Asynchronous Development of Honey Bee Host and *Varroa destructor* (Mesostigmata: Varroidae) Influences Reproductive Potential of Mites

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ABSTRACT A high proportion of nonreproductive (NR) *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae), is commonly observed in honey bee colonies displaying the varroa sensitive hygienic trait (VSH). This study was conducted to determine the influence of brood removal and subsequent host reinvasion of varroa mites on mite reproduction. We collected foundress mites from stages of brood (newly sealed larvae, prepupae, white-eyed pupae, and pink-eyed pupae) and phoretic mites from adult bees. We then inoculated these mites into cells containing newly sealed larvae. Successful reproduction (foundress laid both a mature male and female) was low (13%) but most common in mites coming from sealed larvae. Unsuccessful reproductive attempts (foundress failed to produce both a mature male and female) were most common in mites from sealed larvae (22%) and prepupae (61%). Lack of any progeny was most common for mites from white-eyed (83%) and pink-eyed pupae (92%). We also collected foundress mites from sealed larvae and transferred them to cells containing newly sealed larvae, prepupae, white-eyed pupae, or pink-eyed pupae. Successful reproduction only occurred in the transfers to sealed larvae (26%). Unsuccessful reproductive attempts were most common in transfers to newly sealed larvae (40%) and to prepupae (25%). Unsuccessful attempts involved the production of immature progeny (60%), the production of only mature daughters (26%) or the production of only a mature male (14%). Generally, lack of progeny was not associated with mites having a lack of stored sperm. Our results suggest that mites exposed to the removal of prepupae or older brood due to hygiene are unlikely to produce viable mites if they invade new hosts soon after brood removal. Asynchrony between the reproductive status of reinvading mites and the developmental stage of their reinvasion hosts may be a primary cause of NR mites in hygienic colonies. Even if reinvading mites use hosts having the proper age for infestation, only a minority of them will reproduce.

KEY WORDS asynchronous development, *Varroa destructor*, mating failure, resistance, nonreproduction

The parasitic mite *Varroa destructor* Anderson & Trueman (Mesostigmata: Varroidae) is regarded as one of the greatest threats facing the honey bee, *Apis mellifera* L., worldwide (Le Conte et al. 2010). Its rapid spread has resulted in the loss of almost all wild or feral honey bee populations in its invaded range (Oldroyd 2007). In addition, treatment of managed colonies has added to the overall cost of beekeeping, enabled the development of strains of mites resistant to treatment (Thompson et al. 2002) and could lead to negative synergistic effects of in-hive miticides on the bees themselves (Johnson et al. 2009). As a result, the breeding of bees that have greater resistance to the mite has become an area of significant research. Several characteristics of both the honey bee, and the varroa mite have been found to interact to reduce mite populations and enable coexistence without chemical treatment (reviewed in Rinderer et al. 2010).

The reproductive cycle of *V. destructor* is closely synchronized with that of its honey bee host. Timing of infestation is essential to maximize both varroa mite's fecundity and the viability of progeny. The sex ratio of the mite is spanandrous with many more females being produced than males (Martin et al. 1997), and only the adult females survive outside of the brood cells. Varroa mites are reported to be monandrous, that is, females only mate with one male but multiple mating is required to fill the spermatheca (Donzé et al. 2006). These matings only occur within the natal cell before host emergence. Offspring that do not mate in the natal cell are thought to be infertile and thus fail to contribute to overall mite population growth (Harbo and Harris 2005). In all infested honey bee colonies, a certain

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proportion of mites fail to reproduce (Harbo and Harris 1999a). This situation can be partly related to environmental factors (Martin et al. 1997). de Guzman et al. (2008) showed that combs built by Russian honey bees contributed to an increased rate of nonreproductives (NRs), which may be enhanced by semiochemicals present in the cocoons (Donzé and Guerin 1994). However, in colonies exhibiting resistance to the varroa mite, the proportion of NR mites is significantly higher than in susceptible stock (Rosenkranz and Engels 1994, de Guzman et al. 2007). In field trials carried out in the United States, the number of NR mites was found to have a strong negative correlation with the final mite population of test colonies (Harbo and Harris 1999a). This higher proportion of NR mites also was found to have a genetic basis in resistant colonies and termed suppression of mite reproduction ([SMR]; Harbo and Harris 1999b). Mites from colonies with this trait will enter a cell in the process of reproduction but the mites either 1) die in the cell without reproducing, 2) produce no progeny, 3) produce a male only, or 4) produce progeny that fails to reach maturity (Harbo and Harris 1999a). SMR became the basis for a breeding program to develop a line of bees in the United States. with resistance to the mite (Harbo and Harris 1999b). In their study, Ibrahim and Spivak (2006) found that bees bred solely for the SMR trait were also highly hygienic and, in fact, removed more infested brood than the Minnesota hygienic line. It was therefore hypothesized that bees preferentially removed brood cells that were infested with reproductive mites (Harbo and Harris 2005). The trait was later renamed varroa sensitive hygiene ([VSH]; Harris 2007).

Another possible cause of NR is the interruption or disturbance in the mite's reproductive cycle resulting from honey bee's hygienic behavior (Harris et al. 2010). Hygienic behavior involves three steps: detection of infested cells, uncapping; and, the subsequent removal of infested pupae. Recently, bees with the VSH trait were found to remove brood that also are infested with NR varroa mites (Harris et al. 2010). These bees are reported to preferentially uncap cells between 1 and 5 d postcapping (Harris 2007). This period incorporates the prepupae to pink-eyed pupal stages of brood development. Under this scenario, the foundress should have begun her normal reproductive cycle in the host cell before being removed or freed. The exposed varroa is therefore forced to either enter a new cell where they may or may not continue to reproduce or to become phoretic. In our previous trials wherein marked mites were inoculated into brood, some of the unrecovered mites were later found in other brood cells. It could be assumed that having begun reproducing, the female must resume reproduction upon invading another host, as oogenesis has already been activated (Rosenkranz and Garrido 2004). These experiments were designed to evaluate the reproductive success of these potentially reinvading mites.

Materials and Methods

These experiments were conducted at the USDA–ARS, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, LA, in July 2010.

Experiment 1. Reproduction of Foundress Varroa Mites That Have Been Removed From Different Stages of Honey Bee Development and Inoculated Into Newly Sealed Larvae. Varroa mites used in this investigation were obtained from four stages of honey bee brood development, based on the brood stages (prepupae to pink-eyed pupal stages) that are most likely to be targeted by hygienic bees (Harris et al. 2010). The brood stages considered were newly sealed larvae, prepupae, and white-eyed and pink-eyed pupae. Adult female varroa mites were collected by opening brood cells with a pair of forceps, removing the developing bee from the cell and then removing the mites from the bee or inside the cell wall with an insect brush. Phoretic mites also were collected from adult bees using the sugar-shake technique (Macedo et al. 2002). Mites collected using this technique were allowed to walk on damped filter paper and gently brushed off with an insect brush to clean the mites. Approximately 400 mites (80 mites representing each stage) were collected from two highly infested colonies of *Apis mellifera ligustica* Spinola. These mites were then inoculated into cells of newly sealed larvae using a variation of the well-established transfer technique (Garrido and Rosenkranz 2003). In brief, a small opening on the capping was made at the edge of a capped brood using a minute pin and then a mite was introduced using the tip of an insect brush. The capping was pressed back to close the opening using the insect brush. We used this technique to duplicate what may occur with mites when bees perform hygienic activities. Mites from different stages of brood that are exposed due to hygienic activities may escape grooming by worker bees, and reinvade newly sealed larvae that are uncapped by bees.

A Russian honey bee colony (brood infestation, 2.3%; seven of 300 cells and four cells with reproductive foundresses) was chosen as the host colony to prevent the transfer of mites to cells that were already infested. The colony never received any chemical treatment. Two frames of newly sealed larvae were used for this study. By the time mite inoculation was completed, the age of the test brood was estimated to be $\leq$24 h postcapping (ninth day after egg-laying). For each frame, a test section of brood consisting of $\approx$20 rows with 20–25 brood cells per row was isolated by removing brood that surrounded the section. The section of brood was digitally photographed and numbers were assigned to all cells within each row. Earlier trials indicated that there was no increased brood removal due to this technique. For example, one trial showed five out of 37 unmanipulated cells, two of 30 opened and closed cells without mite inoculation, and nine of 36 mite-inoculated cells were removed by worker bees. Hence, only the inoculated and noninoculated groups were assessed in this study. Mites (n = 40 mites per source per frame) were introduced

Table 1. Reproduction parameters of foundress varroa mites that have been removed from different stages of honey bee development and inoculated into newly sealed larvae

Trial	Source of mites	Total inoculated	Total recovered	Reproductive foundresses	Avg. progeny per foundress	Nonreproductive foundresses	Foundress, immature progeny only	Foundress, mature daughter, no male	Foundress, mature male, no daughter
	Phoretic	40	14	0 (0)	0	100 (14)	0 (0)	0 (0)	0 (0)
1	Larvae	40	23	4.3 (1)	3.25	95.6 (22)	8.7 (2)	17.4 (4)	4.3 (1)
	Pre-pupae	40	23	0 (0)	2.4	100 (23)	30.4 (7)	13 (3)	0 (0)
	White-eyed	40	17	0 (0)	1.8	100 (17)	23.5 (4)	0 (0)	5.9 (1)
	Pink-eyed	40	22	0 (0)	2	100 (22)	9.1 (2)	0 (0)	0 (0)
	Phoretic	40	23	8.7 (2)	3.25	91.3 (21)	4.3 (1)	0 (0)	4.3 (1)
2	Larvae	40	22	2.3 (5)	3.63	77.3 (17)	4.5 (1)	9.1 (2)	0 (0)
	Pre-pupae	40	18	0 (0)	3.1	100 (18)	33.3 (6)	50 (9)	0 (0)
	White-eyed	40	23	4.3 (1)	4	95.6 (22)	0 (0)	4.3 (1)	0 (0)
	Pink-eyed	40	25	4 (1)	3	96 (24)	4 (1)	0 (0)	0 (0)
	Phoretic	80	37	5.4 (2)	1.625	94.6 (35)	2.7 (1)	0 (0)	2.7 (1)
Combined	Larvae	80	45	13.3 (6)	3.44	86.7 (39)	6.7 (3)	13.3 (6)	2.2 (1)
	Pre-pupae	80	41	0 (0)	2.75	100 (41)	31.7 (13)	29.3 (12)	0 (0)
	White-eyed	80	40	2.5 (1)	2.9	97.5 (39)	10 (4)	2.5 (1)	2.5 (1)
	Pink-eyed	80	47	2.1 (1)	2.5	97.9 (46)	6.4 (3)	0 (0)	0 (0)

Foundress data are presented as percentage (number).

randomly among the test brood cells, whereas some of the cells were not inoculated with mites (trial 1, 23 cells; trial 2, 82 cells). The location of the brood cells and the treatments they received were recorded. A print of the photo was also used as a map of brood cells with their randomly assigned treatments. According to Ibrahim and Spivak (2006), varroa mites inoculated into brood and kept in an incubator had a lower reproduction rate than those kept in the hive. Hence, test frames were returned and remained in the colony for the duration of the experiment.

Cells were examined at the tan-bodied stage, at least 9 d postcapping, and reproductive output of the foundress mite was determined. We considered a varroa mite to be reproductive when an infested cell consisted of a mature male and at least one mature daughter, similar to the distinction used by Correa-Marques et al. (2003). If more than one mature (darker due to degree of sclerotization) female mite was found in a cell, the exuvia was located to confirm that one was a daughter mite. In cases wherein a foundress produced immature progeny (eggs only, eggs and protonymphs, or eggs to early deutonymphal stages) only, the sex of eggs and protonymphs was not determined.

Experiment 2. Reproduction of Foundress Varroa Mites That Have Been Removed From Newly Sealed Larvae and Inoculated Into Different Capped Brood Stages of Honey Bee Development. Foundress varroa mites (n = 320) were collected from newly sealed larvae in each of the two source colonies used in experiment 1. Forty of these mites were inoculated into each of the following brood stages: sealed larvae, prepupae, and white-eyed and pink-eyed pupae. This condition imitated what may occur if exposed varroa mites invade different stages of brood that have been opened by hygienic bees and then recapped by the bees. All other procedures were carried out in the same way as experiment 1. There were 56 and 94 cells that were not inoculated with mites for trial 1 and trial 2, respectively. The same host colony was used in both experiments.

Spermatozoa Count of NR Mites That Did Not Produce Progeny. While examining test brood at the end of the experiments, we noticed that a high proportion of the inoculated mites, from one colony in particular, were NR. Because the presence of spermatozoa in the spermathecae serves as an important tool in determining mating success, a subset of the NR mites was dissected to confirm the presence or absence of mature spermatozoa. NR mites were grouped as 1) phoretic mites that were collected from adult bees and did not reproduce when transferred to cells; 2) brood mites that were collected from either newly sealed larvae, prepupae, white-eyed pupae, or pink-eyed pupae and did not reproduce when transferred; and 3) brood mites that had laid one or more eggs in their original cells but did not reproduce when transferred.

Mites were dissected using the method of Akimov and Yastrebtsov (1984) and Steiner et al. (1994). In brief, the ventral side of each mite was glued onto a glass slide and allowed to dry. Thereafter, a drop of mite saline solution was placed over the mite, and the dorsal shield was removed to expose reproductive organs.

Data Analysis. The data were analyzed with chi-square tests on multiple contingency tables. The results are presented in Tables 1–3.

Results

Experiment 1. Of the 400 mites inoculated into brood cells, 53% were recovered and 47% were removed by bees in the host colony (Table 1). The majority of the mites (95%) that were recovered were NR (no progeny, immature progeny, male progeny only, or a mature daughter but no male). Only one mite collected from colony 774 reproduced (at least one mature daughter and a son), whereas nine mites from colony 924 reproduced. Overall, mites collected

Table 2. Reproduction parameters of foundress varroa mites taken from newly sealed larvae and inoculated into different brood stages

Trial	Mite receiver	Total inoculated	Total recovered	Reproductive foundresses	Avg. progeny per foundress	Nonreproductive foundress	Foundress, immature progeny only	Foundress, mature daughter, no male	Foundres, mature male, no daughter
1	Larvae	40	28	10.7 (3)	2.58	89.3 (25)	21.4 (6)	14.3 (4)	10.7 (3)
	Prepupae	40	29	0 (0)	2.85	75.9 (22)	17.2 (5)	3.4 (1)	3.4 (1)
	White-eyed	40	31	0 (0)	2	100 (31)	3.2 (1)	0 (0)	0 (0)
	Pink-eyed	40	24	0 (0)	1	100 (24)	0 (0)	0 (0)	4.2 (1)
2	Larvae	40	22	45.5 (10)	3.76	54.5 (12)	9.1 (2)	18.2 (4)	4.5 (1)
	Prepupae	40	26	0 (0)	1.7	100 (26)	26.9 (5)	7.7 (2)	0 (0)
	White-eyed	40	25	0 (0)	3	100 (25)	19.2 (1)	0 (0)	0 (0)
	Pink-eyed	40	29	0 (0)	1	100 (29)	20.7 (6)	0 (0)	0 (0)
Combined	Larvae	80	50	26 (13)	3.17	74 (37)	16 (8)	16 (8)	8 (4)
	Prepupae	80	55	0 (0)	2.275	100 (55)	18.2 (10)	5.5 (3)	1.8 (1)
	White-eyed	80	56	0 (0)	2.5	100 (56)	3.6 (2)	0 (0)	0 (0)
	Pink-eyed	80	53	0 (0)	1	100 (53)	11.3 (6)	0 (0)	1.9 (1)

Foundress data are presented as percentage (number).

from phoretic, pink-eyed pupae, and white-eyed pupae and inoculated into sealed larvae yielded the highest proportions of mites that did not produce any progeny ($\chi^2 = 12.3$, df = 4, $P = 0.05$). Most (61%) of the mites in the prepupae to sealed larvae inoculation group produced progeny ($\chi^2 = 29.89$, df = 4, $P = 0.001$), but only in NR categories. This group also had the highest proportion of foundresses that produced mature daughters but no mature males ($\chi^2 = 28.52$, df = 4, $P = 0.001$). The highest proportion of reproductive mites resulted from sealed larvae to sealed larvae inoculation ($\chi^2 = 11$, df = 4, $P < 0.05$), and this also produced the highest average number of progeny per foundress, but this result was not significant. Inoculating phoretic mites into sealed larvae supported the lowest average number of progeny per foundress. Only six foundresses produced mature males but no mature daughters across all inoculation groups.

Approximately 15% (16/105) of the noninoculated brood were removed by the worker bees. Two of the 89 (2%) noninoculated brood that were not removed were infested; one was reproductive. No recapped cell was observed.

Experiment 2. A similar trend of reproductive success was observed when varroa mites collected from sealed larvae were inoculated into different stages of capped brood. Of 320 inoculated mites, 214 (67%) were recovered and 106 (33%) were removed by bees in the host colony (Table 2). Mites originating from mite source 774 showed lower rates of reproduction than source 924. Only the sealed larvae to sealed larvae inoculation group resulted in successful reproduction ($\chi^2 = 39$, df = 3, $P = 0.001$). All foundress mites in the sealed larvae to prepupae, white-eyed and pink-eyed pupae inoculation groups were NR, most of which did not produce any progeny ($\chi^2 = 19.75$, df = 3, $P = 0.001$). The average number of progeny per reproductive foundress was also highest in the sealed larvae to sealed larvae inoculation group with the least progeny recorded from the sealed larvae to pink-eyed inoculation group.

Of the 150 noninoculated brood, 27 (18%) were removed by the bees. Only one of the 123 (0.8%) cells in which brood was not removed was infested with a reproductive mite.

Number of Spermatozoa in the Spermatheca of NR Mites That Produced No Progeny. At the end of the experiment, it became apparent that most of the recovered mites were NR. Thus, dissections of spermathecae were conducted to determine the presence or absence of mature spermatozoa. In total, 116 NR mites (no progeny) were dissected (Table 3). There was a noticeable difference between the two mite sources in the number of spermatozoa. Despite the failure of the mites to lay eggs after transfer, the majority of the mites (68%) collected from source colony 924 had ≈20 or more mature spermatozoa in their spermathecae. Approximately 8% had one to 10

Table 3. Percentage of varroa mites having mature spermatozoa in three groups of nonreproductive mites

Colony	Mite source	Total mites dissected	No. of sperm				
			0	1–5	≈10	≈20	≥25
774	Phoretic (NR)	9	66.66 (6)	0	0	22.22 (2)	11.11 (1)
	Brood (NR)	23	47.82 (11)	13.04 (3)	13.04 (3)	0	26.09 (6)
	Brood (egg)	9	33.33 (3)	22.22 (2)	0	33.33 (3)	11.11 (1)
924	Phoretic (NR)	14	21.43 (3)	7.14 (1)	0	14.29 (2)	57.14 (8)
	Brood (NR)	47	25.53 (12)	2.13 (1)	6.38 (3)	23.4 (11)	42.55 (20)
	Brood (egg)	14	21.43 (3)	0	7.14 (1)	28.57 (4)	42.86 (6)

Phoretic (NR) refered to mites collected from adult bees and did not produce any progeny after transfer. Brood (NR) was a group of mites collected from either newly sealed larvae, prepupae, white-eyed pupae, or pink-eyed pupae and did not produce any progeny after transfer. Brood (egg) represented mites that produced at least one egg in the original host cell but did not reproduce after transfer. Data are presented as percentage (number) of mites for each source.

spermatozoa, and 24% had no spermatozoa. In contrast, source colony 774 had more mites that did not have spermatozoa (49%), 20% had one to 10, and ≈31% had ≥20 spermatozoa.

Discussion

A high proportion of NR varroa mites were recovered in test colonies. This study reveals both a behavioral and a physiological reason why this may be the case, and in turn how it could be related to the hygienic activities of the colony's adult bee population. Having already begun oviposition in their original cell, varroa mites removed from prepupae, white-eyed, or pink-eyed stages had lower reproductive success upon entering a new host cell. As the foundress had laid her first male egg in the original host cell, she continued her reproductive program and laid only female eggs after the transfer. This finding was supported by a low number of mature male varroa mites in these inoculated cells. Alternatively, even if a foundress had not begun oviposition in the original cell but was forced to enter a stage other than freshly sealed larvae, and in particular white-eyed or pink-eyed pupae, she did not produce offspring. These mites may have had mature spermatozoa in their spermathecae but nevertheless failed to produce any young, perhaps owing to a lack of cues appropriate to egg-laying. Interestingly, a high proportion of phoretic mites inoculated into sealed larvae failed to reproduce. This may have resulted from the phoretic mites not having been phoretic for a period sufficient to prepare for reproduction. When inoculations that involved stages other than freshly sealed larvae did produce young, these were more likely to only produce immature young and therefore be classed as NR. Thus, synchronization with the life cycle of its honey bee host is essential for varroa reproduction. Any disturbance to this synchronization will result in a disruption to the normal reproductive cycle of the mite.

The underlying causes of NR in foundress varroa mites are not well understood. Martin et al. (1997) linked NR of varroa mites to the premature death of male mites within the cell, whereas Harbo and Harris (1999) associated infertility with the complete absence of matured males within cells. Although we found that the majority of the recovered mites were NR (experiment 1, 95%; experiment 2, 94%) as defined by Harbo and Harris (1999), we did not observe dead males in this study. Rather, we observed the absence of males in cells with mature daughters (experiment 1, 9%; experiment 2, 5%). Our results suggest that the lack of synchronization between the reproductive status of the reinvading mite and its reinvasion host also may contribute to the pool of NR mites. This observation may provide the crucial link between the VSH trait and NR. The removal of foundress mites through hygienic activities may interrupt the normal reproductive cycle of varroa mites by causing the male and female offspring to be laid in separate cells, thereby resulting to mating failure.

Synchronization of the bee host and varroa mite development is crucial to maximize fecundity and to ensure mating success. Our results showed that the majority of the mites that were transferred from white-eyed pupae, pink-eyed pupae, and phoretic stages to sealed larvae, and from sealed larvae to prepupae, white-eyed pupae, and pink-eyed pupae were NR. Also, nearly all the mites removed from pupal stages of brood and transferred into sealed larvae did not produce mature males, but foundress mites continued their normal reproductive program and laid females in the new hosts. We found one case of male production in the white-eyed to sealed larvae group, but this may have been a natural resident mite because the host colony had 2% varroa infestation. The 23 noninoculated cells that were not removed were uninfested. Nevertheless, our observation is in disagreement with the findings of Garrido and Rosenkranz (2003) who found very high (55%, 17/31) numbers of mites that laid males using the white-eyed to sealed larvae transfer. This disparity in our findings may be due to a higher natural varroa infestation of their host colony. They did not report the natural infestation of their host colony and relied on the effectiveness of chemicals applied one year before the conduct of their experiment to suppress varroa infestations.

According to Rosenkranz and Garrido (2004), the initiation of oogenesis is regulated by a "cascade" of stimuli, during which larval volatiles are crucial. Also, it has been shown that larval cuticular hydrocarbons do play a role in varroa reproduction (Trouiller and Milani 1999). Indeed, only the sealed larvae to sealed larvae inoculation group supported reproductive success in this study. This group also produced the highest average number of progeny per foundress probably because of the longer postcapping duration for larvae thereby allowing the mites to reproduce and their progeny mature. Although larval feeding is a prerequisite for varroa reproduction, it did not always result in viable females as we found out in this study. The female varroa mite lays her first egg 60 h after the cell is capped, which develops into a male (Rehm and Ritter 1989). Subsequent female eggs are laid at 30 h intervals (Martin 1994) allowing males to emerge before the females, a phenomenon called protandry (Donzé et al. 2006). However, hygienic activities generally peak during these periods (Harris 2007). Therefore, the removal of capped brood during this time may have some negative effects on the reproductive success of the mites. Being protandrous, exposed mites that have laid male eggs in their original cell can no longer produce a viable family even if the mites reinvade a suitable larval host. Males and females being laid in separate cells will result in mating failure because of the lack of opportunity to mate.

Even if a male and a female have reached maturity within a cell, they may not have an opportunity for multiple mating if brood is removed. This may be the cause for the lack of spermatozoa in some of the NR mites we found from colony 774. According to Donzé et al. (2006), several matings are required before a daughter mite's spermathecae are filled and complete

mating with the older daughter is rare. Further study is required to determine the level to which this contributes to the proportion of NR mites in a colony. In addition, exposed mites that reenter older pupae will not reach maturity before bees emerge. Because of this asynchrony in the development of bees and mites, even a successfully reproducing mite will produce fewer progeny, as we discovered, in inoculations involving older brood. This decrease in the number of progeny minimizes the effect of feeding activities of mites on the honey bee host because the mobile nymphs are known to be the active feeders. In colonies that are partially hygienic (detection and uncapping) however, mating success can still be achieved because some mites do not actually leave the cell and continue reproduction and development.

Our results also showed high proportions of NR mites that did not have progeny including those that were collected from the brood. However, ≈69% of the brood mites that were dissected actually had spermatozoa in their spermathecae, the majority of which had high numbers. This failure to reproduce may be related to the availability of space within the cell and the changes in bee morphology during metamorphosis. Placement of the eggs within the host cell is a key factor in the survival of varroa offspring (Donzé and Guerin 1997). Therefore, it is possible that mites entering cells at stages other than larvae would not be prompted to reproduce in the normal manner as the space within the cell may not permit it. Our observation also may explain why NR mites inoculated into newly sealed brood by Weller (2008) reproduced. Similarly, ≈39% (9/23) of the NR phoretic mites we dissected did not have mature spermatozoa. This observation was in disagreement with the findings of Garrido (2004) claiming that nearly all phoretic mites had spermatheca full of spermatozoa. DeRuijter (1987) found that mites may interrupt egg-laying for one or more periods when inoculated into brood cells, and that very young mites may not lay in the first period. Although most of the phoretic mites we used were dark and therefore mature, some were light in color. The high proportion of NR mites that did not have any spermatozoa may suggest that 1) the mites were virgin because of the absence of matured male in the natal cells, 2) the spermatozoa are not mature or still in the process of migration from solenostomes down the rami and into the seminal receptacle (Alberti and Hänel 1986), or 3) the stored spermatozoa have been depleted (Harbo and Harris 1999). We did not examine for the presence of prosperms in this study. Hence, we cannot deduce from our available data whether a sperm count of zero indicates mating failure, an issue of sperm maturation or old age.

Unfortunately, a high proportion of the brood deliberately inoculated with varroa was removed by worker bees. Russian honey bees are known to be hygienic toward freeze-killed brood (de Guzman et al. 2002). However, it is uncertain whether the Russian honey bee colony we used as a host was also hygienic toward varroa-infested brood. For future studies, known nonhygienic bees should be used as host colonies. Caging inoculated brood and keeping them in the hive also may provide more conclusive results. Nevertheless, this study clearly demonstrates the effect of synchronization on the reproductive output of foundress mites and in particular a failure to produce male off-spring in reinvaded cells, which will lead to nonreproduction in the subsequent generation. Therefore, the ability of honey bees to remove varroa-infested brood may enhance the pool of NR mites. These findings suggest that breeding honey bees with hygienic traits will significantly impede mite population growth.

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