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A comparison of the reproductive ability of *Varroa destructor* (Mesostigmata:Varroidae) in worker and drone brood of Africanized honey bees (*Apis mellifera*)

Rafael A. Calderón · Luis G. Zamora ·
Johan W. Van Veen · Mariela V. Quesada

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Abstract Colony infestation by the parasitic mite, *Varroa destructor* is one of the most serious problems for beekeeping worldwide. In order to reproduce varroa females, enter worker or drone brood shortly before the cell is sealed. To test the hypothesis that, due to the preference of mites to invade drone brood to reproduce, a high proportion of the mite reproduction should occur in drone cells, a comparative study of mite reproductive rate in worker and drone brood of Africanized honey bees (AHB) was done for 370 mites. After determining the number, developmental stage and sex of the offspring in worker cells, the foundress female mite was immediately transferred into an uninfested drone cell. Mite fertility in single infested worker and drone brood cells was 76.5 and 79.3%, respectively. There was no difference between the groups ($X^2 = 0.78$, $P = 0.37$). However, one of the most significant differences in mite reproduction was the higher percentage of mites producing viable offspring (cells that contain one live adult male and at least one adult female mite) in drone cells (38.1%) compared to worker cells (13.8%) ($X^2 = 55.4$, $P < 0.01$). Furthermore, a high level of immature offspring occurred in worker cells and not in drone cells ($X^2 = 69$, $P < 0.01$). Although no differences were found in the percentage of non-reproducing mites, more than 74% ($n = 85$) of the mites that did not reproduce in worker brood, produced offspring when they were transferred to drone brood.

Keywords *Varroa destructor* · Reproductive ability · Comparison · Africanized honey bees · Worker brood · Drone brood

Introduction

Colony infestation by the parasitic mite, *Varroa destructor* (Anderson and Trueman 2000) is one of the most serious problems for beekeeping worldwide (De Jong 1997; Garrido

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R. A. Calderón (✉) · L. G. Zamora · J. W. Van Veen · M. V. Quesada
Centro de Investigaciones Apícolas Tropicales, Universidad Nacional, 475-3000, Heredia, Costa Rica
e-mail: rcalder@una.ac.cr

et al. 2003). In order to reproduce varroa females, enter worker or drone brood shortly before the larva is sealed with a wax capping (Camazine 1986). The reproduction of varroa mites is a significant factor determining its virulence and has a major impact on the persistence of both host and parasite.

The reproductive ability of *V. destructor* varies among the honey bee races and bee brood (worker or drone brood) (Camazine 1986; Martin and Cook 1996; Martin et al. 1997). Drone brood is more frequently infested and preferred by mites (Santillan-Galicia et al. 2002). In European honey bees (EHB), varroa mites invade drone cells up to 11.6 times more frequently than they invade the worker brood cells (Boot et al. 1995) and were found to have a reproductive rate of 2.2 new female mites in drone brood (De Jong 1988). In worker brood fewer offspring is produced and higher rates of non-reproduction were found than in drone brood (Boot et al. 1995).

A critical factor related to varroa infestation level in colonies is mite fertility (De Jong 1997). The high percentage of infertile mites observed in worker brood has been proposed to be related with the tolerance of Africanized honey bee colonies (AHB) to varroa mites in Brazil, where colony losses are not recorded (Aumeier et al. 2000).

Another factor that influences varroa reproductive capacity is related to the mites that fail to produce viable female offspring despite entering a suitable host cell. This can be classed into non-fertile female mites, foundresses that produced only immature stages or only one adult sex, and the absence or premature mortality of the male offspring before it is able to mate with its sisters (Martin et al. 1997).

Varroa was first detected in Costa Rica on September 1997 (van Veen et al. 1998). It was confirmed that the mites belonged to the Korea haplotype (De Guzman personal communication 1999; Anderson and Trueman 2000), which is considered to be more virulent than the Japan/Thailand haplotype found predominantly in South America (Anderson and Trueman 2000).

The comparison of mite reproduction in worker and drone brood requires artificial infestation of single brood cells with female mites. So far no experiments under such well-defined comparative conditions have been carried out with Africanized bees. In previous studies, we found a low mite reproductive ability in worker brood of AHB colonies, in which less than 30% of the foundress mites produced viable female offspring (Calderon et al. 2003). Meanwhile in drone brood more than half of the fertile mites produced viable female offspring. We found that varroa reproductive ability in drone brood of AHB colonies was limited by the absence of male or male mortality in a considerable percentage of cells (Calderon et al. unpublished data). To test the hypothesis that, due to the preference of mites to invade drone brood to reproduce, a high proportion of the mite reproduction should occur in drone cells, we compare the reproductive rate of *V. destructor* in worker and drone cells of AHB colonies under the tropical conditions of Costa Rica.

Materials and methods

Varroa reproduction was studied in AHB colonies at CINAT of the Universidad Nacional in Heredia, Costa Rica. The experiments were carried out from January to August 2005 using 10 colonies, in which both worker and drone brood were reared.

Mite reproduction in worker brood cells

In order to measure mite reproduction in worker brood, open cells likely to be capped within few hours were marked on a transparent sheet temporarily placed over the frames.

The brood combs were put back into the colony and the cells were checked after 4 h. Mature female mites were collected from capped worker brood (IV stage of sealed brood = pupae with brown eyes). To collect these mites, brood was removed from cells with forceps and adult mites were transferred to plastic petri dishes containing pupae using a fine paint brush. Those cells that had been capped in the meantime were artificially infested with a single mite. Mites were introduced into worker cells by opening the host cell carefully with a scalpel introducing the mite and resealing the host cell. The positions of the test cells and times of cell sealing were recorded on transparent sheets. This method is widely used, even though the manipulation of the brood cells may elicit higher removal rates compared to naturally invaded brood cells (Aumeier et al. 2000; De Ruijter 1987). The combs were replaced into the experimental colonies for a further 10 days before being re-examined. To compare reproduction of individual mites only data from cells invaded by one mite were analyzed, because otherwise offspring of different mother mites cannot be distinguished. At the moment the cells were opened, only a few offspring had developed into adult females, and these mites could be distinguished from their mothers by their light pigmentation.

Mite reproduction in drone brood cells

The reproduction of the same mother mite in worker cell was compared with what they produced in their subsequent reproductive cycle in drone brood. After determining the number, developmental stage and sex of the offspring in worker cells, the foundress female mite was immediately transferred into uninfested drone cell by carefully making and then resealing a small hole in the side of the cell cap through which the mite was introduced (Martin et al. 1997). We assured to have drone cells prepare for mite transfer from worker brood into the new drone brood cell (an approximate synchronization for both worker and drone cell capping times was necessary). Only drone cells, which had been sealed within 6 h, were used.

The position of the experimental cells was recorded by placing transparent sheets over the brood area and by marking the cells. The combs were replaced into the colony for 10 days before being examined.

Mite reproduction in worker and drone brood was analyzed approximately 240 h after cell sealing. The cells were opened and the number of offspring was determined using a stereoscope microscope. Offspring were classified as egg-larva, protonymph (mobile and immobile stages), deutonymphs (mobile and immobile stages), male and female adults. Examining honey bee worker and drone brood 10 days after capping (240 h) is more accurate since it takes into account mite offspring mortality, which greatly affects the number of viable female offspring produced. Furthermore, the mature female offspring mites can be distinguished from their foundress mothers by their lighter pigmentation.

Several reproduction parameters were measured for foundress female mites in both, worker and drone cells: fertility (successful production of offspring), production of viable female offspring (cells that contain one live adult male and at least one adult female mite: females that can reach the adult stage and have a mate available), fecundity (number of progeny), production of only immature offspring, production of only female or only male offspring (one adult sex) and no reproduction at all (mites producing no offspring) (Boot et al. 1995).

To estimate the total viable female offspring in drone brood cells, the deutonymphs (mobile and immobile) were also considered, since under natural conditions the drone cell remains capped 96 h more, which is enough time for both stages to reach adulthood (Ifantidis 1983). Cells with only egg-larvae or protonymphs were considered to be immature offspring producing mites.

Statistical analysis

Differences of the mite reproductive parameters in worker and drone brood of AHB were analyzed using the chi-square test. Data are given as percentage values and mean ± standard deviation.

Results

A comparison of the reproductive ability of *V. destructor* in worker and drone brood of AHB was done for 370 mites.

Mite fertility in single infested worker and drone brood cells was 76.5 and 79.3%, respectively (Table 2). No significant differences were found between the groups (CHI-test: $X^2 = 0.78$, $P = 0.37$, $n = 370$).

There was a clear difference in the percentage of mother mites producing viable female offspring in worker and drone brood (CHI-test: $X^2 = 55.4$, $P < 0.01$, $n = 370$), being drone brood more suitable for mite reproduction (Table 2). A total of 68 and 287 viable females offspring were produced from 370 worker and drone cells, respectively (Table 1). More than 23.0% of the foundress mites that produced immature offspring or only an adult female or male offspring (one adult sex) in worker brood produced viable females when they were transferred to drone brood.

The average fecundity of reproducing mites was 2.9 ± 1.4 ($n = 290$) and 3.8 ± 1.6 ($n = 293$) descendants per foundress in worker and drone brood, respectively (Table 1).

A significant difference was found in the percentage of mothers producing immature offspring in worker and drone brood (CHI-test: $X^2 = 69$, $P < 0.01$, $n = 370$) (Table 2). A greater proportion of mites produced only a female offspring in drone brood cells (Table 2). Meanwhile, the presence of only male in the cells was not different between the groups (CHI-test: $X^2 = 0.74$, $P = 0.40$) (Table 2). A maximum of six offspring was found in worker brood, most frequently, the number of eggs laid was two and three. Meantime in drone brood, a maximum of seven offspring was found; being more frequently three and four eggs.

Table 1 Reproductive rate of *V. destructor* in worker and drone brood of AHB

Characteristic	Worker	Drone
Number of progeny produced per all adult females	2.3 ± 1.7 (*n* = 370)	3.0 ± 2.1 (*n* = 370)
Number of eggs produced per reproductive female	2.9 ± 1.4 (*n* = 290)	3.8 ± 1.6 (*n* = 293)
Number of viable females produced per foundress producing viable offspring	1.4 ± 0.5 (*n* = 49)	2.1 ± 1.0 (*n* = 140)
Number of viable females produced per reproductive female mite	0.3 ± 0.6 (*n* = 290)	1.0 ± 1.2 (*n* = 293)
Number of viable females produced per mite taken into account all adult females	0.2 ± 0.5 (*n* = 370)	0.8 ± 1.1 (*n* = 370)

Data are given as mean values ± SD

Table 2 Comparison of reproductive parameters of varroa foundress mites in worker and drone brood of AHB

Parameter	Worker (%) ($n = 370$)	Drone (%) ($n = 370$)	Statistical test
Non-reproductive	23.5	20.7	$X^2 = 0.78$, $P = 0.37$
Fertile	76.5	79.3	$X^2 = 0.78$, $P = 0.37$
Viable female offspring	13.8	38.1	$X^2 = 55.4$, $P < 0.01$
Immature offspring	40.6	13.2	$X^2 = 69.0$ $P < 0.01$
Males only	14.9	12.8	$X^2 = 0.74$, $P = 0.40$
Females only	7.2	15.2	$X^2 = 12.3$, $P < 0.01$

Table 3 Non-reproducing mites in worker brood cells transferred to drone brood cells of AHB

Parameter	Drone (%) ($n = 85$)
Non-reproductive	25.9
Fertile	74.1
Viable female offspring	36.5
Immature offspring	14.1
Males only	9.4
Females only	14.1

Non-reproductive mites in worker cells

A total of 85 foundress mites did not reproduce at all in worker cells. The reproductive behaviour of the majority of these non-reproducing mites became different between subsequent reproductive cycles in drone brood. A 74.1% of the non-reproducing mites produced offspring when were transferred to drone brood (Table 3). The production of offspring was influenced by the brood type. The average fecundity of reproducing mites was 3.6 ± 1.5 ($n = 63$). Of the mites that reproduced in drone brood, 36.5% produced viable females offspring. The number of viable females produced per foundress producing viable offspring was 2.3 ± 1.4 ($n = 31$). Only 25.9% of the mites remained the same with no reproduction between transfers (Table 3).

Discussion

Mites reproduction rate in the previous cycle in worker brood was compared with what they produce in the next reproductive cycle in drone brood. This comparison required artificial infestation of single worker brood cells with female mites and the transfer of these mites into uninfested drone cell. This method allows the possibility to compare the reproductive ability of varroa mites in worker and drone brood cells and analyze if mite reproduction behaviour is influenced by the brood type. No experiments under these conditions have been conducted with Africanized bees. According to De Ruijter (1987), the female mother mites are able to reproduce up to seven times in successive reproductive cycles in artificial infested worker cells. Nevertheless, it should be consider that the age of the mite is not known and it can influence the reproductive success.

In this study, removal of capped infested brood did occur. From approximately one third of brood cells infested with varroa mites were found completely emptied (brood completely removed by the bees). The removal response to infested cells may be due to the artificial introduction of the mites. By artificial introduction, the cell is damaged and the mites may have an alien odor (Aumeier et al. 2000). Rosenkranz et al. (1993) showed that the removal response of bees was strongly affected by scent cues adhering to the mites. Thus, mites in artificially infested cells may be more easily detected by bees than mites in naturally infested cells.

Mite fertility in worker and drone brood was about 79.0% (every female which had laid at least one egg was considered fertile). In Africanized bees in Brazil, mite fertility in singly infested cells was 82.0% in worker and 77.0% in drone brood (Garrido et al. 2003). These results represent an increase of the proportion of fertile mites in Brazil, where previous studies had demonstrated less than 50% average mite fertility (Camazine 1986). In Mexico, the annual mean percentage of mother mites that reproduce in worker brood was 85.5% with a range from 74.1 to 91.5% (Mondragon et al. 2006). The foundress producing viable offspring in worker cells falls to around 40.0% on AHB in Mexico (Medina and Martin 1999).

The number of eggs produced per reproductive female and the number of viable females produced per foundress producing viable offspring were higher in drone brood compared to worker brood. Medina and Martin (1999) indicated that a direct consequence of higher rates of offspring mortality in AHB worker cells results in only 0.73 viable adult females offspring being produced per mother mite. Moreover, a mean fecundity of 4.1 mites per mother was found in worker brood of AHB in Mexico (Mondragon et al. 2006).

One of the most significant differences in mite reproduction was the higher percentage of mites producing viable offspring in drone cells compared to worker cells. This indicates that brood has an influence in the host–parasite relationship. The number of viable female offspring produced by invading mother mites depends, in part, upon the type of cell the mite enters, whether it is worker or drone (Boot et al. 1991; Santillan-Galicia et al. 2002). Drone brood is more frequently infested and preferred by mites, and the average number of mites entering a cell with drone brood is higher than the number entering a cell with worker brood (Boot et al. 1991). In addition, mites prefer drone larvae to worker larvae when given the choice in laboratory tests (Le Conte et al. 1989). Mite preference appears to be based, in part, on chemical attractants secreted by the larvae and present on the cuticle (Trouiller et al. 1991). Koeniger et al. (1981) found that varroa on *A. cerana* in Sri Lanka reproduced only in drone cells. Mites can enter worker brood cells but no offspring are produced (Boot et al. 1997).

The high level of immature offspring occurred in the worker cells (40.6%) and not in the drone cells (13.2%), indicates that what is occurring in the worker cells is generating a significant differences in mite reproductive ability in AHB colonies. This factor influences the production of viable female mites because immature stages remain in the cell when a bee emerges, and these immature mites are removed by nurse bees (De Jong 1997).

About 15.0% of mites produced only a female offspring in drone brood cells. The absence of the male or premature death of the male offspring within the host cell before it is able to mate with its sisters leads to unfertilized adult females offspring emerging from the cell. Since only one male is produced per batch of eggs, its absence or death will result in all the female offspring being unmated and so unable to produce offspring although they do enter cells and attempt to reproduce (Harris and Harbo 1999).

A maximum of six and seven offspring was found in worker and drone brood respectively. According to Ifantidis (1984), varroa females are biologically capable of laying up to six eggs in worker cells and seven in drone cells.

Although no differences were found in the percentage of non-reproducing mites, a significant finding was that the reproductive behaviour of the majority of non-reproducing mites in worker brood become different between subsequent reproductive cycle in drone brood. More than 74.0% of the non-reproducing mites produce offspring when were transferred from worker to drone brood, and 36.0% of these mites produce viable females offspring with an average of 2.3 viable females. Therefore, the production of offspring was influenced by the brood type. Termed as behavioral non-reproduction, since although these mites are capable of reproduction, they fail to reproduce in some cycles (Martin et al. 1997). The reasons for non-reproduction are as yet unknown, but this study suggest that it appears to occur more commonly in worker than drone cells. De Ruijter (1987) reported that some mites interrupted egg laying for one period or more and resumed egg laying in the next period. Lack of a stimulus, as a result of the haemolymph composition of the pupa, has been discussed as one of the most important factors.

In conclusion, mite fertility was similar between worker and drone brood. Nevertheless, a significant difference in the number of foundress mites producing viable female offspring and immature offspring was found, being drone brood more suitable for mite reproduction. The number of eggs and viable females produced per foundress mites was higher in drone brood compared to worker brood. Furthermore, a high percentage of the non-reproducing mites in worker brood produce offspring when they were transferred to drone brood.

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