Exp Appl Acarol (2018) 74:43–54
https://doi.org/10.1007/s10493-018-0216-4

# Spermatozoa production in male *Varroa destructor* and its impact on reproduction in worker brood of *Apis mellifera*

**Claudia Katharina Häußermann[1] · Bettina Ziegelmann[1] · Peter Rosenkranz[1]**

Received: 18 October 2017 / Accepted: 23 January 2018 / Published online: 1 February 2018


**Abstract** Reproduction in *Varroa destructor* exclusively takes place within the sealed honey bee brood cell and is, therefore, limited by the duration of the postcapping period. Oogenesis, ontogenetic development and mating must be optimized to ensure the production of as many mated daughter mites as possible. One adult male mite has to mate with up to five sister mites and transfer 30–40 spermatozoa to each female. We analyzed the production and transfer of male spermatozoa during a reproductive cycle by counting all spermatozoa in the genital tracts of the male and daughter mites in 80 worker brood cells at defined times after cell capping. We could show that spermatozoa production in male mites is an ongoing process throughout their adult lifetime starting after the adult molt. The spermatozoa are transferred to the females in an early non-capacitated stage and require further maturation within the female's genital tract. Our study points out that a *Varroa* male has at any time in the brood cell enough spermatozoa to inseminate all daughter mites but does not waste energy in producing a big surplus. In total one male produced, on average, 125 spermatozoa during a reproductive cycle in worker brood which is sufficient for successful matings with at least three daughter mites. Spermiogenesis in *Varroa* males represents therefore a further adaptation to the limited time available for reproduction.

**Keywords** *Varroa destructor* · Spermatozoa · Reproduction · Spermatozoa production

## Introduction

*Varroa destructor* Anderson and Trueman (Acari, Mesostigmata: Varroidae) is the major threat for beekeeping worldwide (Genersch et al. 2010; Genersch 2010; Rosenkranz et al.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10493-018-0216-4) contains supplementary material, which is available to authorized users.

✉ Claudia Katharina Häußermann
Claudia.Haeussermann@uni-hohenheim.de

[1] Apicultural State Institute, University of Hohenheim, 70599 Stuttgart, Germany

2010; vanEngelsdorp et al. 2011; Dietemann et al. 2013). The life cycle of *V. destructor* is divided into a phoretic phase on adult honey bees and a reproductive phase inside the sealed honey bee brood cell (Rosenkranz et al. 2010). Mite reproduction is strictly limited by the duration of the capping period of the honey bee brood: Within about 12 days (worker brood) or about 15 days (drone brood), respectively, the mother mite has to lay the eggs, the offspring has to develop into adult mites and the adult male has to mate with all mature daughter mites (Dietemann et al. 2013). Approximately 70 h after the capping of the brood cell, the mother mite lays its first egg which is haploid. Due to haplodiploid sex determination the haploid egg develops into a male mite (Rehm and Ritter 1989). After the first egg is laid the mother mite lays an additional 3–5 eggs in 30-h intervals that are diploid and will develop into female mites (Ifantidis 1983, 1990; Martin 1994). During the ontogenetic development the mite offspring pass through different stages, starting with an egg-larva and followed by protonymph, protochrysalis, deutonymph and deutochrysalis to the adult stage. The whole development takes approximately 5.8 days for female mites and 6.6 days for male mites (Donzé and Guerin 1994; Ifantidis 1990; Martin 1994; Rehm and Ritter 1989). With the last molt the adult *Varroa* mites become sexually mature.

The mating of the mature offspring is a crucial step in mite reproduction (Ziegelmann et al. 2013a, b). Generally, the male mite mates with its sisters immediately after they have finished their adult molt. During one reproductive cycle a mother mite can—depending on the duration of the capping period—produce up to five adult female daughters which have to be mated by a single male (Ifantidis 1983, 1990). The typical mating behavior of the male mite includes several behavioral steps from walking around the adult female, mounting the dorsum and finally moving sideward to the females' venter to transfer the spermatozoa (Ziegelmann et al. 2013a). This final behavioral step is triggered by the female sex pheromone which is exclusively elicited by attractive young females (Ziegelmann et al. 2013b) and perceived by the male by the sensory pit organ on the front leg tarsi (Häußermann et al. 2015). During multiple matings male mites transfer 30–40 spermatozoa per female mite (Alberti and Hänel 1986; Donzé et al. 1996; Ziegelmann and Rosenkranz 2014). Considering the comparatively large size of the spermatozoa (Alberti and Coons 1999), the timely production of a sufficient number of spermatozoa for all daughter mites represents a challenge to the male's physiology.

So far, little is known about the spermiogenetic process in male mites. The male genital tract consists of one single dense testis at both anterior-lateral sides the tubular vas deferentia originate. The vas deferentia lead to the unpaired ductus ejaculatorius which has an external opening. Beyond that an unpaired accessory genital gland releases its products into the ductus ejaculatorius (Alberti and Hänel 1986). Alberti and Hänel (1986) showed that spermiogenesis of *Varroa* males takes place in cysts composed of somatic cells inside the male testis. The most developed spermatozoa are close-by or inside the vas deferentia. The spermatozoa have to pass the ductus ejaculatorius before they are released into the genital tract of the female mites. Unfortunately, the final step of the *Varroa* copulation—i.e. the transmission of sperm—has never been observed in detail. It is assumed that male mites produce a spermatophore and take it with their spermatodactyls and put it into the paired genital pores of the female mites (Rosenkranz et al. 2010). Spermatozoa get stored inside a specialized female storage organ—the spermatheca—inside the female genital tract (Alberti and Hänel 1986). The spermatozoa stored within the spermatheca have to last the whole life time of the female mite. Spermatozoa are transferred by the male mite in a roundish form that is not yet able to fertilize the egg cells. For this purpose, the spermatozoa still have to go through a maturation phase inside the female genital tract—the so-called spermatozoa capacitation (Alberti and Hänel 1986; Häußermann et al. 2016).

During this process spermatozoa undergo substantial morphological changes until they are fusiform (Häußermann et al. 2016).

Importantly, male mites have to finish the mating activities before the hatching of the host bee, as they are unviable outside the sealed brood cell (Donzé et al. 1996; Ziegelmann and Rosenkranz 2014) and unmated female mites are incapable to lay fertilized female eggs (Martin et al. 1997). The reproductive success of a *Varroa* female therefore depends not least on the timely production of a sufficient number of spermatozoa by the male mite.

In view of the limited time for the production of the spermatozoa and their comparatively large size we here tested two hypotheses: (1) male *Varroa* mites produce constantly over their whole adult lifetime spermatozoa that are ready for the transmission into the female genital tract and (2) a *Varroa* male has at any time in the brood cell enough spermatozoa to inseminate all daughter mites. For this purpose, we focused on the time-dependent production of spermatozoa within male mites and analyzed the total number of spermatozoa that have been produced at different time points after brood cell capping.

## Materials and methods

### Infestation of brood cells and *Varroa* mite stages considered for spermatozoa counts

The mite stages were collected from *Apis mellifera* L. hives at the Apicultural State Institute at the University of Hohenheim. For the identification of sex and age of the *Varroa* mites see Dietemann et al. (2013). Briefly, adult male mites were characterized by their pear shaped body (Fig. 1c) with a size of 0.8 × 0.7 mm (Fernandez and Coineau 2006). Daughter mites differ in the intensity of sclerotization: Freshly molted female mites are characterized by their bright dorsal shield and the fine red line around their dorsum (Fig. 1b); with increasing age of the daughter mites the sclerotization becomes more and more intense (Fig. 1d) but can still be clearly distinguished from the dark reddish-brown mother mite. The size of adult female mites is 1.1 × 1.7 mm (Anderson and Trueman 2000). From the immature stages, which are not sexually active, only the male deutochrysalis was analyzed to verify whether mature spermatozoa already are present in the male's genital tract before the final adult molt. The male deutochrysalis as the last stage before the adult molt is immobile and extends its legs forwards in a typical way (Fig. 1a), its length is 0.7 mm (Fernandez and Coineau 2006).

To analyze all adult *Varroa* stages (i.e., male, mother mite and daughter mites) at exactly 10, 11 and 12 days after cell capping, we infested brood cells artificially with phoretic *Varroa* mites. We only used female mites with a dark reddish-brown coloration, as they are at least several days old and do not need further stimulation on adult bees before the start of oogenesis (Häußermann et al. 2016). Phoretic mites were sampled directly before by the powdered sugar method (Dietemann et al. 2013). Brood cells just before capping were marked on a transparency sheet and only brood cells closed within a 4-h period ('freshly capped brood cells') were opened with a scalpel and one *Varroa* mite ('mother mite') was introduced with fine forceps and closed again. Thereafter, the brood comb was put back to the honey bee colony until analysis of the artificially infested brood cells after 10, 11 or 12 days. At these time points we exclusively analyzed the number of spermatozoa in 'complete' *Varroa* mite families containing a certain composition of living family members (see Table 1). In total, 342 brood cells were opened to receive 80 mite families according to

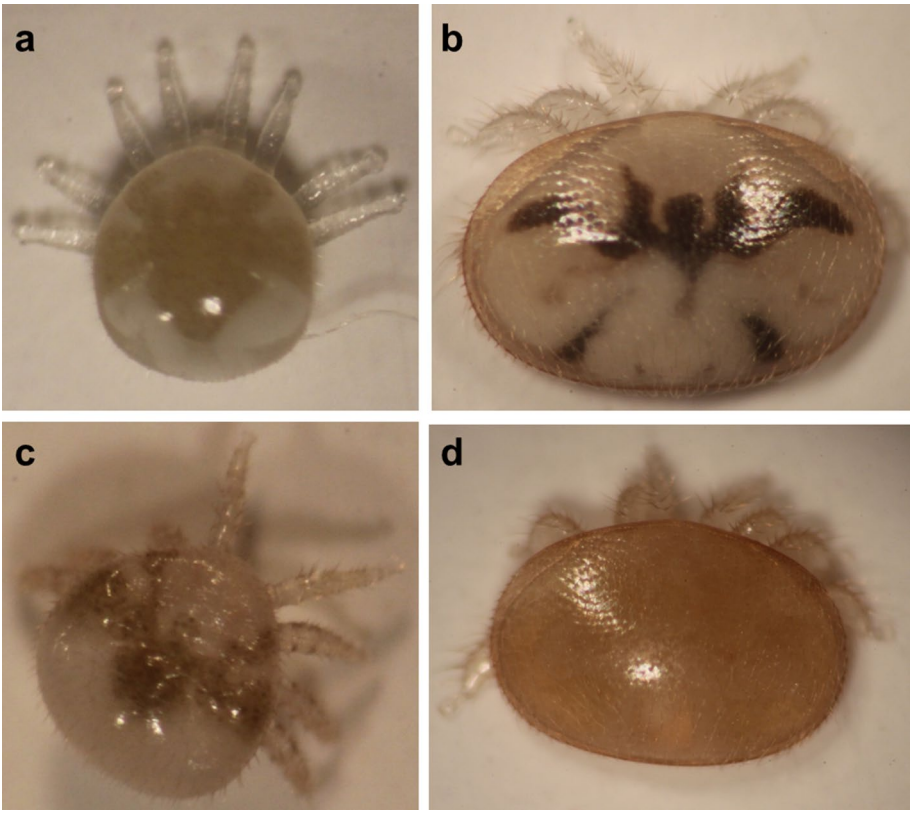


**Fig. 1** *Varroa destructor* mite stages used in this study. **a** immature male deutochrysalis (length 0.7 mm), **b** freshly molted adult female mite characterized by its bright dorsum and the fine red line around it (size 1.1 × 1.6 mm), **c** adult male mite (size 0.8 × 0.7 mm), **d** adult female mite with reddish dorsal shield (size 1.1 × 1.7 mm)

**Table 1** Minimum requirements for the composition of the analyzed *Varroa destructor* families on day 10, 11 and 12 after cell capping

| Days after cell capping | | |
|---|---|---|
| 10 | 11 | 12 |
| Mother mite | Mother mite | Mother mite |
| Male mite | Male mite | Male mite |
| 1st daughter | 1st daughter | 1st daughter |
| | 2nd daughter | 2nd daughter |
| | | 3rd daughter (optional) |

As immature stages are not sexually mature they were not considered. All adult stages had to be alive

Table 1. The rationale behind this practice was that the spermatozoa produced by an individual male must either be stored in the male's genital tract and/or in the genital tract of the adult female mites within the same brood cell. By adding together the numbers of non-capacitated spermatozoa from male mite, daughter mites, and mother mites we were able

to determine the total number of spermatozoa that has been produced by the male mite at a distinct time point. To examine the chronological sequence of spermatozoa production, we analyzed the mites in honey bee worker brood cells at 10, 11 and 12 days after cell capping, which approximately coincide with the adult molt of the first, second, and third daughter mite.

## Dissection and spermatozoa analysis

The genital tract of female and male mites were dissected in phosphate-buffered saline (PBS, ingredients: 1.000 ml $H_2O$, 8 g NaCl, 0.2 g KCl, 1.25 g $Na_2HPO_4 \cdot 2H_2O$ and 0.2 g $KH_2PO_4$) under a stereo-zoom-microscope (VWR: SZT 100) with a magnification of about 30×, using DUMONT 5 tweezers. For the dissection of female mites the female ventral shields were opened and the spermatheca and rami were isolated. For the dissection of the male mites (deutochrysalis and adults) the idiosoma was carefully opened and the male genital tract including the testis, the vas deferentia and the ductus ejaculatorius were isolated. All the other body parts like intestine and legs were removed.

The male and female genital tracts were analyzed with a VWR TR 500 light microscope (magnification 100–400×). Images were taken with a CANON EOS 60 D camera. The maximum diameter of the roundish stage-0 spermatozoa inside the male genital tract was measured by analyzing the pictures with the software GIMP 2 (v.2.8.10). The genital tract of the adult male mites contains roundish 'stage 0' spermatozoa (Häußermann et al. 2016, Fig. 5). These stage-0 spermatozoa are transmitted during mating and can be clearly distinguished from other stages. They are exclusively found within the male's testis, the male's vas deferentia and in the rami of recently mated adult females (Fig. 2b–d). As stage-0 spermatozoa represent the final stage of spermatozoa production we here exclusively counted these stage-0 spermatozoa ready for transmission during mating. In daughter mites we counted all spermatozoa stages found in the rami and in the spermatheca as these daughter mites could have exclusively mated with the male (= brother) of the respective brood cell (Fig. 2e, f). In the mother mite, however, only 'young' spermatozoa up to stage VI (see Häußermann et al. 2016) that have not yet completed the capacitation process in the female genital tract were counted. Considering the fact that the capacitation process lasts about 5 days (Häußermann et al. 2016), these spermatozoa must have been transferred during the past 1–4 days and therefore derive from the own son as the only available male during this time period. Fully capacitated spermatozoa (= stage VII) in the spermatheca, however, must have been transferred by matings during previous reproductive cycles.

## Data analysis

All data were analyzed using the statistic software SPSS (v.22.0). All reported means are ± standard deviation (SD). Data were checked for normal distribution by the Kolmogorov–Smirnov-test and for homogeneity of variance by the Levene-test. If normal distribution and homogeneity of variances could be confirmed, we used ANOVA analysis with Bonferroni correction for the pairwise post hoc tests. If normal distribution or homogeneity of variances could be not confirmed we used ANOVA according to Kruskal–Wallis. Statistical analyses were used for comparisons of the size of the spermatozoa and for the time-dependent numbers of spermatozoa. Differences between groups with $\alpha = 0.05$ were considered statistically significant.

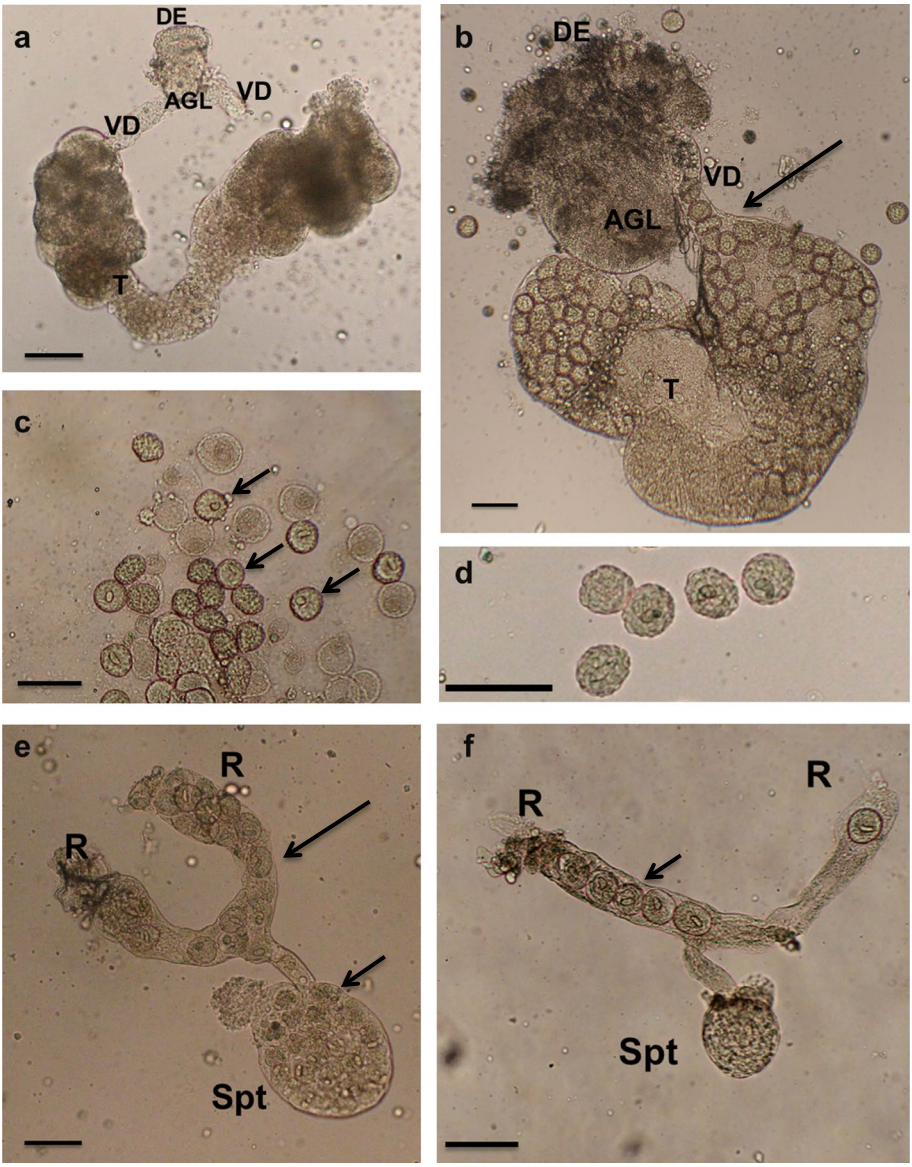


**Fig. 2** Occurrence of spermatozoa in adult male and female *Varroa destructor* mites. **a** Genital tract of male deutochrysalis, *T* testis, *VD* vas deferens, *AGL* Accessory gland, *DE* ductus ejaculatorius. No roundish stage-0 spermatozoa are visible. **b** Genital tract of adult male mite in the T and the VD several roundish stage-0 spermatozoa are visible (arrow). **c** Spermatozoa stages found in the male's genital tract. Stage-0 spermatozoa are characterized by the roundish cell shape and the nubby cell surface (e.g. arrows). **d** Stage-0 spermatozoa in detail. **e** Spermatheca and rami of the 1st daughter mite 11 days after cell capping with several stage II spermatozoa in rami and spermatheca (arrows). **f** Spermatheca and rami of the 1st daughter mite 10 days after cell capping with a couple of stage II spermatozoa inside the rami (arrow). Scale bar is 100 μm

## Results

### Occurrence of spermatozoa in deutochrysalis and adult *Varroa* males

In total we analyzed the genital tract of 12 male deutochrysalis. In none of the analyzed male genital tract the stage-0 spermatozoa that are ready for transmission during mating were present (Fig. 2a). However, the cysts in which spermiogenesis takes place were clearly visible.

In adult male mites, however, these typical roundish stage-0 spermatozoa were frequently found in the vas deferentia and in the testis (Fig. 2b). Such spermatozoa are characterized by their roundish cell shape and the nubby cell surface that reminds of a raspberry (Fig. 2c, d). The nucleus was mostly visible.

There were no significant differences in the mean diameter of these stage-0 spermatozoa singly measured in the genital tract of one to two males each at 10 (n = 16), 11 (n = 11) and 12 (n = 18) days after cell capping (Fig. 3; $F_{2,55} = 0.637$, $p = 0.87$, ANOVA with Bonferroni correction). Total mean (± SD) diameter (n = 58) of theses stage-0 spermatozoa was 44.9 ± 4.83 µm, with a range of 32–56 µm.

### Numbers of spermatozoa at different times after cell capping

In total we analyzed the genital tract of adult mites from 80 *Varroa* mite 'families' consisting of at least one mother mite, one adult daughter mite and one adult male mite at three distinct time points after cell capping (10, 11 and 12 days, respectively). In male mites we exclusively counted the roundish stage-0 spermatozoa as these spermatozoa are ready to be transferred into the genital tract of the female mites. In the daughter mites we counted all spermatozoa inside the genital tract because daughter mites must have received all spermatozoa from their brother as single male available during this time period. In mother mites we only counted non-capacitated spermatozoa (up to stage VI, Häußermann et al. 2016) as these spermatozoa must have been transferred during the past 1–4 days. Fully capacitated fusiform spermatozoa (= stage VII) must have been transferred in matings in previous reproductive cycles, and thus were not considered into account.

The mean total number of spermatozoa reveals highly significant differences between all three time points (Fig. 4, Kruskal–Wallis ANOVA, $F_{2,77} = 51.74$, $p < 0.0001$). Ten days after cell capping already 31 ± 16 (= mean ± SD) spermatozoa, on average, were counted. At 11 days after cell capping this number increased to 85 ± 42 spermatozoa, on average,

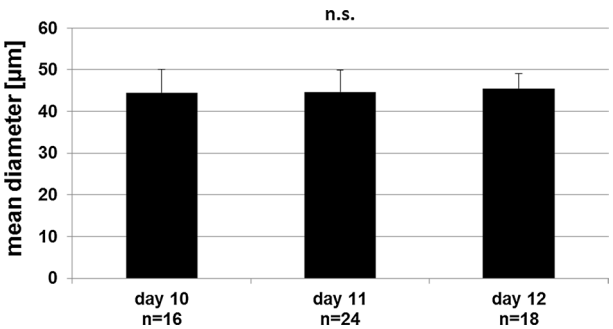


**Fig. 3** Mean (+ SD) maximum diameter of the roundish stage-0 spermatozoa in the *Varroa destructor* male genital tract (n, number of individual spermatozoa measured). There were no significant differences between days 10, 11 and 12 after cell capping

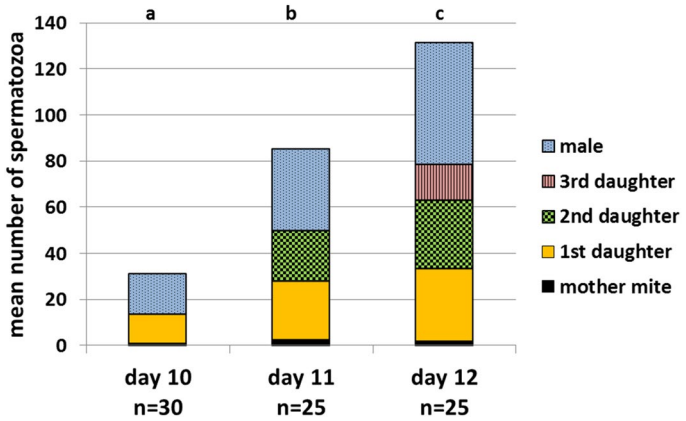


**Fig. 4** Mean total number of non-capacitated spermatozoa (stage 0–stage VI) per *Varroa destructor* family itemized by individual mite stages (male, daughter and mother mites) at 10, 11 and 12 days after cell capping. Different letters mark significant differences. Mean total number of non-capacitated spermatozoa per *Varroa* family was highly significant between day 10, 11 and 12 respectively (Kruskal–Wallis ANOVA, $F_{2,77} = 51.74$, $p < 0.0001$). For single mean values and respective standard deviations of each group see Online Resource S1

and reached the maximum number at 12 days (shortly before the hatching of the adult bee) with an average 125 ± 38 spermatozoa (Fig. 4).

The distribution of the spermatozoa among male and daughter mites changes over time with significant increase in spermatozoa counts in both, the male and the female genital tract. In the first female spermatozoa counts significantly increases over time (Kruskal–Wallis ANOVA, $F_{2,77} = 30.798$, $p < 0.0001$). At day 10, the time when the first daughter mite attains maturity, more spermatozoa were present in the male (18 ± 12) compared to the first daughter (13 ± 10). At day 12 the first and the second *Varroa* daughter stored significantly more spermatozoa 32 ± 8 and 30 ± 19 spermatozoa, respectively, in their genital tract compared to only 16 ± 10 spermatozoa in the 3rd daughter (Kruskal–Wallis ANOVA, $F_{2,62} = 14.348$, $p = 0.001$). At day 12 the male mite still has 53 ± 26 stage-0 spermatozoa for further matings (Online Resource S1). Only in a few cases (18.8%) also the mother mite gets mated with her son (n = 15 from a total of 80 analyzed families) with a low number of non-capacitated spermatozoa (9 ± 5) in her genital tract that has been transferred during these matings.

## Discussion

Our results on the production of spermatozoa in male *Varroa* mites give a further example for the adaptation of the parasite to the host reproductive cycle which is predefined by the capping period of the host brood. For the female mite we could already demonstrate how oogenesis and copulation behavior are optimized in order to make sure that mated female daughters can be produced under this extreme time pressure. An immediate activation of oogenesis after the invasion of a honey bee brood cell (Cabrera Cordon et al. 2013; Frey et al. 2013; Garrido et al. 2000), a rapid preimaginal development (Steiner et al. 1994; Häußermann et al. in prep.) and mating immediately after the adult female molt (Ziegelmann

et al. 2013a, b) are examples for the close synchronization of the parasite reproduction with the development of the honey bee host.

Certainly, the production of sufficient roundish stage-0 spermatozoa ready for transmission during mating is an additional major challenge for a successful *Varroa* reproduction. The big size of these spermatozoa with a maximum diameter of more than 50 µm in comparison to male body size of 800 × 700 µm confirms the high physiological effort involved in the spermatozoa production. Already a chain of 18 of these spermatozoa ready for transmission during mating side by side would be as long as one adult male. We could clearly show that the *Varroa* males continuously produce these stage-0 spermatozoa, obviously starting immediately after the adult molt. In the deutochrysalis stage the cysts in which spermiogenesis takes place are already visible, but we could not identify any stage-0 spermatozoa. Each adult male mite produces about 125 roundish stage-0 spermatozoa that are ready for transmission during mating in worker brood. Considering that only adult males can produce the respective spermatozoa, the sequence of sexes in *Varroa* mite offspring within the sealed brood cell (Garrido and Rosenkranz 2003) is consequential. The male egg is laid approximately 30 h before the first female egg, giving the male a timing edge for the preimaginal development (Rosenkranz et al. 2010). This temporal advance enables the production of a sufficient number of spermatozoa before the adult molt of the first female daughter.

The adult molt of the first female takes place approximately 10 days after the sealing of the brood cell and about half a day after the adult molt of the male. Immediately after the adult molt of the first female mite mating takes place. Our study indicates that at day 10—and thus shortly after the adult molt of the female mite—the freshly molted daughter mite already has received some spermatozoa while the male's genital tract contains about 20 spermatozoa ready for transmission during mating which might be sufficient for the next matings. The relatively low number of available spermatozoa at the beginning of the mating period might be one reason for the multiple matings of the male with the different daughter mites (Donzé et al. 1996). Interestingly, freshly molted adult male mites that do not yet have produced spermatozoa ready for transmission did not reveal the typical copulation behavior of older males (Häußermann 2014). We do not know how this age-dependent mating behavior is triggered, but it ensures that the first mating will only take place when spermatozoa are available.

After the start of sexual activity, the production of spermatozoa in the adult males is an ongoing process with a consistent productivity. Already on day 11 after cell sealing, the male had doubled the stock of spermatozoa within his genital tract. At this time, the number of transferred spermatozoa in the first daughter mite had also doubled to an average of more than 25, while the second daughter has already received about 20 spermatozoa. Wendling et al. (2014) suggest that 0.99 spermatozoa are needed to fertilize one oocyte. As their data are based on a calculation it is possible that unknown parameters may have an influence on this number. However, it is likely that not many spermatozoa are needed to fertilize one oocyte. In literature, the mean number of altogether transferred spermatozoa per female mite varies between 30 and 40 spermatozoa (Alberti and Hänel 1986; Donzé and Guerin 1994; Donzé et al. 1996; Ziegelmann and Rosenkranz 2014). This should be sufficient for the insemination of all female eggs during the average number of two to three reproductive cycles per female mite (Fries and Rosenkranz 1996; Martin and Kemp 1997). On day 12 after cell sealing and thus just before hatching of the host bee, both, the first and second daughter mites have, on average, reached the required number of more than 30 spermatozoa. However, the third daughter received a lower amount of spermatozoa. It is noticeable, that in the first daughter mite the number of spermatozoa hardly increased from

day 11 to 12 after cell sealing indicating that (1) the male does not transfer more spermatozoa and/or (2) the mating frequency significantly decreased in older daughter mites. The latter is in line with the age dependent production of the female sex pheromone (Ziegelmann et al. 2013b) with the consequence that daughter mites are attractive to males almost exclusively during the first 24 h after the adult molt (Ziegelmann et al. 2013a).

The fact that the third daughter receives a significant lower number of spermatozoa compared to her sister mites might limit the possible number of successful reproductive cycles. Possibly, such mites have to mate during one of the next reproductive cycles with their son to 'fill up' the stock of spermatozoa. This could explain the low frequency of matings between sons and mother mites in our study with a relatively low number of transferred spermatozoa. This finding is consistent with previous behavioral experiments in which a relatively low frequency and duration in mating attempts of male mites towards the mother mites was observed indicating that mother mites are less attractive compared to her daughters (Ziegelmann and Rosenkranz 2014). Additionally, mother mites move faster than the young adult daughter mites hindering male mites mounting the female's dorsum for mating (Ziegelmann and Rosenkranz 2014). Probably, mother mites that run out of spermatozoa change their behavior supporting matings with their own son. As we did not count the number of fusiform (= capacitated) spermatozoa in the spermatheca of the mother mites, this hypothesis has to be confirmed in future studies.

The total number of about 125 roundish stage-0 spermatozoa produced by the males during a reproductive cycle in worker brood seems to be more than needed for the matings with the maximum of three daughter mites in our study. However, this overspill of about 50 spermatozoa ready for transmission during mating in the male mite could serve as a reserve for the parasitation of drone brood where the capping period is extended and more daughters are produced by the mother mite (Oldroyd 1999). Additionally, in case of brood cells that are double infested with *Varroa* females the male mites could not only mate with their sisters but also with the daughters from the second mother mite. Therefore, this spermatozoa surplus might also be an adaptation to such situations when sperm competition might occur (Witalinsky 1998). Further studies are needed to support this assumption. In any case the production of about 125 spermatozoa ready for transmission during mating confirms our second hypothesis that a male mite produces enough spermatozoa to inseminate all daughter mites.

A shortened duration of the male's spermiogenesis with the production and transfer of non-capacitated spermatozoa seems to be a further factor to optimize the course of mite reproduction. So far, the details of spermiogenesis within the cysts of *Varroa* males are unknown and require further studies. Only Alberti and Hänel (1986) analyzed sections of the male's genital tract and described six different stages of spermiogenesis resulting from male germ cells. For this study, we only recorded the roundish spermatozoa which are transmitted by the male into the genital tract of the female mites. These spermatozoa are still not able to fertilize the female egg cell. They are characterized by the roundish cell shape and the nubby cell surface that reminds of a raspberry and the nucleus is mostly visible. We found these spermatozoa in male mites especially in the anterior-lateral sides of the testis and in the two vas deferentia. In a previous study we detected these spermatozoa in very low amounts in the genital tract of female mites dissected immediately after mating (Häußermann et al. 2016) and defined this stage as 'stage 0' spermatozoa that gets transferred into the female genital tract during copulation. In the genital tract of the females these roundish spermatozoa still have to undergo the so-called spermatozoa capacitation. During this process sperm cells change their morphology substantially accompanied by an immense elongation until they appear fusiform and reach a length of more than 200 µm

and finally are able to fertilize the female egg cell (Häußermann et al. 2016). Interestingly, these stage-0 spermatozoa did neither change their size nor their morphology within the female's genital tract. However, immediately after the transfer to the female's genital tract the capacitation process starts (Häußermann et al. 2016).

Our results also raise the question of how the spermatozoa are finally transmitted during mating. It is assumed that male mites produce a spermatophore, take it with their spermatodactyls and put it into the paired genital pores of the female mites (Alberti and Hänel 1986, Rosenkranz et al. 2010). However, the female genital pores have a size of only 7.5 µm (De Ruijter and Kaas 1983) compared to a diameter of approximately 45 µm of the spermatozoon that needs to be transferred. It is an intriguing question how the *Varroa* male pushes this huge spermatozoon through the small genital opening. In other gamasid mites like *Hattena cometis* and *Veigaia* sp., Di Palma et al. (2013) could show that male chelicera feature a basal opening in the movable digit that is supposed to allow the sperm shift of single sperms from the spermatophore into the lumen of the movable digit. The lumen leads through a sperm transfer tube up to the tip of the spermatodactyl. Male mites inaugurate the spermatodactyl into the female genital pores and thus transmitting the spermatozoa (Di Palma et al. 2006, 2013). We assume that spermatozoa get transmitted from male to female *Varroa* mites in a similar way. Perhaps male *Varroa* mites don't even create spermatophores; however, this is very speculative and needs to be confirmed.

Our study clearly shows that in *Varroa* reproduction not only the female oogenesis and copulation behavior are optimized but also the timely production of male spermatozoa. Hence we could confirm both of our initial hypotheses, because this optimization includes (1) a rapid and continuous production of non-capacitated spermatozoa, (2) in an amount that is sufficient to inseminate all daughter mites at any time in the brood cell.

## References

Alberti G, Coons LB (1999) Acari—Mites. In: Harrison FW (ed) Microscopic anatomy of invertebrates, vol 8c. Wiley, New York

Alberti G, Hänel H (1986) Fine structure of the genital system in the bee parasite, *Varroa jacobsoni* (Gamasida: Dermanyssina) with remarks on spermiogenesis, spermatozoa and capacitation. Exp Appl Acarol 2:63–104

Anderson DL, Trueman JWH (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp Appl Acarol 24:165–189

Cabrera Cordon AR, Shirk PD, Duehl AJ, Evans JD, Teal PEA (2013) Variable induction of vitellogenin genes in the Varroa mite, *Varroa destructor* (Anderson & Trueman), by the honeybee, *Apis mellifera* L, host and its environment. Insect Mol Biol 22(1):88–103

De Ruijter A, Kaas JP (1983) The anatomy of the Varroa mite. *Varroa jacobsoni* oud affecting honey bees: present status and needs. AA Balkema, Rotterdam, pp 45–47

Di Palma A, Alberti G, Nuzzaci G, Krantz GW (2006) Fine structure and functional morphology of the mouthparts of a male *Veigaia* sp. (Gamasida: Veigaiidae) with remarks on the spermatodactyl and related sensory structures. J Morphol 267(2):208–220

Di Palma AD, Seeman O, Alberti G (2013) Ultrastructure of the male chelicerae of *Hattena cometis* Domrow (Acari: Gamasida: Ameroseiidae) functioning as gonopods. J Morphol 274(4):404–411

Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, Quentin W, Tannahill C, Frey E, Ziegelmann B, Rosenkranz P, Ellis JD (2013) Standard methods for *Varroa* research. J Apic Res 52(1):1–54

Donzé G, Guerin PM (1994) Behavioral attributes and parental care of Varroa mites parasitizing honeybee brood. Behav Ecol Sociobiol 34:305–319

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

Fernandez N, Coineau Y (2006) *Varroa*: the serial bee killer mite—to be able to combat her, one must properly understand her. Atlantica, pp 43–62
Fries I, Rosenkranz P (1996) Number of reproductive cycles of *Varroa jacobsoni* in honey-bee (*Apis mellifera*) colonies. Exp Appl Acarol 20(2):103–112
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(1):56–62
Garrido C, Rosenkranz P (2003) The reproductive program of female *Varroa destructor* mites is triggered by its host, *Apis mellifera*. Exp Appl Acarol 31:269–273
Garrido C, Rosenkranz P, Stürmer M, Rübsam R, Büning J (2000) Toluidine blue staining as a rapid measure for initiation of oocyte growth and fertility in *Varroa jacobsoni* Oud. Apidologie 31(5):559–566
Genersch E (2010) Honey bee pathology: current threats to honey bees and beekeeping. Appl Microbiol Biot 87(1):87–97
Genersch E, Von Der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen 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(3):332–352
Häußermann C (2014) Paarungsverhalten der Varroa-Männchen (*Varroa destructor*) in Abhängigkeit von Alter und Begattungsstatus der Weibchen sowie der Zusammensetzung des weiblichen Sexualpheromons, Master thesis at the Apicultural State Institute of the University of Hohenheim
Häußermann CK, Ziegelmann B, Bergmann P, Rosenkranz P (2015) Male mites (*Varroa destructor*) perceive the female sex pheromone with the sensory pit organ on the front leg tarsi. Apidologie 6:771–778
Häußermann CK, Ziegelmann B, Rosenkranz P (2016) Spermatozoa capacitation in female *Varroa destructor*. Exp Appl Acarol 69(4):371–387
Ifantidis MD (1983) Ontogenesis of the mite *Varroa jacobsoni* in worker and drone honey bee brood cells. J Apicult Res 22:200–206
Ifantidis MD (1990) Re-examination of some parameters concerning reproduction of the mite *Varroa jacobsoni* Oud. Proceedings of the International Symposium on Resent Research on Bee Pathology, Ghent, Belgium, pp 20–26
Martin SJ (1994) Ontogenesis of the mite *Varroa jacobsoni* Oud. In worker brood of the honeybee *Apis mellifera* L. under natural conditions. Exp Appl Acarol 18:87–100
Martin SJ, Kemp D (1997) Average number of reproductive cycles performed by *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. J Apicult Res 36:113–123
Martin S, Holland K, Murray M (1997) Non-reproduction in the honeybee mite *Varroa jacobsoni*. Exp Appl Acarol 21(8):539–549
Oldroyd B (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of the western honeybees. Trends Ecol Evol 14(8):312–315
Rehm SM, Ritter W (1989) Sequences of the sexes in the offspring of *Varroa jacobsoni* and resulting consequences for the calculation of the developmental period. Apidologie 20:339–343
Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. J Invertebr Pathol 103:96–119
Steiner J, Dittmann F, Rosenkranz P, Engels W (1994) The first gonocycle of the parasitic mite (*Varroa jacobsoni*) in relation to preimaginal development of its host, the honey bee (*Apis mellifera carnica*). Invertebr Reprod Dev 25(3):175–183
VanEngelsdorp D, Hayes J Jr, Underwood RM, Caron D, Pettis J (2011) A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. J Apicult Res 50(1):1–10
Wendling S, Guillet B, Roy L, Kreiter S, Colin ME (2014) Fertilization and fertility in the female of *Varroa destructor*, a key point for the parasite population dynamics. Apidologie 45(6):722–732
Witalinsky W (1998) Sperm competition in the Acari. Exp Appl Acarol 23:455–465
Ziegelmann B, Rosenkranz P (2014) Mating disruption of the honeybee mite *Varroa destructor* under laboratory and field conditions. Chemoecology. https://doi.org/10.1007/s00049-014-0155
Ziegelmann B, Lindenmayer A, Steidle J, Rosenkranz P (2013a) The mating behavior of *Varroa destructor* is triggered by a female sex pheromone. Apidologie 44:314–323
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