Reproduction of *Varroa destructor* and offspring mortality in worker and drone brood cells of Africanized honey bees

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Abstract   *Varroa destructor* is known to be the most serious parasite of *Apis mellifera* worldwide. In order to reproduce varroa females enter worker or drone brood shortly before the cell is sealed. From March to December 2008, the reproductive rate and offspring mortality (mature and immature stages), focusing on male absence and male mortality of *V. destructor*, was investigated in naturally infested worker and drone brood of Africanized honey bees (AHB) in Costa Rica. Data were obtained from 388 to 403 single infested worker and drone brood cells, respectively. Mite fertility in worker and drone brood cells was 88.9 and 93.1%, respectively. There was no difference between the groups ($X^2 = 3.6$, $P = 0.06$). However, one of the most significant differences in mite reproduction was the higher percentage of mites producing viable offspring in drone cells (64.8%) compared to worker cells (37.6%) ($X^2 = 57.2$, $P < 0.05$). A greater proportion of mites in worker brood cells produced non-viable female offspring. Mite offspring mortality in both worker and drone cells was high in the protonymph stage (mobile and immobile). A significant finding was the high rate of male mortality. The worker and drone brood revealed that 23.9 and 6.9%, respectively, of the adult male offspring was found dead. If the absence (missing) of the male and adult male mortality are taken together the percentage of cells increased to 40.0 and 21.3% in worker and drone cells, respectively ($X^2 = 28.8$, $P < 0.05$). The absence of the male or male mortality in a considerable number of worker cells naturally infested with varroa is the major factor in our study which reduces the production of viable daughters in AHB colonies in Costa Rica.

Keywords   *Varroa destructor* · Reproductive ability · Comparison · Africanized honey bees · Worker brood · Drone brood · Mite offspring mortality · Male mortality

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Introduction

Varroa destructor Anderson and Trueman (2000) (formerly named *Varroa jacobsoni*) is known to be the most serious parasite of *Apis mellifera* worldwide. Most western honey bee colonies do not survive infestation by the mite when left untreated (Bailey and Ball 1991). Enormous losses of managed honey bee colonies in Europe (Ritter and De Jong 1984) and the United States (Bailey and Ball 1991) have been reported.

Because varroa populations increase when brood is present, it would be expected that in tropical climates, where brood rearing takes place year-round, the effect of varroa would be even more devastating. However, in Brazil varroa was introduced more than 30 years ago and established itself at low levels of infestation (approximately less than 3.0%), without causing apparent damage to apiculture with Africanized bees (De Jong 1997; Moretto and Leonidas 2001, 2003). Some reports suggest that these bees are tolerant or resistant to varroa and that colony losses are not a problem (De Jong 1997). The low fertility of female mites in worked brood (less than 50.0%) is the most important factor associated to this phenomenon. Nevertheless, regional differences in weather conditions and mite genotypes make it difficult to characterize the mite reproductive ability and the varroa population dynamics over a wide-spread area (De Guzman et al. 2007). In Costa Rica, some bee-keepers have reported loss of hives and reduced production of honey as a consequence of *V. destructor* introduction. Newly emerged bees with damaged wings, bees crawling in front of the hive and unusually severe adult bee mortality were observed in AHB colonies in Costa Rica. A large proportion of the adult bee population in most of these colonies was infested with *V. destructor*. The analysis of adult bee samples showed the occurrence of deformed wing virus and Kashmir bee virus (Calderón et al. 2003).

The reproductive ability of *V. destructor* varies according to the type of honey bee brood (worker or drone) being parasitized (Calderone and Kuenen 2001; Camazine 1988; Fuchs 1992; Ifantidis 1988; Martin et al. 1997). 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; Trouiller et al. 1992). Varroa mites are present on adult bees prior to invasion of brood cells. In order to decide whether to invade a brood cell or stay on a bee, mites should obtain information about the content of the cell, e.g., a volatile chemical coming from the larvae (Boot 1994; Rickli et al. 1992, 1994). Such chemical information could be different for worker and drone brood, enabling discrimination by the mites (Boot et al. 1992; Calderone and Lin 2001). In relation to the chemical attractants related to cell invasion by varroa mites, there are a number of physical factors correlated to cell invasion (Calderone and Kuenen 2003; Kuenen and Calderone 2000). The shape of the brood cell appears to influence preference for drone cells (De Ruijter and Calis 1988). The surface of a drone cell is 1.7 times bigger than that of a worker cell, which increases the chance for a mite on a bee to come close enough to a suitable cell. Worker larvae in shallow cells are infested and capped sooner than larvae in control cells (Boot et al. 1995; Goetz and Koeninger 1992), whereas larvae reared in elongated cells are capped later than unmanipulated cells (Goetz and Koeninger 1993).

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). Furthermore, the mites have a much higher reproductive success in drone brood cells (Martin 1995), an average of 1.4 new female mites per original mother mite in worker cells and 2.2 in drone cells are reported for European bees (Fuchs and Langenbach 1989). In *A. cerana* varroa reproduces only in drone cells (97–100% of the mites in drone brood produced daughters),

though the mites occasionally enter worker cells. The low tendency of varroa to invade and reproduce in worker brood cells of *A. cerana* reduces the principal cause of damage found in *A. mellifera* colonies. It has been suggested that AHB tolerance to varroa mites is due to the drone brood been more infested at certain times of the year (Martin and Medina 2004).

To evaluate the effect of brood type in mite reproduction success, we compared the reproductive rate of *V. destructor* in artificially infested worker and drone cells of AHB colonies. We had previously found that a much higher percentage of mites produced viable offspring in drone cells than in worker cells (Calderón et al. 2007).

The death of male mites plays a central role in explaining a decrease in the production of viable daughters. When mites produce only female offspring these will remain unmated in single infested cells, because varroa mites mate just after their final molt inside the cell (Boot et al. 1997). The lack of male offspring, probably due to mortality will result in large numbers of non-reproductive females in the mite population (Martin et al. 1997). The mortality suffered by the first (specifically the male protonymphs) mite offspring found in AHB, is thought to contribute in part to the tolerance of the bees (Medina and Martin 1999).

We hypothesize that, due to the low number of viable females offspring found in worker brood cells of AHB, a higher rate of male absence or male mortality should occur in worker brood than in drone brood cells. Therefore, the aim of the present study was to investigate the mite reproductive ability and offspring mortality, focusing in male absence or male mortality, in naturally infested worker and drone brood cells of AHB colonies under tropical conditions.

Materials and methods

The study was conducted at the Centro de Investigaciones Apícolas Tropicales (CINAT) of the Universidad Nacional located in Heredia in the Central Valley of Costa Rica (10°01′N, 84°07′W; 1,130 m altitude). The experiments were carried out from March to December 2008 using 15 chemically untreated Africanized honey bee (AHB) colonies (none of the colonies examined had recently been exposed to acaricides) (all honey bee colonies in Costa Rica have become Africanized) (Spivak 1991), in which the worker and drone brood was naturally infested by the mite. The months of March and April corresponded to the dry season, whereas from May to December to the rainy season.

Mite reproduction in worker and drone brood cells

The reproductive ability of the foundress mite was evaluated in sealed worker and drone brood collected from the experimental colonies. The reproductive rate of varroa was determined in 18 to 20-day-old worker and drone brood (pupa has a dark tan or gray appearance and some movements of the legs were observed, 240 h post-capping for both). Examining honey bee worker and drone brood 10 days after capping (240 h) is 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.

Comb sections (10 × 10 cm approximately) containing capped brood were collected from the colonies and immediately analyzed for mite reproduction (we used only fresh brood samples).

At the laboratory, sealed worker and drone brood cells were carefully opened and the developmental stage of the bee and infestation level determined (from 75 to 150 cells were opened in each comb sample). All of the infested brood cell contents were removed and

examined using a stereoscope microscope (10× magnification). The offspring of the mites were classified as eggs-larva, protonymph (mobile and immobile stages), deutonymph (mobile and immobile stages), male and female adults, using the ontogenic developmental charts in Martin (1994) and pictures in Ifantidis (1983). Only those cells infested by a single female mite were used, since mites are less productive as the number of mother mites per cell increases (Medina et al. 2002).

Several variables were measured for foundress female mites: fertility (production of any offspring), fecundity (number of progeny), production of viable female offspring (cells that contain an adult male mite and at least one adult female mite, both alive), production of only immature offspring (cells with eggs-larvae, protonymphs or deutonymphs), production of only female or only male offspring (no adult female, immature offspring possible), 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 72–96 h more (in average), 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. In addition, non-viable female offspring was analyzed for both, worker and drone brood, and it corresponded to the cells with the presence of the adult daughter and male, but one of them or both were found dead (Martin and Kryger 2002).

Mite offspring mortality

A detailed analysis of the mite offspring mortality (mature and immature stages) was conducted, special attention was paid to male absence or male mortality. Mite offspring mortality was determined only in cells invaded by a single mother mite. The offspring was considered dead if it was crumpled or shrunk. Furthermore, offspring mortality was considered using death criteria indicated by Ifantidis (1997).

– Heavy body deformations indicate death of individuals in every stage.
– Death of intact mobile phases is recognized simply for the lack of motion in fresh brood comb samples.
– The death in immobile female, especially for deutonymphs was recognized by the lack of the spontaneous and periodically peristaltic movements of Malpigian tubules. Malpigian tubules are well formed and easily visible through the transparent integument.
– Immobile deutonymphs which die during the moulting process can be recognized by the lack of local movements of legs. The legs are no more out-stretched, they are contracted under opisthosoma.

Foundress mite mortality was also determined for worker and drone cells. Since the number of viable female offspring produced per foundress mite in worker and drone brood is a vital value for mite reproduction, the total viable female offspring in a cell was calculated only when the male and female offspring were alive.

Statistical analysis

Mite reproductive parameters and offspring mortality in worker and drone brood of AHB were analyzed using the R-Program (95% confidence). Differences between worker and drone for various mite reproductive factors were analyzed using the T-student and Chi-square tests. Data are given as percentage values and mean ± standard deviation.

Results

A comparison of the reproductive ability of *V. destructor* was done for 388 and 403 naturally infested worker and drone brood cells of AHB, respectively. The number of cells analyzed by each colony, depended on the availability of worker and drone brood and the level of varroa mites infestation. Cells infested by two mites or more were not analyzed.

Varroa fertility and fecundity

Mite fertility in worker and drone brood cells was 88.9% (n = 388) and 93.1% (n = 403), respectively. No significant differences were found between the groups. The average fecundity of reproducing mites was 3.6 ± 1.2 (n = 345) and 4.3 ± 1.6 (n = 375) descendants per foundress in worker and drone brood, respectively (Table 1). A maximum of six offspring was found in worker brood. Most frequently the number of eggs laid was four and five. In drone brood, a maximum of seven offspring was found being more frequently five and six eggs.

Viable female offspring

There was a clear difference in the percentage of mother mites producing viable female offspring in worker and drone brood ($X^2 = 57.2, P < 0.05$), being drone brood more suitable for mite reproduction (Table 2). A total of 210 and 862 viable females offspring were produced from 388 worker and 403 drone brood cells, respectively (Table 1). A greater proportion of mites in worker brood cells produced non-viable female offspring (Table 2).

Varroa that produced only immature offspring or one adult sex

A significant difference was found in the percentage of mothers producing immature offspring in worker and drone brood ($P < 0.05$) (Table 2). Meanwhile, the presence of only female in the cells was not different between the groups ($X^2 = 0.05, P = 0.83$) (Table 2). A greater proportion of mites in worker brood cells produced only a male offspring that was identifiable when the cells were sampled 10 days after capping (Table 2). Eggs, larvae and protonymphs were not sexed and could have been female or male.

Table 1 Reproductive rate of *Varroa destructor* in worker and drone brood (240 h post capping) of Africanized honey bees (AHB) (data are given as mean values $\pm$ S.D.)

Characteristic	Worker	Drone	Mean difference (IC 95%)	Statistical test (t)	P
Number of progeny produced per all adult females	3.2 ± 1.6	4.0 ± 1.9	-0.8 (-1.0 to -0.5)	-6.2	<0.05
Number of eggs produced per reproductive female	3.6 ± 1.2	4.3 ± 1.6	-0.7 (-0.9 to 0.5)	-6.3	<0.05
Number of viable females produced per foundress producing viable offspring	1.4 ± 0.6	3.3 ± 1.2	-1.9 (-2.0 to 1.7)	-21.1	<0.05
Number of viable females produced per reproductive female mite	0.6 ± 0.8	2.3 ± 1.8	-1.7 (-1.9 to 1.5)	-16.3	<0.05
Number of viable females produced per mite taken into account all adult females	0.5 ± 0.8	2.1 ± 1.9	-1.6 (-1.8 to -1.4)	-15.9	<0.05

Table 2 Comparison of reproductive parameters of varroa foundress mites in worker and drone brood of Africanized honey bees (AHB)

Parameter	Worker (%) (n = 388)	Drone (%) (n = 403)	Mean difference (IC 95%)	Statistical test (X^2)	P
Fertility	88.9	93.1	−4.2 (−8.4 to 0.1)	3.6	0.06
Non-reproductive	11.1	6.9	4.2 (−0.1 to 8.4)	3.6	0.06
Viable female offspring	37.6	64.8	−27.2 (−34.1 to −20.2)	57.2	<0.05
Non-viable female offspring	14.7	5.2	9.5 (5.1–13.9)	18.9	<0.05
Immature offspring	4.6	1.0	3.6 (1.1–6.2)	8.4	<0.05
Males only	17.8	8.7	9.1 (4.2–14.0)	13.5	<0.05
Females only	14.2	13.4	0.8 (−4.3 to 5.8)	0.05	0.83

Mite mortality

The mean proportion of dead foundress mites was 8.1 and 3.4% in worker and drone brood cells, respectively, with some of them found trapped in the bottom of the cell (there was insufficient data to reveal any pattern). The data concerning mite offspring mortality in both worker and drone cells show a high mortality in the protonymph stage (mobile and immobile) (Table 3). An interesting finding was the high rate of male mortality. The worker and drone brood analyzed revealed that 23.9 and 6.9%, respectively (Table 3), of the adult male offspring was found dead. If both the absence (missing) of the male and male mortality are considered the percentage of cells increased to 40.0 and 21.3% in worker and drone cells, respectively ($X^2 = 28.8$, $P < 0.05$). In some cases the male offspring die before reaching the adult stage (early stages), and can be missed. If this was the case, a closer look within the cell, on the pupa or on the bee exuviae, revealed the presence of the dead mite or confirmed its absence.

Effect of male absence or male mortality in the amount of viable females

The number of viable female offspring produced per foundress mite is a crucial value since it represents the average reproductive ability of the population of mites being studied. In

Table 3 Comparison of the foundress mite mortality and mite offspring mortality in worker and drone brood cells of Africanized bees

Mite/offspring	Worker (%)	Drone (%)	Mean difference (IC 95%)	Statistical test (X^2)	P
Foundress mite	8.1 (n = 422)	3.4 (n = 417)	4.7 (1.3–8.1)	7.74	0.01
Offspring	23.3 (n = 1249)	12.5 (n = 1605)	10.8 (7.9–13.8)	57.2	<0.05
Protonymph mobile	66.4 (n = 110)	78.4 (n = 51)	12.0 (−27.8 to 3.7)	1.88	0.17
Protonymph immobile	45.2 (n = 157)	42.6 (n = 61)	2.6 (−13.2 to 18.4)	0.04	0.85
Deutonymph mobile	17.6 (n = 68)	5.9 (n = 34)	11.7 (−2.5 to 26.0)	1.75	0.19
Deutonymph immobile	6.9 (n = 261)	7.3 (n = 220)	−0.4 (−5.4 to 4.6)	0.01	1.0
Females	14.1 (n = 368)	10.3 (n = 915)	3.8 (−0.4 to 8.1)	3.50	0.06
Males	23.9 (n = 272)	6.9 (n = 317)	17.0 (10.8–23.1)	32.1	<0.05

this study, we observed a significant decrease in the number of cells with viable females because the absence of the male or male mortality. The absence of the male in worker brood cells affected the production of 83 viable females (22.5%; n = 368). In addition, in some cells there were two new adult daughters but the male was absence or dead resulting in non-viable female offspring.

Discussion

A critical aspect concerning varroa parasitism is the extent to which existing differences in reproduction among host honey bee species and subspecies are due to characteristics of the host or the parasite. A significant factor, which is strongly correlated with the population dynamics of varroa mites, is the number of fertile mites developing in worker and drone brood cells (Fries et al. 1994; Correa-Marquez et al. 2003). It is quite evident that geographic and climatic differences as well as host specific effects play an important role in mite fertility (De Jong et al. 1984; Moretto 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 question remains as to why varroa mites refrain from reproduction in worker cells of *A. cerana*. Apparently, reproduction is not the only reason for mites to invade a brood cell. They may invade worker cells of *A. cerana* to hide in safety from the grooming behavior of adult bees (Fries et al. 1996) or because residing in worker cells may lead to higher reproductive success in a following cycle in drone brood cells (Boot et al. 1997).

In European colonies *V. destructor* reproduces both in drone and in worker cells. Reproduction in worker cells appears essential for the high susceptibility of European bees to varroa mites, because it implies that the mite population grows rapidly (exponentially) (Boot et al. 1999). Meanwhile, the high percentage of infertile mites observed in worker brood has been proposed to be related with the tolerance of Africanized bee colonies to varroa mites in Brazil (Rosenkranz 1999), where colony losses are not recorded (Aumeier et al. 2000).

We found that mite fertility was similar between worker and drone brood (every female which had laid at least one egg was considered fertile). In previous studies using AHB in Costa Rica, mite fertility in artificially infested worker and drone brood cells was about 79.0% (Calderón et al. 2007). In Mexico, the annual mean percentage of mother mites that reproduce in worker brood of Africanized bees was 85.5% with a range from 74.1 to 91.5% (Mondragon et al. 2006). 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). Nevertheless, despite the increased fertility, no increase in mite infestation rates in the colonies has been detected so far. Currently, the rate of infestation on adult bees in Africanized bees in Blumenau has remained low (Carneiro et al. 2007). As the low fertility of mites in AHB worker brood has been discussed as one of the most significant factors contributing to varroa tolerance, the new status of mite fertility in Brazil demands for further attention. In addition, this results indicates that mite fertility in worker and drone brood in Africanized bees is currently at European levels.

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 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 (brood type has an influence in the host–parasite relationship) (Boot et al. 1991; Santillan-Galicia et al. 2002). The percentage of mites with viable offspring reported from worker brood cells was similar in Africanized bees in Mexico (Medina and Martin 1999) and Brazil (falls to around 40.0%) (Correa-Marquez et al. 2003), and was slightly lower in Costa Rica. This is unexpected when we consider that varroa population increase in Africanized colonies in Mexico and Costa Rica, and not so in Brazil.

Male mortality

Mite offspring mortality and the mortality rates of the male offspring have not been widely reported in Africanized bees. We found that mite offspring mortality was higher in worker brood than in drone cells. In addition, a higher rate of male absence or male mortality occurred in worker brood cells. The death of a male plays a central role in explaining a decrease in the production of viable daughters. A direct consequence of male mortality in worker cells results in only 0.73 viable female offspring produced per mother mite, which rises to 0.85 if both mated (viable) and non-mated females are considered from AHB in Mexico (Medina and Martin 1999). For AHB in Costa Rica the number of viable females produced per reproductive female mite in worker cells was 0.30. If only the foundress producing viable offspring is considered this rises to 1.44 (Calderón et al. 2007).

Eguaras et al. (1995) found that a high infertility was associated with a greater lack of males. When mites produce daughters but no living son, the daughters mites will remain unmated in single infested cells (Boot et al. 1997). These unfertilized mites cannot mate once they have emerged from the cell and so never produce viable offspring, although they do enter cells and attempt to reproduce. The considerable higher rate of mortality suffered by the first (specifically the male) and second (female) mite offspring found in AHB, is thought to contribute in part to the tolerance of these bees (Medina and Martin 1999).

The mortality of the male offspring within the bee brood cells may be due to physical problems, i.e., being crushed or dislodged by bee movement during pupation or a failure to locate the feeding site established by the mother (Donzé and Guerin 1994). The origin of the absence of the male still has to be elucidated. We observed in transparent cells containing infested worker brood in Africanized bees that in a considerable number of cases the first egg was disturbed and damaged when the prepupa molts into a pupa. The first egg is generally male and will not have any chance to get the adult stage. We consider that the absence of male in an important number of worker cells could be explained by this effect (Calderon et al. unpublished data).

The male mortality affects mite population growth in a negative way. However, since varroa completes between two and three reproductive cycles (Martin and Kemp 1997), positive population growth is possible (infestation rates of up to 22.0% in adult bees were observed in AHB colonies in Costa Rica) although at a reduced rate in AHB compared to EHB. In addition, a high proportion of the mite reproduction should occur in drone cells.

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 was found, being drone brood more suitable for mite reproduction. The number of eggs and viable females produced per foundress mite was higher in drone brood compared to worker brood. The absence of the male or male mortality in a considerable number of worker cells naturally infested with varroa is the major factor in our study which reduces the production of viable daughters in AHB colonies in Costa Rica.

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