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Low fertility, fecundity and numbers of mated female offspring explain the lower reproductive success of the parasitic mite *Varroa destructor* in African honeybees

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Research Article

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Abstract

Although *Varroa destructor* is the most serious ecto-parasite to the honeybee, *Apis mellifera* L., some honeybee populations such as *Apis mellifera scutellata* in Kenya can survive mite infestations without treatment. Previously, we reported that grooming behaviour could be a potential tolerant mechanism expressed by this honeybee subspecies towards mite infestation. However, both hygienic and grooming behaviours could not explain the lower mite-infestation levels recorded in these colonies. Here, we investigated the involvement of other potential resistant mechanisms including suppression of mite reproduction in worker brood cells of *A. m. scutellata* to explain the low mite numbers in their colonies. High infertility rates (26–27%) and percentages of unmated female offspring (39–58%) as well as low fecundity (1.7–2.2, average offspring produced) were identified as key parameters that seem to interact with one another during different seasons to suppress mite reproduction in *A. m. scutellata* colonies. We also identified offspring mortality in both sexes and absence of male offspring as key factors accounting for the low numbers of mated daughter mites produced in *A. m. scutellata* colonies. These results suggest that reduced mite reproductive success could explain the slow mite population growth in *A. m. scutellata* colonies.

Introduction

Varroa destructor Anderson and Trueman is the most serious ecto-parasitic mite that has significantly contributed to the decline of the Western honeybees (*Apis mellifera* L.), both wild and managed, particularly in Europe and North America (Neumann and Carreck, 2010; Francis *et al.* 2013; Smith *et al.* 2014; Kielmanowicz *et al.* 2015). The mite invaded *A. mellifera* colonies outside its native host range in Southeast Asia where it was originally restricted only to its natural host *Apis cerana* (reviewed in Nazzi and Le Conte, 2016). The infestations by the mites can have significant negative effects on susceptible *A. mellifera* populations, especially the ones of European origin, mainly because they lack or poorly express the behavioural mechanisms displayed by the mite's original host to counter infestation (Ritter, 1981; Fries *et al.* 1996). These behavioural mechanisms include: efficient hygienic behaviour (the ability of nurse honeybees to detect, uncap and remove dead or diseased/parasitized brood) and grooming behaviour (the ability of individual honeybees to remove mites off their bodies or from those of their nest mates thereby sometimes inflicting physical injuries to the mites during the removal process) as well as entombing of drone broods (Peng *et al.* 1987; Boecking and Spivak, 1999; Rath, 1999). Additionally, the mite reproduces only in the less abundant and seasonally occurring drone brood in colonies of *A. cerana*, whereas its reproduction takes place in both drone brood and the more abundant worker brood which occurs throughout the breeding season in *A. mellifera* colonies (Rath, 1999). As a result, beekeepers in the affected countries practice periodic miticide treatment to prevent the collapse of honeybee colonies within 1 or 2 years (Lee *et al.* 2010; Neumann and Carreck, 2010; Rosenkranz *et al.* 2010).

The reproductive cycle of *Varroa* mite takes place entirely in sealed brood cells and synchronizes with the sealed brood development time of the host larvae (Martin, 1994). A foundress mite invades a worker brood cell shortly before it is capped and lays her first unfertilized egg, ~60–70 h following cell capping (Ifantidis, 1983; Martin, 1994). This unfertilized egg develops into a male while the subsequent three to four fertilized eggs which are laid at approximately 30 h interval each develop into females (Ifantidis, 1983; Martin, 1994). A mite can lay up to five eggs in worker brood and up to six eggs in drone brood (Martin, 1994). It takes about 6 and 7 days for female and male mites, respectively, to develop into adults (Martin, 1994). Mating between the mite's offspring occurs within the sealed brood cells once they reach adulthood with the male *Varroa* mite dying shortly afterwards. The foundress mites together with one or two viable, mature and mated daughter mites attach

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themselves to the honeybee that emerges from the cell leaving behind all immature mites which ultimately die inside the cells. Therefore, a foundress mite is considered to reproduce successfully when one or two viable, mature and mated daughter mites emerge from the cell during each reproductive cycle (Ifantidis, 1983; Martin, 1994). Thus, the duration of the post-capping stage of worker brood and the mite offspring mortality in these cells are factors which can potentially influence the reproductive success of foundress mites (Martin, 1994; Rosenkranz *et al.* 2010; Ardestani, 2015). Alternatively, mites could be considered non-reproductive because they die in the cell without reproducing, produce no offspring, produce only male offspring or produce offspring that fail to reach maturity before the developing honeybee pupa hatches as an adult (Harbo and Harris, 1999). While reproducing inside the brood cells, the mite and her offspring feed on the fat body of the developing pupae and the foundress together with the mature female offspring continue to feed on the adult honeybee after emergence from the cells (Ramsey and VanEngelsdorp, 2017). In the course of feeding, the mites can/often transmit lethal pathogens to the individual honeybee (Rosenkranz *et al.* 2010), which affects the individual honeybee physically and physiologically (Aronstein *et al.* 2012; VanDooremalen *et al.* 2012; Annoscia *et al.* 2015).

However, some *A. mellifera* populations are reported to display behavioural mechanisms including hygienic and grooming behaviours and suppression of mite reproductive success which allow these honeybee populations to coexist with the mite for longer periods without requiring any in-hive miticide treatment (Peng *et al.* 1987; Fries *et al.* 1996; Calderón *et al.* 2010; Calderón *et al.* 2012; Locke *et al.* 2012; Strauss *et al.* 2013; Strauss *et al.* 2016). For example, previously we had shown that, the surviving African savannah honeybee, *Apis mellifera scutellata* (Lepeletier) in Kenya maintains a lower mite colony infestation (~3-fold lower) than their susceptible *A. mellifera* hybrids of European origin found in the USA (Nganso *et al.* 2017). Furthermore, they also express a higher grooming behaviour towards the mite than their European counterparts, although both honeybee subspecies express similar levels of hygienic behaviour. However, both hygienic and grooming behaviours could not explain the lower mite infestation levels recorded in *A. m. scutellata* colonies. Grooming behaviour was identified as a potential tolerant mechanism displayed by the African savannah honeybee towards infestation by the mite, suggesting that other resistant mechanisms such as suppression of mite reproduction might explain the lower mite population growth observed in colonies of the savannah honeybee. The suppression of the reproductive success of *Varroa* mite in the worker brood cells by *A. mellifera* populations is considered a crucial adaptive resistant mechanism (Fries *et al.* 1994; Harris *et al.* 2003; Martin and Medina, 2004; Mondragón *et al.* 2006). It explains the slow rate of mite population growth within their colonies and slight variations in this trait could underline resistance development towards the mite. The suppression of the mite reproductive output which translates into lower mite fertility, fecundity and reproductive success in worker brood cells has been found to explain honeybee resistance towards the mites in various populations. These populations include *A. m. scutellata* in South Africa (Strauss *et al.* 2016), Africanized honeybees in Brazil (Calderón *et al.* 2012), the oldest *Varroa* tolerant European honeybee populations, *A. m. ligustica* in the island of Fernando de Noronha in North-eastern Brazil (Brettell and Martin, 2017), Avignon and Gotland honeybee populations in France and Sweden, respectively (Locke and Fries, 2011; Locke *et al.* 2012), the Russian honeybee population in the USA (de Guzman *et al.* 2008) and the Norwegian honeybee population (Oddie *et al.* 2017). In the present study, we aimed to investigate mite reproduction in worker brood cells of *A. m. scutellata* to explain the low mite numbers recorded in their colonies.

Materials and methods

Study sites

The study was conducted in Nairobi, Kenya in November 2015 (the short rainy season), January 2016 and February 2018 (the hot dry season). The hot dry season is characterized by a drastic reduction or cessation in brood rearing while the short rainy season is characterized by increased brood rearing in savannah honeybee colonies (Raina and Kimbu, 2005). All the colonies were housed in standard Langstroth hives containing 3–4 brood combs and were not treated with acaricides to reduce mite infestations.

Four and 14 (14 = 7 colonies used in each hot dry season) queen right colonies of *A. m. scutellata* were selected at an apiary in Kithimani (1°8′S, 37°25E) during the short rainy and hot dry season, respectively, while three colonies were selected at an apiary in Kilimanbogo (1°8′S, 37°21′E) during the short rainy season. Both apiaries are located within the county of Machakos and hosted *A. m. scutellata* colonies that originated from locally captured swarms (Hepburn and Radloff, 1988; Raina and Kimbu, 2005; Muli *et al.* 2014).

Assessment of Varroa mite reproduction in worker brood cells

To quantify *Varroa* mite reproductive output, we used the method described by Strauss *et al.* (2016) with slight modifications. Briefly, 200 worker brood cells containing pupae at the molting stage were inspected in each colony (Martin, 1994). All the colonies in each of the apiary were screened for brood at this stage and only positive colonies were used. These were four colonies in November 2015, seven colonies in January 2016, seven colonies in February 2018 at the apiary in Kithimani and three colonies in November 2015 at the apiary in Kilimanbogo. We used this stage because at the time of emergence of the young honeybees from the worker cells, the foundress mites have already completed their reproduction and it becomes easy to estimate their reproductive output. To determine *Varroa* mite reproduction, we initially generated count data on the number of foundresses, mature daughter mites, immature daughter mite and males in each infested cell. We used only singly infested cells to determine the reproductive success of the mites in worker brood cells of *A. m. scutellata* (Rosenkranz *et al.* 2010). For each infested cell, we further collected data on infertility (alive and dead foundresses with no offspring), fertility (production of offspring), fecundity (number of offspring produced), number of viable, mated and mature daughters and presence (alive and dead) or absence of adult males. The mating status of the daughter mites was determined by the simultaneous presence of one live mature daughter and one live adult male in a worker brood cell during an inspection of infested cells (Rosenkranz *et al.* 2010; Locke *et al.* 2012; Strauss *et al.* 2016; Brettell and Martin, 2017). We also determined the fecundity and number of mature mated female offspring produced in cells infested by two or more foundress mites.

Assessment of the post-capping duration of worker brood

The duration of the post-capping stage of worker brood was determined in three colonies at the apiary in Kithimani. Two frames containing approximately 300 mature worker larvae prior to capping were removed from the central region of each colony and marked. Snap shots were taken to record the position

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of all sealed and unsealed worker broods after which the marked frames were returned to their colonies. The frames were then inspected twice a day (morning and evening) to record worker cells that were capped and monitored those until the honeybees emerged from the cells. A total of 657 worker brood cells were recorded in the savannah honeybee colonies. During each inspection period, photographs were taken. The number of brood that emerged from the worker cells and the number of days they took to emerge were recorded to determine the average duration of the sealed worker brood stage of *A. m. scutellata* through a thorough analysis of the photographs.

Statistical analysis

Statistical analyses were performed using R-Software version 3.2.5 (R Development Core Team, 2015) and the alpha level was set at 0.05 (Pirk *et al.* 2013). The generalized linear model (GLM) with logit link and binomial distribution error was used to examine the differences in the percentage of fertile and infertile foundress mites, and the percentage of foundress mites with viable mated daughter mites, unmated daughter mites and only male produced per cell and per foundress among the short rainy (November 2015) and hot dry seasons (January 2016 and February 2018) at the apiary in Kithimani. To compare the average number of offspring and mated daughter produced per cell and per foundress among the short rainy and hot dry seasons at the apiary in Kithimani, we used the GLM with log link and binomial distribution error. We also used the GLM with log link and binomial distribution error to compare the average number of offspring and mated daughter produced per cell and per foundress in worker cells infested by 1 or 2–4 foundresses in each season in the colonies of the African savannah honeybee.

Results

Assessment of Varroa mite reproduction in worker brood cells

Reproduction in singly infested cells

The patterns of *Varroa* mite reproduction during the different seasons of assessment in colonies of *A. m. scutellata* are presented in Tables 1 and 2.

The percentage of infertile mites was significantly lower during the hot dry season (January 2016) than the short rainy (November 2015) and hot dry (February 2018) seasons at the apiary in Kithimani (df = 16: $\chi^2 = 0.64$; $P = 0.001$, Table 1). However, there were no significant differences in the average number of offspring produced per cell (df = 16: $\chi^2 = 0.02$; $P = 0.89$, Table 1) and foundress (df = 16: $\chi^2 = 0.07$; $P = 0.80$, Table 1) and the average number of mated daughter mites produced per cell (df = 16: $\chi^2 = 1.63$; $P = 0.20$, Table 1) and foundress (df = 16: $\chi^2 = 2.45$; $P = 0.12$, Table 1) among these seasons at the same apiary. Likewise, there were no significant differences in the percentage of viable mated daughter mites produced per cell (df = 16: $F = 0.002$; $P = 0.97$, Table 1) and foundress (df = 16: $F = 0.002$; $P = 0.97$, Table 1) and the percentage of only male produced per cell (df = 4: $\chi^2 = 0.33$; $P = 0.57$, Table 1) and foundress (df = 4: $\chi^2 = 0.28$; $P = 0.60$, Table 1) among these seasons at the apiary in Kithimani. Furthermore, the percentage of unmated daughter mites produced per cell (df = 13: $\chi^2 = 12.13$; $P = 0.001$, Table 1) and foundress (df = 13: $\chi^2 = 12.11$; $P = 0.001$, Table 1) was significantly lower during the hot dry season (February 2018) than the short rainy (November 2015) and hot dry (January 2016) seasons at the apiary in Kithimani.

Reproduction in multiply infested cells

During the hot dry season (January 2016) at the apiary in Kithimani, the mites reproduced in all the 9 cells infested with 2

Table 1. Comparison of the reproductive parameters of *Varroa* foundress mites produced per cell and per fertile foundress in singly infested worker brood cells in *A. m. scutellata* during the hot dry and short rainy seasons at the apiary in Kithimani, Kenya

Parameters	Hot dry season (January 2016)	Hot dry season (February 2018)	Short rainy season (November 2015)	*P*-value[a]
Per single infested cell, Fertile and infertile (Total inspected cells)	*n* = 39 (1400)	*n* = 99 (1400)	*n* = 41 (800)	
Fertility	92%	74%	73%	
Infertility	8%	26%	27%	**0.001**
Viable and mated female offspring	62%	54%	29%	0.97
Unmated female offspring	39%	16%	49%	**0.001**
Non-viable female offspring due to adult daughter and male dead, adult male dead and missing	23%	13%	29%	**0.04**
Immature offspring	16%	3%	20%	**0.002**
Male only	8%	5%	7%	0.57
Average number of offspring produced (mean ± S.D)	2.2 ± 1.0	1.9 ± 0.6	1.7 ± 0.3	0.89
Average number of mated daughter produced (mean ± S.D)	0.5 ± 0.3	0.5 ± 0.2	0.3 ± 0.1	0.20
Per fertile foundress only	*n* = 36	*n* = 73	*n* = 30	
Viable and mated female offspring	67%	73%	40%	0.97
Unmated female offspring	42%	22%	66%	**0.001**
Non-viable female offspring due to adult daughter and male dead, adult male dead and missing	25%	18%	40%	**0.04**
Immature offspring	17%	4%	26%	**0.002**
Male only	9%	7%	10%	0.60
Average number of offspring produced (mean ± S.D)	2.7 ± 1.5	2.7 ± 0.5	2.4 ± 0.2	0.80
Average number of mated daughter produced (mean ± S.D)	0.5 ± 0.3	0.7 ± 0.2	0.4 ± 0.1	0.12

[a]*p* values were calculated by generalized linear model (GLM) with log and logit links.

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Table 2. Reproductive parameters of *Varroa* foundress mites produced per cell and per fertile foundress in singly infested worker brood cells in *A. m. scutellata* during the short rainy season at the apiary in Kilimanbogo, Kenya

Parameters	Short rainy season (November 2015)
Per single infested cell, Fertile and infertile (Total inspected cells)	*n* = 35 (600)
Fertility	91%
Infertility	9%
Viable and mated female offspring	49%
Unmated female offspring	58%
Non-viable female offspring due to adult daughter and male dead, adult male dead and missing	52%
Immature offspring	6%
Male only	3%
Average number of offspring produced (mean ± S.D)	2.1 ± 0.3
Average number of mated daughter produced (mean ± S.D)	0.6 ± 0.6
Per fertile foundress only	*n* = 32
Viable and mated female offspring	53%
Unmated female offspring	62%
Non-viable female offspring due to adult daughter and male dead, adult male dead and missing	56%
Immature offspring	6%
Male only	3%
Average number of offspring produced (mean ± S.D)	2.4 ± 0.5
Average number of mated daughter produced (mean ± S.D)	0.7 ± 0.8

live foundresses and a total of 34 offspring were produced, with 3.8 ± 0.3 (mean ± S.D) offspring produced per cell (Fig. 1A). There was no significant difference in the average number of offspring produced per cell (df = 10: $\chi^2 = 1.46$; $P = 0.23$) and per foundress (df = 10: $\chi^2 = 2.45$; $P = 0.12$) as well as, the average number of mated daughter produced per foundress (df = 10: $\chi^2 = 0.70$; $P = 0.40$) between multiply and singly infested worker cells (Fig. 1A). However, the average number of mated daughter produced per cell was significantly higher in multiply infested worker cells than in singly ones (df = 10: $\chi^2 = 5.07$; $P = 0.02$) (Fig. 1A).

During the hot dry season (February 2018) at the apiary in Kithimani, the mites reproduced in 62 out of the 64 cells infested with 2–4 live foundresses and a total of 170 offspring were produced, with 2.7 ± 1.4 (mean ± S.D) offspring produced per cell (Fig. 1B). There was no significant difference in the average number of offspring (df = 12: $\chi^2 = 0.36$; $P = 0.55$) and the average number of mated daughter (df = 12: $\chi^2 = 0.0$; $P = 1$) produced per cell between multiply and singly infested worker cells (Fig. 1B). However, the average number of offspring (df = 12: $\chi^2 = 9.64$; $P = 0.002$) and the average number of mated daughter (df = 12: $\chi^2 = 9.70$; $P = 0.002$) produced per foundress were significantly lower in multiply than singly infested worker cells (Fig. 1B).

During the short rainy season (November 2015) at the apiary in Kithimani, there was reproduction in 10 out of the 11 worker cells infested with 2–3 live foundresses and a total number of 26 offspring were produced, with 2.6 ± 1.0 (mean ± S.D) offspring produced per cell (Fig. 1C). There was no significant difference in the average number of offspring produced per cell (df = 6: $\chi^2 = 1.33$; $P = 0.25$) and per foundress (df = 6: $\chi^2 = 1.97$; $P = 0.16$) as well as, the average number of mated daughter produced per cell (df = 6: $\chi^2 = 1.05$; $P = 0.31$) and per foundress (df = 6: $\chi^2 = 0.0$; $P = 1$) between multiply and singly infested worker cells (Fig. 1C).

During the short rainy season (November 2015) at the apiary in Kilimanbogo, the mites reproduced in all the 8 worker cells infested with two live foundresses and a total of 27 offspring were produced, with 3.4 ± 0.5 (mean ± S.D) offspring produced per cell (Fig. 1D). There was no significant difference in the average number of offspring produced per cell (df = 4: $\chi^2 = 0.53$; $P = 0.47$) and per foundress (df = 4: $\chi^2 = 0.08$; $P = 0.78$) as well as, the average number of mated daughter produced per cell (df = 4: $\chi^2 = 0$; $P = 1$) and per foundress (df = 4: $\chi^2 = 0.2$; $P = 0.65$) between multiply and singly infested worker cells (Fig. 1D).

Assessment of the post-capping duration of worker brood

The average duration of the post-capping developmental time of *A. m. scutellata* worker brood was 265.2 ± 0.04 h.

Discussion

Mite reproduction in singly infested worker cells

In colonies of the African savannah honeybee, we recorded a higher infertility rate for the mites during the short rainy (November 2015) and the hot dry (February 2018) seasons which are characterized by increased and reduced brood rearing, respectively, at the apiary in Kithimani (26–27%). In contrast, a lower infertility rate of the mites was recorded during the hot dry season (January 2016) at the same apiary (8%) which was similar to the infertility rate recorded during the short rainy season at the apiary in Kilimanbogo (9%). The amount of brood present in honeybee colonies is a host feature that is known to significantly influence the fertility and the population dynamic of the mites (Lodesani *et al.* 2002). It appears that when brood is available in the colonies, features of the mites such as the reproductive capacity during their lifetime and lifespan might also influence their reproductive rate and population dynamics in honeybee colonies (Rosenkranz *et al.* 2010). Despite the variability in the fertility rates of the mites observed in worker brood cells of *A. m. scutellata*, the reproductive success of foundress mites remained similar to those reported in other surviving honeybee populations (Medina and Martin, 1999; Locke and Fries, 2011; Calderón *et al.* 2012; Locke *et al.* 2012; Strauss *et al.* 2016; Brettell and Martin, 2017; Oddie *et al.* 2017). Thus, these results suggest a strong suppression of mite reproduction in worker brood cells of *A. m. scutellata* in Kenya and this could be a plausible explanation for the low mite numbers recorded previously in colonies of this honeybee subspecies (Nganso *et al.* 2017).

In this study, we found that the post-capping duration of worker brood of *A. m. scutellata* could not explain the lower reproductive success of the mites recorded in their colonies. Up to 3–5 eggs were laid and 1–2 viable, mature and mated daughter mites emerged in worker brood cells of this honeybee subspecies. This finding suggests that when oviposition is initiated, up to five eggs are laid and there is sufficient time for one and sometimes two daughter mites to emerge from the worker cells of *A. m. scutellata* according to *Varroa* developmental charts (Martin, 1994). Interestingly, we identified high infertility rates (26–27%) and percentage of unmated female offspring (39–58%) as well as low fecundity (1.7–2.2, mean number of eggs laid) as exciting parameters that appears to explain the lower mite reproductive success in colonies of the savannah honeybee studied herein (Tables 1 and 2). These parameters seem to

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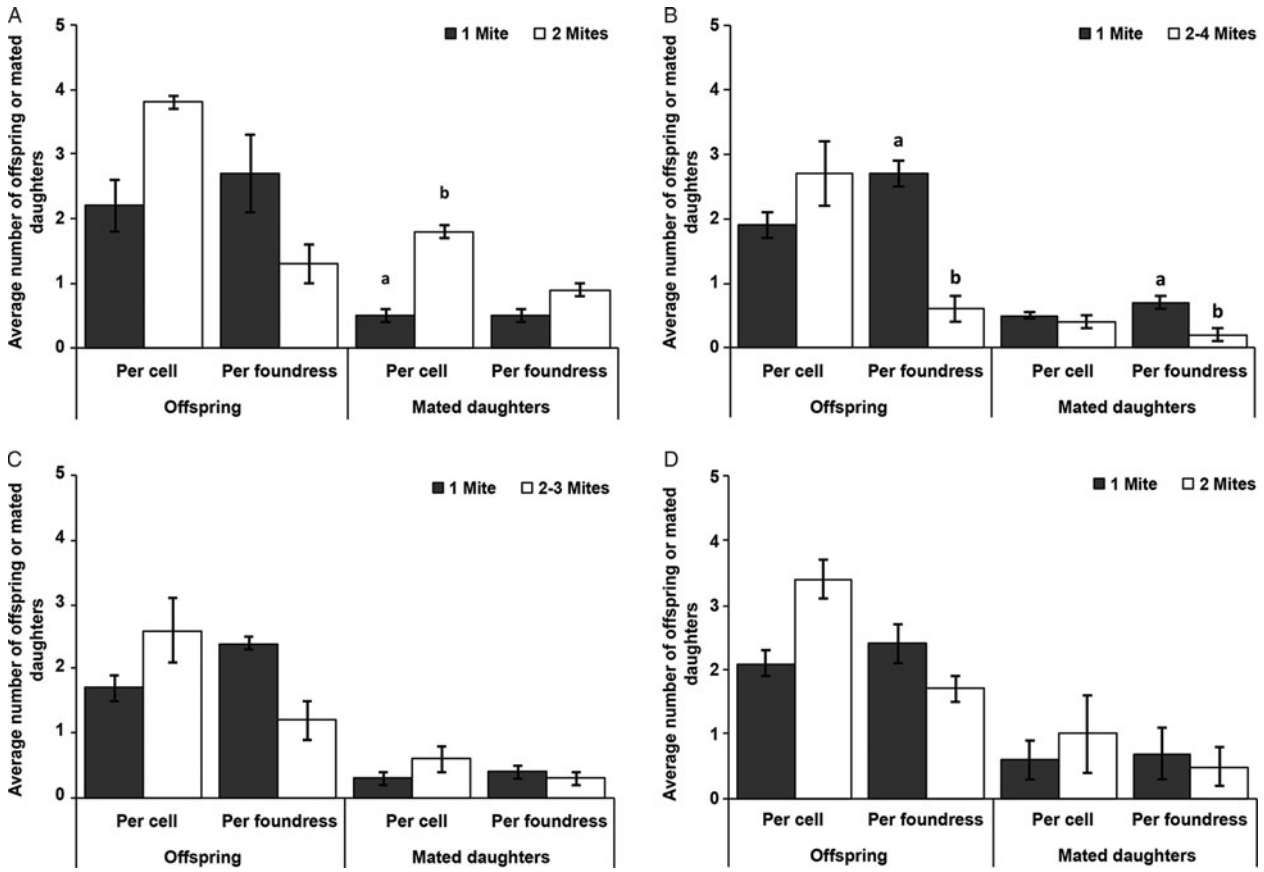

Fig. 1. The average number of offspring and mated daughters (mean ± S.E.) produced per cell and per foundress in singly and multiply infested worker brood cells in *A. m. scutellata* during the hot dry seasons (January 2016 and February 2018) at the apiary in Kithimani (A) and (B) respectively, short rainy season (November 2015) at the apiary in Kithimani (C) and short rainy season (November 2015) at the apiary in Kilimanbogo (D). Only fertile foundresses were considered. Pair of bars with letters indicates significant effects for each category.

interact with one another during different seasons to reduce the number of viable female offspring produced in worker brood cells of the African savannah honeybee. The low mite fecundity recorded in this study was similar to those reported in worker brood cells of the surviving *A. m. scutellata* population in South Africa (1.7 ± 0.3, mean ± S.D) (Strauss *et al.* 2016); though it is much lower than those reported in other surviving or susceptible honeybee populations (3.1–4.9, mean number of eggs laid) (Medina and Martin, 1999; Martin, 2001; Alattal *et al.* 2006; Locke and Fries, 2011; Calderón *et al.* 2012; Locke *et al.* 2012; Brettell and Martin, 2017). Also, an increase in the percentage of infertile mites over time (from 13 to 30%) has been reported as a parameter that suppresses the mite reproduction in worker brood cells of the surviving *A. m. scutellata* population in South Africa (Martin and Kryger, 2002; Strauss *et al.* 2016). Furthermore, we identified offspring mortality for both sexes and absence (missing) of male offspring as key factors that appear to be responsible for the high number of unmated daughters produced in the African savannah honeybee colonies (23–52%). Mite offspring mortality has also been reported as a major factor that accounts for the lower mite reproductive output and population growth in the surviving Africanized honeybee colonies in Brazil; despite the fact that the fertility of the mites is currently reported to be at the same level as in European honeybee colonies (Mondragón *et al.* 2006; Calderón *et al.* 2010; Calderón *et al.* 2012). Offspring mortality or absence (missing) within the worker brood cells has been reported to be due to failure to locate the single feeding site established by the foundress mite on the developing honeybee brood and the disturbance or damage of the first egg which is usually male when the pre-pupae molts into pupae, respectively (Donzé and Guerin, 1994; Donze *et al.* 1996; Calderón *et al.* 2010; Calderón *et al.* 2012).

Mite reproduction in multiply infested cells

The reproduction of mites in multiply infested cells can also influence their reproductive success and population growth in honeybee colonies (Rosenkranz *et al.* 2010). In this study, we observed that the number of offspring produced per individual mite in multiply infested cells was generally lower than those produced in singly infested cells in *A. m. scutellata* colonies though the difference was only significant during the hot dry season (February 2018) (Fig. 1). Additionally, there was a general reduction in the number of female offspring produced per foundress in multiply than singly infested cells in colonies of this honeybee subspecies though the difference was only significant during the hot dry season (February 2018) (Fig. 1). However, the number of female offspring produced per cell was generally higher in multiply than singly infested cells in the savannah honeybee colonies though the difference was only significant during the hot dry season (January 2016) (Fig. 1). In multiply infested cells where competition for food resources is expected, the fecundity and reproductive success of individual mites is generally reduced compared with those of singly infested cells (Fuchs and Langenbach, 1989; Martin, 1995; Martin and Medina, 2004; Mondragón *et al.* 2006). The higher reproductive success of the mites recorded in multiply infested cells in this study might be due to the lower incidence of offspring mortality and absence recorded in multiply infested cells than those of singly infested cells (Strauss *et al.* 2016). Moreover, daughter mites have a greater

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chance to mate successfully before emerging from multiply infested cells because more than one adult male can be produced (Martin, 1995). In this study, however, only a single male offspring was produced in all multiply infested cells of *A. scutellata*. Therefore, the probability that all the daughter mites produced in these cells will receive sufficient sperms before emerging from the cell is questionable. Hence, though the reproductive success of mites remains high in these cells, there could be a chance that not all the daughter mites will receive sufficient sperm from the male before emerging from the cell (Donze *et al.* 1996; Wendling *et al.* 2014). Our findings corroborate results of a previous study which also reported a significant reduction in the number of offspring produced per individual mite in multiply infested worker cells compared to singly infested ones; though the number of mated daughters produced per cell was higher in multiply infested cells compared to singly infested cells in *A. m. scutellata* colonies in South Africa (Strauss *et al.* 2016).

In conclusion, the *A. m. scutellata* population studied herein showed evidence of resistance towards mite attack. This translates into the strong suppression of the mite reproductive success recorded in worker brood cells. This lower reproductive output was mainly due to the high mite infertility rates and percentage of unmated daughter mites as well as low mite fecundity recorded in infested cells of *A. m. scutellata*. The mortality of adult male and female offspring and the absence (missing) of male offspring in a considerable number of worker brood cells were identified as major factors responsible for the lower production of mated daughters in the savannah honeybee colonies. The consistency of results regarding mite reproduction in two geographically distinct *A. m. scutellata* populations (South Africa, Strauss *et al.* 2016 and Kenya, this study) suggests general adaptations towards *V. destructor* within African honeybees, most likely due to the higher number of wild colonies and lack of miticide use in their colonies (Pirk *et al.* 2017). Nonetheless, because the number of multiply infested cells recorded in this study was low, we recommend that the data should be treated with caution. We recommend further verification of the reproductive values of the mites obtained herein in other *A. m. scutellata* populations distributed in other climatic zones in Africa to help shed more light on the evolution of tolerance and resistance mechanisms towards *Varroa* mites.

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Conflicts of interest. None

Ethical standards. Not applicable

References

Alattal Y, Rosenkranz P and Zebitz CPW (2006) Reproduction of *Varroa destructor* in sealed worker bee brood cells of *Apis mellifera carnica* and *Apis mellifera syriaca* in Jordan. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* **15**, 315–319.

Annoscia D, *et al.* (2015) Mite infestation during development alters the in-hive behaviour of adult honeybees. *Apidologie* **46**, 306–314.

Ardestani MM (2015) Investigating the influence of postcapping period on *Varroa* mite infestation. *Journal of Apicultural Research* **54**, 335–341.

Aronstein KA, *et al.* (2012) How *Varroa* parasitism affects the immunological and nutritional status of the honeybee, *Apis mellifera*. *Insects* **3**, 601–615.

Boecking O and Spivak M (1999) Behavioral defenses of honeybees against *Varroa Jacobsoni* Oud. *Apidologie* **30**, 141–158.

Brettell LE and Martin SJ (2017) Oldest *Varroa* tolerant honeybee population provides insight into the origins of the global decline of honeybees. *Scientific Reports* **7**, 1–7.

Calderón RA, *et al.* (2010) Reproductive biology of *Varroa destructor* in Africanized honeybees (*Apis mellifera*). *Experimental and Applied Acarology* **50**, 281–297.

Calderón RA, Urena S and van Veen JW (2012) Reproduction of *Varroa destructor* and offspring mortality in worker and drone brood cells of Africanized honeybees. *Experimental and Applied Acarology* **56**, 297–307.

de Guzman LI, Rinderer TE and Frake AM (2008) Comparative reproduction of *Varroa destructor* in different types of Russian and Italian honeybee combs. *Experimental and Applied Acarology* **44**, 227–238.

Donzé G and Guerin PM (1994) Behavioral attributes and parental care of *Varroa* mites parasitizing honeybee brood. *Behavioral Ecology and Sociobiology* **34**, 305–319.

Donze G, *et al.* (1996) Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni*. *Ecological Entomology* **21**, 17–26.

Francis RM, Nielsen SL and Kryger P (2013) *Varroa*-Virus interaction in collapsing honeybee colonies. *PLoS ONE* **8**, e57540.

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

Fries I, *et al.* (1996) Grooming behavior and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. *Apidologie* **27**, 3–11.

Fuchs S and Langenbach K (1989) Multiple infestation of *Apis mellifera* L. brood cells and reproduction in *Varroa jacobsoni* Oud. *Apidologie* **20**, 257–266.

Harbo JR and Harris JW (1999) Selecting honeybees for resistance to *Varroa jacobsoni*. *Apidologie* **30**, 183–196.

Harris JW, *et al.* (2003) Variable population growth of *Varroa destructor* (Mesostigmate: Varroidae) in colonies of honeybees (Hymenoptera) during a 10-year period. *Environmental Entomology* **32**, 1305–1312.

Hepburn HR and Radloff SE (1988) *Honeybees of Africa*. Berlin, Heidelberg, New York: Springer Verlag.

Ifantidis MD (1983) Ontogenesis of the mite *Varroa jacobsoni* in worker and drone honeybee brood cells. *Journal of Apicultural Research* **22**, 200–206.

Kielmanowicz MG, *et al.* (2015) Prospective large-scale field study generates predictive model identifying major contributors to colony losses. *PLoS Pathogens* **11**, e1004816.

Lee KV, *et al.* (2010) Practical sampling plans for *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies and apiaries. *Journal of Economic Entomology* **103**, 1039–1050.

Locke B and Fries I (2011) Characteristics of honeybee colonies (*Apis mellifera*) in Sweden surviving *Varroa destructor* infestation. *Apidologie* **42**, 533–542.

Locke B, *et al.* (2012) Host adaptations reduce the reproductive success of *Varroa destructor* in two distinct European honeybee populations. *Ecology and Evolution* **2**, 1144–1150.

Lodesani M, Crailsheim K and Moritz RFA (2002) Effect of some characters on the population growth of mite *Varroa jacobsoni* in *Apis mellifera* L. colonies and results of a bi-directional selection. *Journal of Applied Entomology* **126**, 130–137.

Martin SJ (1994) Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. *Experimental and Applied Acarology* **18**, 87–100.

Martin SJ (1995) Reproduction of *Varroa jacobsoni* in cells of *Apis mellifera* containing one or more mother mites and the distribution of these cells. *Journal of Apicultural Research* **34**, 187–196.

Martin SJ (2001) *Varroa destructor* reproduction during the winter in *Apis mellifera* colonies in UK. *Experimental and Applied Acarology* **25**, 321–325.

Martin SJ and Kryger P (2002) Reproduction of *Varroa destructor* in South African honeybees: does cell space influence *Varroa* male survivorship? *Apidologie* **33**, 51–61.

Martin SJ and Medina LM (2004) Africanized honeybees have unique tolerance to *Varroa* mites. *Trends in Parasitology* **20**, 112–114.

https://doi.org/10.1017/S0031182018000616 Published online by Cambridge University Press

Medina LM and Martin SJ (1999) A comparative study of *Varroa jacobsoni* reproduction in worker cells of honeybees (*Apis mellifera*) in England and Africanized bees in Yucatan, Mexico. *Experimental and Applied Acarology* **23**, 659–667.

Mondragón L, Martin S and Vandame R (2006) Mortality of mite offspring: a major component of *Varroa destructor* resistance in a population of Africanized bees. *Apidologie* **37**, 67–74.

Muli E, *et al.* (2014) Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honeybee (*Apis mellifera*) populations in East Africa. *PLoS ONE* **9**, e94459.

Nazzi F and Le Conte Y (2016) Ecology of *Varroa destructor*, the major ectoparasite of the Western honeybee, *Apis mellifera*. *Annual Review of Entomology* **61**, 417–432.

Neumann P and Carreck NL (2010) Honeybee colony losses. *Journal of Apicultural Research* **49**, 1–6.

Nganso BT, *et al.* (2017) Hygienic and grooming behaviors in African and European honeybees – New damage categories in *Varroa destructor*. *PLoS ONE* **12**, e0179329.

Oddie MAY, Dahle B and Neumann P (2017) Norwegian honeybees surviving *Varroa destructor* mite infestations by means of natural selection. *PeerJ Preprints* **5**, e3956.

Peng YS, *et al.* (1987) The resistance mechanism of the Asian honeybee, *Apis cerana* fabr., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. *Journal of Invertebrate Pathology* **49**, 54–60.

Pirk CW, Crewe RM and Moritz RF (2017) Risks and benefits of the biological interface between managed and wild bee pollinators. *Functional Ecology* **31**, 47–55.

Pirk CWW, *et al.* (2013) Statistical guidelines for *Apis mellifera* research. *Journal of Apicultural Research* **52**, 1–24.

Raina SK and Kimbu DM (2005) Variations in races of the honeybee *Apis Mellifera* (Hymenoptera: Apidae) in Kenya. *International Journal of Tropical Insect Science* **25**, 281–291.

Ramsey SD and VanEngelsdorp D (2017) Varroa destructor Feed Primarily on Honeybee fat Body not Haemolymph. In Simone-Finstrom M (ed). Proceedings of the American Bee Research Conference; Paper presented at the 2017 American Bee Research Conference (ABRC). Galveston TX: Galveston Island Convention Center, Bee World.

Rath W (1999) Co-adaptation of *Apis cerana* Fabr. and *Varroa jacobsoni* Oud. *Apidologie* **30**, 97–110.

R Development Core Team (2015) *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ritter W (1981) *Varroa* disease of the honeybee *Apis mellifera*. *Bee World* **62**, 141–153.

Rosenkranz P, Aumeier P and Ziegelmann B (2010) Biology and control of *Varroa destructor*. *Journal of Invertebrate Pathology* **103**, S96–S119.

Smith KM, *et al.* (2014) Pathogens, pests, and economics: drivers of honeybee colony declines and losses. *EcoHealth* **10**, 434–445.

Strauss U, *et al.* (2013) Seasonal prevalence of pathogens and parasites in the Savannah honeybee (*Apis mellifera scutellata*). *Journal of Invertebrate Pathology* **114**, 45–52.

Strauss U, *et al.* (2016) Resistance rather than tolerance explains survival of Savannah honeybees (*Apis mellifera scutellata*) to infestation by the parasitic mite *Varroa destructor*. *Parasitology* **143**, 374–387.

VanDooremalen C, *et al.* (2012) Winter survival of individual honeybees and honeybee colonies depends on level of *Varroa destructor* infestation. *PLoS ONE* **7**, e36285.

Wendling S, *et al.* (2014) Fertilization and fertility in the female of *Varroa destructor*, a key point for the parasite population dynamics. *Apidologie* **45**, 722–732.

https://doi.org/10.1017/S0031182018000616 Published online by Cambridge University Press