

Remating in *Varroa* : for which purpose ?

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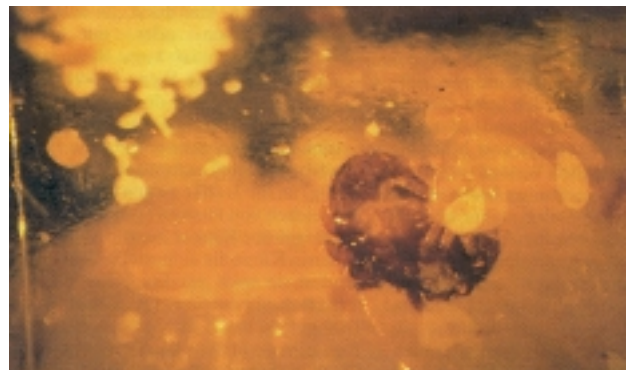
The mite Varroa jacobsoni reproduces in the capped brood cells of the honeybee Apis mellifera. Only the females infest the brood cells and can proceed to several reproduction cycles. However, like every parasite, Varroa has to cope with the constraints of the host to which it has to adapt itself.

The reproduction in brief

Varroa's reproduction is characterised by the oviposition and the development of 5-6 descendants. Oviposition starts at 60-70 hours after capping with a male egg followed by 4-5 female eggs laid at regular 30 hours intervals. Thanks to this, the *Varroa* male matures first and the oldest daughter moults to adulthood some 20 hours later. By laying only one male egg, *Varroa* increases the number of females which can reproduce at the next generation. But since males do not survive outside the cell, females must be fertilised before bee emergence. If this does not occur, female *Varroas* will remain sterile. So the duration of each reproductive cycle is limited by the duration of bee development. In the first article, we described how the mite structures its time and space in the capped brood cell. Here we would like to explain the last stage of *Varroa's* reproductive cycle, in other words, the mating of young females.

Our observations on *Varroa's* reproduction with transparent polystyrol brood cells show that the infesting mother forms a rendezvous site with her faeces on the cell wall. All individuals present in the cell aggregate on this site so that 90 % of the 287 matings we observed occurred on or just beside the faecal accumulation. This ensures that adult daughters and males meet to copulate (fig.1).

Fig. 1 *Varroa's* mating. The male (bright, back view) samples the female's venter (dark) which hang to the cell wall. It is rare to be able to observe matings so clearly as most of them occur on the faecal accumulation (in the corner, on the left-hand side).



The mating ritual

The only preliminary to mating consists of cleaning of the pedipalps (sensory mouth part) and chelicerae by the male.

On the figure 2, we can see that the male's chelicerae are modified in the tube-like spermadactyls, which allow him to transfer his sperm from his genital opening into the female's copulatory pores. When he encounters a female, the male climbs onto her dorsal shield and reaches the gnathosomal region (mouth part). Then he invariably hugs the lateral border of her dorsal shield which is equipped with spines. When he encounters the spineless anal region of the female, he crawls under her venter. In order to help him, the female simultaneously lifts off the surface. The male then undertakes lateral criss-crossing (fig. 1) on her sternal shield and intensively samples it with his first leg pair and his pedipalps. He then stops in the centre of the female's venter and orients his gnathosoma in the direction of his genital opening. His pedipalps and chelicerae execute back and forth movements until a moist, glistening ball appears. The male directs himself to one side of the female to the base of legs III and IV where a sperm induction pore is located and injects some sperm in this pore. Sometimes he goes to the other side where the second pore is located and leaves the female.

The spermatozoa which have been deposited in the female's copulatory pores migrate into the spermatheca where they will be stocked until the female's reproductive cycle starts. We observed that 71 % of the matings are interrupted before 3 minutes (figure 3) while we estimate that sperm is only transmitted in the 26% of matings which lasted more than 6 minutes. The different

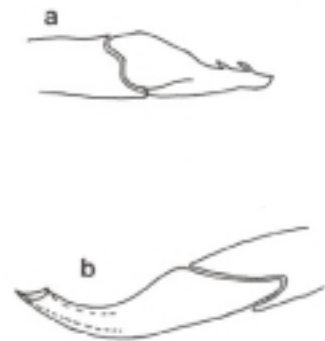


Fig. 2 The females chelicerae are sharp enough to cut the bee's cuticule (a). The male's chelicerae have a tube-like shape (b) allowing the transfer of sperm injected in the sperm induction pores which are located between the base of the legs III and IV in females.

sequences which form the mating event ensure on the one hand that the female is a fairly young female and on the other hand that the male is healthy, since able to perform the complicated sequence. There is no doubt that males are able to differentiate a young female from a foundress female as matings with mother females are all interrupted (fig. 3).

Durations of matings

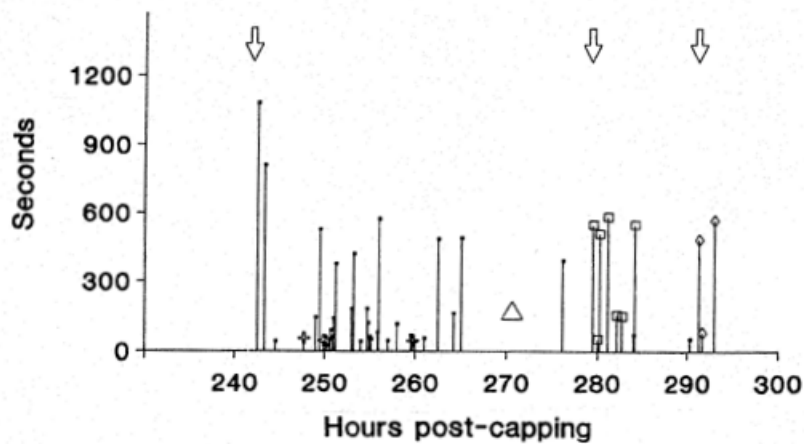


Fig. 3 Duration and frequency of matings between a single male and *Varroa* females in an artificial cell infested by one foundress. The male has moulted 222 hours after the cell capping. The arrows indicate the moulting time of the three successive adult daughters (ff1= \square , ff2= \square , ff3= \square). The \clubsuit symbols indicate mating with the *Varroa* mother. The triangle indicates a period without observation.

In figure 3, one can see that mating starts a short time after the first young female's arrival on the faecal accumulation and that they regularly copulate until the second young female arrives. From that moment, the male mates only the second female. This pattern, which is repeated with the third young female, ensures that all females are mated.

As there is only one male per family, one can expect that, after mating a female, the male saves his energy and sperm for the next females. On the contrary, we observed that *Varroa*'s strategy consists in mating several times (fig. 3). Through remating, the male takes the risk not to be able to mate all the females and through lack of energy, he may be unable to produce sperm. However, we hypothesized that *Varroa* would benefit from remating.

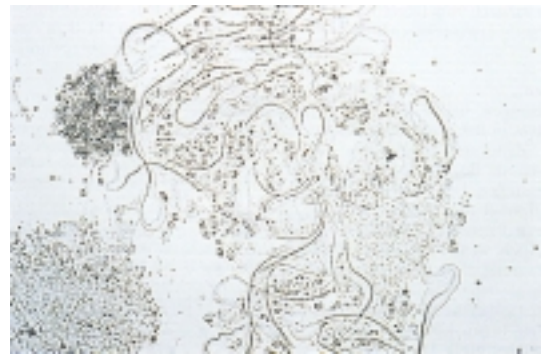
Several matings ensure fertility

There are two arguments in favour of this hypothesis. Firstly, we observed that the male uses the feeding site prepared by the infesting female. Thanks to this site, and despite the fact that his chelicerae have been transformed into spermadactyls, the *Varroa* male often feeds himself, which allows him to mate frequently. Secondly, the mating frequency may influence the spermatozoa number present in the female's spermatheca. Since the females have several reproductive cycles, it was difficult to determine how many eggs a female lays. As a consequence, we used the number of spermatozoa contained in the spermatheca as a potential fecundity criterion. We could do this because of the low number of spermatozoa contained in the spermatheca (less than 40 according to Alberti and Hänel, 1986) which corresponds with the maximum number of 30 eggs laid by a *Varroa* female (de Rujter, 1987).

To estimate the influence of remating, we performed the following experiment in artificial transparent cells containing worker pupae: two males were introduced within an artificial cell with two virgin females having moulted without males. Both males were taken from natural brood cells. Mating was observed in an incubator (34°C, 60 % R.H.). We only took into account complete matings (i.e. those which lasted at least 6 min.). Three test groups were compared (fig. 5): fertilised females after (A) one complete mating, (B) two complete matings and (C) females which

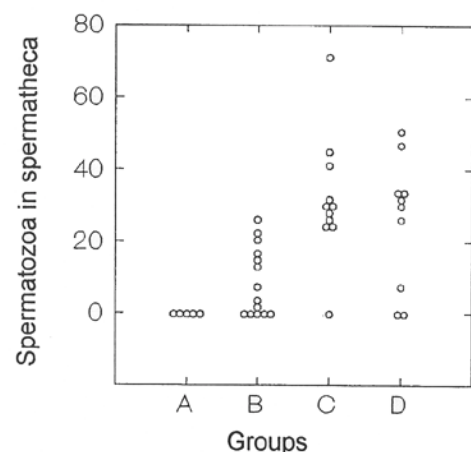
had spent 48 hours with males. Mother females taken from natural capped brood cells were used as control group (D). Three days after mating, spermatozoa have migrated to the spermatheca and have taken on a fusiform shape and progressively a ribbon form (fig. 4). For counting sperms, the females were maintained on their back in a ringier solution and the spermatheca removed. Then a slight pressure was applied to the cover slip to expel the spermatozoa from the spermatheca for counting under the microscope.

Fig. 4: *Varroa*'s spermatozoa in the split spermatheca of a female. Most of the 30 spermatozoa (length 0.2 mm) have not achieved their maturation and are still pear shaped.



No female which had been mated only once (test A) had spermatozoa. Also 5 females mated twice (B) had 0 spermatozoa whereas the other nine had stored between 1 to 26 spermatozoa (fig. 5). When mating proceeded ad libitum for 48 hours (C), the spermatheca contained over 24 spermatozoa in 10 out of 11 females dissected (fig. 5). These results show that with increasing frequency of rematings the number of spermatozoa stored in a female's spermatheca increases. They also indicate how important time is for the mite. For example, the two first daughters in figure 3 are likely to have been better fertilised than the third one since they mated more than 8 and 4 times respectively. Due to a lack of time, the third daughter could only mate twice, as the pupa moulted into an adult bee.

Fig. 5 Number of spermatozoa contained in the female's spermatheca three days after the last mating. The groups represent young females after a complete mating (A), two complete matings (B) or ad libitum for 48 hours (C). The control group (D) contains *Varroa* foundress taken from brood cells and could have reproduced before.



In conclusion

According to our observations, *Varroa* is under time pressure to ensure fertilisation, males and females use a rendezvous site which serves as mating site. In order to mate all females, young mated females are preferred to older ones. As long as no younger female arrives on the aggregation site, males mate the older ones thus increasing their potential fertility.

In the beehive of its original host, the Asian honeybee, *Varroa* reproduces almost exclusively in the drone brood cells, which are less protected by the bees. In the European honeybee colonies,

whose resistance against *Varroa* is low, the mite reproduces with great efficiency causing severe damage to the colonies.

After Donzé G., Fluri P, Imdorf A. (1998) Remating in varroa: for what purpose? American Bee Journal 138 (8) 607-609.

Reference

The complete results have been published in the following papers:

Donzé G., Guerin P.M. (1994) Behavioral attributes and parental care of *Varroa* mites parasitizing honeybee brood. Behav. Ecol. & Sociobiol. **34**: 305-319

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