by a female sex pheromone comprising palmitic acid, stearic acid and oleic acid together with their respective ethyl esters (Ziegelmann et al. 2013b). We could also demonstrate that these compounds, even though they are fairly ubiquitous in many insects are not present within the sealed brood cell during the crucial time when mating takes place (Martin et al. 2002; Frey et al. 2013, Ziegelmann, unpublished data). Our bioassays confirmed that the pheromone is volatile over a short distance of several millimetres and is presumably emitted immediately after the adult molt of the female mite. By the use of this bioassay, we examined the dose–response relationship of each component by determining the copulatory responses of the males. Thereby we could confirm for each component a maximum response when applied in dosages between 10 and 100 ng (Ziegelmann et al. 2013b).

In the current study, we examined whether the *Varroa* mating process can be disturbed by the application of the sex pheromone in order to evaluate the potential of this approach as a biological *Varroa* control.

Materials and methods

Collection of mites

Male and female *V. destructor* were obtained from heavily infested *Apis mellifera* colonies at the Apicultural State Institute, University of Hohenheim, in Germany. Brood combs 10–11 days after host cell capping were removed from the colonies and taken to the laboratory. The suitable combs were identified by the age of the bee pupae which have a dark grayish-brown head and thorax at this stage. The brood cells were opened with tweezers and examined for entire *Varroa* families consisting of mother mite, two mature daughters (i.e. first daughter and second daughter = freshly molted), one female deutochrysalis and one deutonymph as well as one adult male (Fig. 1). The mites were transferred into queen cell cups (Nicot system®, Karl Jenter, Metzingen), with the male separated from the females. As the female mites were used for three tests, additional males were collected. The mites were kept at 28–30 °C for a maximum of 2 h in order to prevent decline in their vitality and mobility.

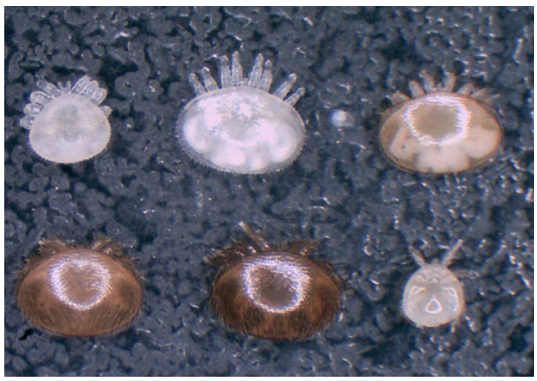

Fig. 1 Composition of the offspring of a *Varroa* female approximately 11 days after host cell capping. From *top left* to *bottom right*: female deutonymph, female deutochrysalis, 2nd mature daughter, 1st mature daughter, foundress (mother), adult male

Synthetic pheromone solutions

We used different applications for the laboratory bioassay and colony test, respectively. For the laboratory bioassay, we used all the so far identified components of the *Varroa* sex pheromone (Ziegelmann et al. 2013b): oleic acid, stearic, acid and palmitic acid, and the respective ethyl esters (Sigma Aldrich ≥99.7 %). These compounds were solved in equal proportions in diethyl ether (Roth, GC grade ≥99.8 %, stabilized with ~1 % ethanol). From a pheromone stock solution with a concentration of 10,000 ng/µL for each of the 6 components, 4 dilutions with concentrations of 1, 10, 100 and 1,000 ng component/µL were prepared and stored at −20 °C until use.

For the test on brood combs we only used the main component oleic acid (Sigma Aldrich, ≥99.7 %), applied as an alcoholic solution with a concentration of 1,000 ng/µL in ethanol (VWR 96 %).

Laboratory bioassay

Male responses toward the different mature and immature females were observed and recorded according to the modified "mating bioassay" described in Ziegelmann et al. (2013a, b). For each test, 1 µL of the synthetic pheromone solutions was applied to a piece of filter paper (size: 1.5 × 15 mm) with a 10-µL Hamilton syringe. According to the different solutions described in 2.2 this resulted in doses of 1, 10, 100 and 1,000 ng. For each dose 24 repetitions were performed. Control tests were performed in the absence of additional pheromone or solvent as former tests did not reveal any influence of the solvent (Ziegelmann et al. 2013a) and as we attempted to ensure the test conditions were as natural as possible.

The filter paper was creased in the middle and placed at the edge of the plastic cell cup (Nicot system®, Karl Jenter, Metzingen) containing the female individuals of the *Varroa* family, so that the treated end of the filter paper reached into the cell cup without touching the bottom or the sides. Afterwards the male was added and the cell cup was sealed with a cover slip to prevent the mites from escaping. The behavior of the male was then observed and recorded over a period of 10 min using the Observer 2.0 software (Noldus Information Technology). The male responses toward the different female stages were categorized in (1) unspecific contacts including movement towards/around the female, palpating it and mounting the dorsal shield of the female and (2) copulation attempts on the venter of the female.

The tests were carried out on a hotplate, with temperatures of 28–30 °C at the bottom of the cell cup which is slightly lower than the temperature of the honeybee brood of 34–35 °C. However, mites showed an optimal activity in the laboratory bioassays at the lower temperature range that we subsequently employed (Le Conte and Arnold 1988; Rosenkranz 1988).

Pheromone application under colony conditions

For the application on brood combs, the alcoholic oleic acid solution (1,000 ng/µL) was sprayed evenly on empty combs which had already been used for one brood cycle within the colony. Each side of the comb was sprayed several times, until a total dose of 5 ml per comb side was achieved. With an average of about 3,000 brood cells per comb side (Zander size, 764 cm^2 comb area), every brood cell was coated with approximately 2,000 ng oleic acid. The comb was then placed in the middle of the brood nest of a bee colony with the queen trapped on the comb. The queen was released 2 days after she had started egg-laying on the comb. On the day of host cell capping, brood cells prior to capping were marked on an overhead projector film. Marked cells that were sealed during the following 4 h were opened with a scalpel and artificially infested with one phoretic mite female that was directly collected from an adult bee. The *Varroa* females' dorsa were marked with paint to distinguish them later from their adult daughters. The artificially infested host cells were opened 11 days after capping and the adult daughter mites as well as the foundress, but not the son, were transferred into a plastic thimble and kept on a bee pupa for four more days in the incubator at 30 °C and approximately 60 % humidity to ensure that all spermatozoa will have migrated from the rami to the spermatheca of the daughter mites, if mated. The spermathecae of the daughter mites were then dissected in PBS buffer and the number of spermatozoa within the spermathecae was counted using a light microscope. In a separate control colony, the same procedure was undertaken with a brood comb treated with the solvent ethanol only.

Data analysis

The data sets were analyzed using the SPSS 20 statistics software. As most data sets were not normally distributed, the non-parametric Kruskal–Wallis one-way analysis of variance with a Dunn–Bonferroni correction procedure was used to evaluate the male behavior toward the females in the absence and in the presence of synthetic pheromone. The number of spermatozoa in spermathecae of daughter mites was compared using Mann–Whitney test. Differences between groups with $P < 0.05$ were considered statistically significant.

Results

Male responses towards females of different ages in the absence and in the presence of synthetic pheromone

In control tests without synthetic pheromone, male mites had long-term contacts exclusively with the two adult daughter mites (Fig. 2, control) with a clear preference for the second (=younger) daughter. The mean duration of contacts with this second daughter mite was significantly longer compared to contacts with all nymphal stages, including the foundress ($P < 0.05$, $n = 24$, Kruskal–Wallis).

Specific mating attempts by males on the females' venter could almost exclusively be observed with the second (=youngest, freshly molted) adult daughter (Fig. 3, control) and only in a few cases with the first (=older) daughter mite. The difference of the mean duration of the copulation attempts between both daughter mites was highly significant ($P < 0.001$, $n = 24$, Kruskal–Wallis). Interestingly, mounting the second daughters' dorsum led in all cases to mating attempts expressed by the typical tip-over behavior and probing the gonopores. When males were exposed to 1 and 10 ng of the synthetic pheromone, their preference behavior was not significantly different from the control tests (Figs. 2, 3). Again, males had on average the longest contacts with the second adult daughter and the copulation attempts with this female lasted significantly longer compared to the first daughter and all other female stages ($P < 0.05$, $n = 24$, Kruskal–Wallis). However, the mean duration of copulation attempts with the second adult daughter was in these test series already about 100 s shorter compared to the control, and some short mating attempts with deutonymphs could be observed (Fig. 3).

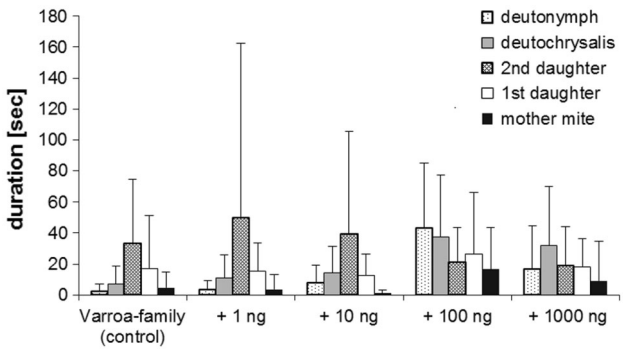

Fig. 2 Mean duration and standard deviation of unspecific contacts (walking around females and mounting the females' dorsum) with mature and immature females of a *Varroa* family (foundress, two receptive daughters and two early instar daughters) 11 days after cell capping in the absence (control) and in the presence of synthetic pheromone. The synthetic pheromone was applied in dosages of 1, 10, 100 and 1,000 ng per pheromone component ($n = 24$ for control and each dose)

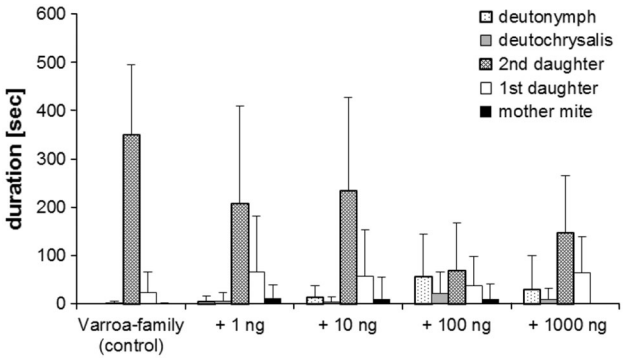

Fig. 3 Mean duration and standard deviation of copulation attempts with different mature and immature females of a *Varroa* family (foundress, two receptive daughters and two early instar daughters) 11 days after cell capping in the absence (control) and in the presence of synthetic pheromone. The synthetic pheromone was applied in dosages of 1, 10, 100 and 1,000 ng per pheromone component

Table 1 Statistical comparisons of the copulatory responses of *V. destructor* males towards the 2nd daughter mite in the absence (control) and in the presence of synthetic pheromone

Dose	*n*	Copulation attempts with 2nd daughter				
		Mean [s] ± SD	Significance			
			1 ng	10 ng	100 ng	1,000 ng
Control	24	350.3 ± 144.5	0.061	0.333	<0.001***	0.004**
1 ng	24	207.6 ± 202.9	–	1.000	0.032*	1.000
10 ng	24	234.7 ± 192.9	–	–	0.004**	1.000
100 ng	24	69.9 ± 97.1	–	–	–	0.328
1,000 ng	24	148.5 ± 116.5	–	–	–	–

Presented is the mean duration of copulation attempts for applications of 1, 10 100 and 1,000 ng of sex pheromone on a filter paper and the corresponding *P* values

P values marked with asterisks were considered statistically significant (Kruskal–Wallis one-way analysis of variance) with * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$

When 100 ng of the synthetic pheromone was applied, a striking change in the preference behavior of the males was recorded. The longest contacts were now observed with deutonymphs followed by deutochrysalis, however, without significant differences among all female stages (Fig. 2, 100 ng). The effect on copulation attempts was even more pronounced: the average duration of copulation attempts with the second daughter was reduced to about 20 % compared to the control (Table 1) and occurred, in addition, less often. In contrast, the number and duration of copulation attempts with the immature deutonymphs and deutochrysalis increased. As a consequence, there were no

significant differences in the duration of the copulation attempts among all female stages ($P > 0.05$, $n = 24$, Kruskal–Wallis) except significant shorter copulation attempts with the foundress ($P < 0.05$, $n = 24$, Kruskal–Wallis; Fig. 3). After the 100-ng application it could also be observed that males did not pause in the venter-to-venter position at one gonopore, but moved from one gonopore to the other of a female or alternated repeatedly between dorsum and venter.

In the presence of 1,000 ng/substance, the mean duration of copulation attempts with the second daughter was not significantly different from the 100 ng applications but still highly significant different from the control (Table 1). Therefore, applications of 100 and 1,000 ng had, under laboratory conditions, a clear and significant effect on both the preference behavior of the males and the duration of the copulation attempts.

Pheromone application under colony conditions

In both colonies, the treated combs were accepted by the queen for egg laying and a normal brood nest with several thousand larvae per comb was produced. Not all phoretic females that were transferred into the brood cells up to 4 h after cell capping reproduced. The percentage of non-reproducing females ranged from 1.4 % in the treated comb to 8.2 % in the control comb which concur with the percentage of non-reproducing females in artificially as well as naturally infested brood cells (Frey et al. 2013). Daughter mites from cells without males (6.8 % in the control comb, 4.1 % in the treated comb) or with dead male offspring (8.2 % in the control comb, 11 % in the treated comb) were not included in further statistical evaluation. Females of both control and experimental combs showed still some single immature spermatozoa within the rami. As it was not always possible to retrieve the spermathecae with uninjured rami these spermatozoa were, therefore, not counted.

Daughter mites taken from the combs treated with oleic acid showed on average a significantly lower number of spermatozoa within the spermatheca (15.4; $n = 40$; Fig. 4) compared to the control (24.5; $P < 0.05$, $n = 33$; Mann–Whitney test). Remarkably, 20 % of the *Varroa* females from treated combs did not have any spermatozoa at all compared to only 6 % in the control combs; however, these differences were not significant ($P = 0.12$, Fisher's exact test).

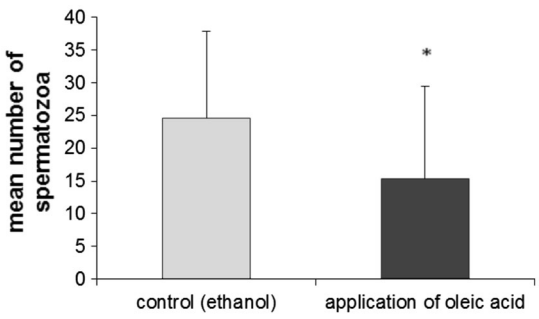

Fig. 4 Mean number and standard deviation of spermatozoa in spermathecae of daughter mites from honeybee brood combs treated with ethanol ($n = 33$) and oleic acid ($n = 40$). Daughter mites were collected from brood cells 11 days after capping and kept in the incubator for 4 days before the spermatozoa were counted. Single asterisk indicates a difference of $P < 0.05$ (Mann–Whitney test) between control (*light gray bar*) and treatment with oleic acid (*dark bar*)

Discussion

The identification of the *Varroa* sex pheromone (Ziegelmann et al. 2013b) offers for the first time a realistic option for a biological approach to reduce the reproductive success of the mite. In this study we determined whether the mating behavior of *V. destructor* can be disturbed by the use of the female sex pheromone (i) under laboratory conditions and (ii) by targeted application within the colony. In order to evaluate the effect of the sex pheromone, we compared male responses toward mature and immature females in the absence and in the presence of synthetic pheromone solutions. For this approach we mimicked the composition of a natural *Varroa* family 11 days after cell capping in our bioassay. At this stage, an infested host brood cell generally contains the foundress, one single adult son, two mature daughters and several immature female stages (Ifantidis 1983). Therefore, the male has the choice for contacts and copulatory attempts with several female stages.

In *V. destructor*, the course of mating is characterized by a distinct sequence of behaviors (Donzé et al. 1996, Ziegelmann et al. 2013a) with the tip-over of the male to the female's venter as the crucial step in copulation. In our bioassay we were able to quantify the general attractiveness of certain female stages (expressed as unspecific contacts) as well as the final mating attempt.

In all control tests, males showed an unmistakable preference for the youngest adult daughter, whereas other females were less attractive or even not attractive at all. This exactly resembles the observations made by Donzé et al. (1996) in tests with artificial polystyrene cells containing naturally reared host brood and mites and also matches the results of our former investigation on the preference behavior of male mites in plastic queen cell cups (Ziegelmann et al. 2013a). Most likely, freshly molted yet unmated female mites elicit higher amounts of the sex pheromone. After about 24 h the attractiveness of these

females decreases, resulting in fewer and shorter copulation attempts by males (Ziegelmann unpublished). Therefore, the most recently molted daughter mite has a greater chance to mate with the male before the host bee emerges from its brood cell. As each *Varroa* foundress usually produces only one male, we consider this a crucial and adaptive behavior to ensure sufficient sperm transfer to all adult daughter mites.

This important sequence of the male mating behavior and preference can be disturbed by the application of an overdose of the female sex pheromone. The application of 1 and 10 ng of synthetic sex pheromone already resulted in a reduced duration of mating attempts with the youngest adult daughter and a slight increase of contacts and mating attempts with other females. An obvious and highly significant change in male behavior was seen with the application of 100 and 1,000 ng of the sex pheromone. These amounts are considerably higher compared to the results of the quantitative analysis of mite extracts (Ziegelmann et al. 2013b) and confirm that a huge overdose of the pheromone is required for significant effects. Under these conditions males were confused and seemed unable to distinguish whether females were receptive or not. The average duration of the copulation attempts with the youngest adult daughter was reduced to about 20 %, even though males had contacted these females and mounted their dorsum. Males often did not show any attempt to copulate but alternated between the ventral and dorsal side of a female or even descended from the dorsum and departed the female mite. In contrast, intense copulation attempts were performed with immature female deutonymphs and deutochrysalis, which are completely unattractive under normal conditions. This is surprising as deutonymphs are much smaller and also their body shape differs from mature females. Consequently, body shape and size might not play a crucial role in receptive mate recognition. The relatively low frequency and duration of mating attempts with the mother mite (foundress) might be due to their more pronounced movement compared to younger developmental stages. These results again confirm that the volatile sex pheromone is the crucial signal for the triggering of the mating behavior (Ziegelmann et al. 2013b), and it confirms that this behavior can be disturbed solely by an overdose of pheromone.

Although we could not achieve a repellent effect or completely hinder the male from mating with the second daughter, we could significantly reduce the time of the mating attempts and instead increase the time the male spent with immature or already mated stages. By spending time with already mated or even immature females, less successful matings with unmated adult daughters might happen. Consequently, the average number of spermatozoa in the spermatheca of daughter mites ought to be reduced.

This hypothesis could be confirmed by our first pheromone application under colony conditions. Instead of using a mixture of all six components, only oleic acid was used due to its favorable physical properties and because in former experiments this main component elicited similar behavioral effects as the mixture (Häußermann 2011). The oleic acid solution was sprayed on a brood comb prior to egg-laying and *Varroa* infestation. Counting the number of spermatozoa in spermathecae of daughter mites taken from this comb showed that indeed the number of transferred spermatozoa was significantly reduced. This result supports the suggestion that the application of pheromone disturbs the mating process and leads to a reduced number of copulations and thus spermatozoa transfer. As the brood cells were opened on day 11 after host cell capping, the time span for mating was shorter than under normal conditions (cells normally remain capped for about 12 days, Rosenkranz and Engels 1994). Thus, the mean number of spermatozoa in females taken from the control comb was somewhat lower than the mean number of spermatozoa given in the literature (Donzé et al. 1996). For an absolute quantification of the effect of the comb treatment on sperm transfer, the comb should be kept in an incubator prior to hatching of the bee. Nevertheless, our results highlight the possibility to disturb *Varroa* mating under colony conditions, which leads to a reduction of transferred spermatozoa and, remarkably, 20 % of the daughters without any spermatozoa at all.

Although this reduced copulatory activity with young females would not completely stop the growth of a *Varroa* population, the higher percentage of unmated female mites and the reduced number of transferred sperm could at least reduce reproductive rates in the following reproductive cycles. Harris and Harbo (1999) already assumed that a low number of sperms within the spermatheca might have a significant effect on the reproductive success of the female mites. However, a valid evaluation of the effect of a reduced sperm transfer has to be performed on the colony level by the treatment of all brood combs and the record of the population dynamic of the mite during the season. If such an application is used as an additional measure throughout the brood season, it might have a significant effect on mite population dynamics. The currently recommended and widely performed *Varroa* treatment concepts mostly start after the honey yield in late summer (Imdorf et al. 1996; Rice et al. 2004; reviewed in Rosenkranz et al. 2010). At that time, the *Varroa* population has often already reached or even exceeded the damage threshold. According to recent models (Fries et al. 1994; Calis et al. 1999; Wilkinson and Smith 2002), even slight reductions in the *Varroa* reproductive rate during the summer season could prevent the exponential growth of the mite population and, therefore, keep the mite infestation rate below this threshold (Vetharaniam 2012).

However, the development of a successful biological *Varroa* control treatment with sex pheromone under beekeeping conditions requires further improvement of the application technique. The effect of the pheromone should preferably last over a longer period and several brood cycles. The normal development of the bee brood in our treated combs indicates that side effects on bees and brood are unlikely; however, this aspect needs to be considered in further experiments. From the perspective of food safety and residues in the human food chain, we do not see unsurmountable obstacles in the application of the component of the sex pheromone we used because fatty acids (and their esters) are commonly of low toxicity and widespread in animal and food material. Finally, we expect that the efficacy revealed in our experiments could be increased. In this first experiment we only used one application method with one dosage of the main component of the *Varroa* sex pheromone (Ziegelmann et al. 2013b). It is likely that mating disruption could be increased by modifications of the pheromone quantity, combined with other components of the sex pheromone and/or application methods.

The entire reproductive cycle of *V. destructor* is triggered by chemical communication, from host finding (Le Conte et al. 1994; Calderone and Lin 2001) via the activation of female oogenesis (Garrido and Rosenkranz 2004; Frey et al. 2013) through to mating (Ziegelmann et al. 2013b). It is surprising that the dependence of the mite's reproductive success on volatile compounds has so far not been used for the development of a biological control method. Our here presented first approach of a sex pheromone application within a colony could not achieve a complete mating disruption of *Varroa* mites. However, our results represent a promising road to the first biological control method based on pheromones and will hopefully encourage further investigations in this direction.

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References

Calderone NW, Lin S (2001) Arrestment activity of extracts of honey bee worker and drone larvae, cocoons and brood food on female *Varroa destructor*. Physiol Entomol 26:341–350

Calis JNM, Fries I, Ryrie SC (1999) Population modelling of *Varroa jacobsoni* Oud. Apidologie 30:111–124

De Jong D, De Jong PH, Goncalves LS (1982) Weight loss and other damage to developing worker honeybees from infestation with *V. jacobsoni*. J Apicult Res 21:165–216

De Miranda J, Genersch E (2010) Deformed wing virus. J Invertebr Pathol 103:S48–S61

Dietemann V, Pflugfelder J, Anderson D, Charrière JD, Chejanovsky N, Dainat B, de Miranda J, Delaplane K, Dillier FX, Fuchs S, Gallmann P, Gauthier L, Imdorf A, Koeniger N, Kralj J, Meikle W, Pettis J, Rosenkranz P, Sammataro D, Smith D, Yañez, Neumann P (2012) *Varroa destructor*: research avenues towards sustainable control. J Apicult Res 51(1):125–132. doi:10.3896/IBRA.1.51.1.15

Dillier FX, Fluri P, Imdorf A (2006) Review of the orientation behaviour in the bee parasitic mite *Varroa destructor*: sensory equipment and cell invasion behaviour. Rev Suisse Zool 113(4):857–877

Donzé G, Herrmann M, Bachofen B, Guerin PM (1996) Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni*. Ecol Entomol 21:17–26

Fahle N, Rosenkranz P (2005) Mate choice in *Varroa destructor*: male mites prefer young females. In: IUSSI-Proceedings of the German Section Meeting at Halle, ISBN 3-901864-02-4

Francis RM, Nielsen SL, Kryger P (2013) *Varroa*-virus interaction in collapsing honey bee colonies. PLoS One 8(3):e57540

Frey E, Odemer R, Blum T, Rosenkranz P (2013) Activation and interruption of the reproduction of *Varroa destructor* is triggered by host signals (*Apis mellifera*). J Invertebr Pathol 113:56–62

Fries I, Camazine S, Sneyd J (1994) Population dynamics of *Varroa jacobsoni*: a model and a review. Bee World 75:5–28

Garrido C, Rosenkranz P (2004) Volatiles of the honey bee larva initiate oogenesis in the parasitic mite *Varroa destructor*. Chemoecology 14:193–197

Genersch E (2010) Honey bee pathology: current threats to honey bees and beekeeping. Appl Microbiol Biotechnol 87:87–97

Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Muehlen W, Gisder S, Meixner M, Liebig G, Rosenkranz P (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie 41:332–352. doi:10.1051/apido/2010014

Guzmán-Novoa E, Eccles L, Calvete Y, Mc Gowan J, Kelly PG, Correa-Benítez A (2010) *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (Apis mellifera) colonies in Ontario, Canada. Apidologie 41:443–450

Harris JW, Harbo JR (1999) Low sperm counts and reduced fecundity of mites in colonies of honey bees (Hymenoptera: Apidae) resistant to *Varroa jacobsoni* (Mesostigmata: Varroidae). J Econ Entomol 92:83–90

Häußermann C (2011) Pheromonwahrnehmung und Repellenteffekte bei der Kopulation der Bienemilbe *Varroa destructor*. Bachelor thesis at the faculty of biology at the University of Hohenheim, p 23

Ifantidis MD (1983) Ontogenesis of the mite *Varroa jacobsoni* in worker and drone honey bee brood cells. J Apicult Res 22:200–206

Imdorf A, Charrière JD, Maquelin C, Kilchenmann V, Bachofen B (1996) Alternative *varroa* control. Am Bee J 136(3):189–193

Le Conte Y, Arnold G (1988) Etude du thermopreferendum de *Varroa jacobsoni* Oud. Apidologie 19:155–164

Le Conte Y, Sreng L, Trouiller J (1994) The recognition of larvae by worker honeybees. Naturwissenschaften 81:462–465

Le Conte Y, Ellis M, Ritter W (2010) *Varroa* mites and honey bee health: can *Varroa* explain part of the colony losses? Apidologie 41:353–363

Martin C, Provost E, Bagnères AG, Roux M, Clèment JL, Le Conte Y (2002) Potential mechanism for detection by *Apis mellifera* of the parasitic mite *Varroa destructor* inside sealed brood cells. Physiol Entomol 27:175–188

Rehm SM, Ritter W (1989) Sequence of the sexes in the offspring of *Varroa jacobsoni* and resulting consequences for the calculation of the developmental period. Apidologie 20:339–343

Rice ND, Winston ML, Higo HA (2004) Integrated Pest Management for the parasitic mite *Varroa destructor* (Anderson and Trueman) in colonies of honey bees (*Apis mellifera*). Am Bee J 144(10):791–795

Richards EH, Jones B, Bowman A (2011) Salivary secretions from the honeybee mite, *Varroa destructor*: effects on insect haemocytes and preliminary biochemical characterization. Parasitology 138:602–608

Rosenkranz P (1988) Temperaturpräferenz der *Varroa*-Milbe und Stocktemperaturen in Bienenvölkern an Tropenstandorten (Acarina: Varroidae/Hymenoptera: Apidae) Entomol Gener 14:123–132

Rosenkranz P, Engels W (1994) Genetic and environmental influences on the duration of preimaginal worker development in eastern (*Apis cerana*) and western (*Apis mellifera*) honey bees in relation to Varroatosis. Rev Brasil Genet 17:383–391

Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. J Invertebr Pathol 103:96–119

Schatton-Gadelmayer K, Engels W (1988) Blood proteins and body weight of newly-emerged worker honeybees with different levels of parasitization of brood mites. Entomol Gener 14:93–101

Schneider P, Drescher W (1987) Einfluss der Parasitierung durch die Milbe *Varroa jacobsoni* aus das Schlupfgewicht, die Gewichtsentwicklung, die Entwicklung der Hypopharynxdrüsen und die Lebensdauer von *Apis mellifera*. Apidologie 18:101–106

Vetharaniam I (2012) Predicting reproduction rate of *Varroa*. Ecol Model 224:11–17

Wilkinson D, Smith GC (2002) A model of the mite parasite, *Varroa destructor*, on honeybees (*Apis mellifera*) to investigate parameters important to mite population growth. Ecol Model 148:263–275

Yang X, Cox-Foster D (2007) Effects of parasitization by *Varroa destructor* on survivorship and physiological traits of *Apis mellifera* in correlation with viral incidence and microbial challenge. Parasitology 134:405–412

Ziegelmann B, Lindenmayer A, Steidle J, Rosenkranz P (2013a) The mating behavior of *Varroa destructor* is triggered by a female sex pheromone. Part 1: preference behavior of male mites in a laboratory bioassay. Apidologie 44:314–323. doi:10.1007/s13592-012-0182-5

Ziegelmann B, Tolasch T, Steidle JLM, Rosenkranz P (2013b) The mating behavior of *Varroa destructor* is triggered by a female sex pheromone. Part 2: identification and dose-dependent effects of components of the *Varroa* sex pheromone. Apidologie 44:481–490. doi:10.1007/s13592-013-0198-5