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Reproductive Biology of *Varroa jacobsoni* Oud. in Worker and Drone Brood of the Honey Bee *Apis mellifera* L. under Michigan Conditions

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Abstract: Reproductive biology of the mite *Varroa jacobsoni* Oud. was studied under Michigan conditions in *Apis mellifera* L. colonies. A total of 353 sealed worker brood cells containing 697 mother mites and 192 sealed drone brood cells containing 498 mother mites were found in 959 sealed worker brood cells and 344 sealed drone brood cells that were examined. Number of offspring were calculated using two different methods, the first one included adult females that either did not produce offspring, or produced only male and dead offspring. While the second method included mother mites producing only live female offspring. It was found that the mean number of females offspring reaching maturity before the bee emerged in worker and drone cells containing a single mother mite are 1.41 and 2.47 offspring, respectively (First method). When the non-reproducing and male only reproducing females (second method) were excluded the mean number increased to 1.82 for workers and 2.69 for drones. In case of the infestation with more than one adult female, the average number of offspring remained 1.09 for workers and 1.87 for drones (First method). These increased to 1.26 and 2.03, respectively, when non-reproducing and male only reproducing mother mites were excluded (Second method. This study found that 86.75 and 93% of the mites were fertile in worker and drone cells, respectively, when including those mother mites that produced male only offspring. Whereas excluding these offspring, the fertility decreased to 82 and 90% in worker and drone cells, respectively. The results also showed that the percentage of non-reproducing females was 11 and 7% in worker and drone cells, respectively. While the mortality percentage of mother mites was recorded 2.29 and 2.7% in worker and drone brood cells, respectively.

Key words: *Varroa jacobsoni, Apis mellifera,* reproduction, acari, fertility and worker and drone brood

Introduction

The ectoparasitic mite *Varroa jacobsoni* Oud. (Acari: Mesostigmata) is considered as one of the most serious pests of *Apis mellifera* L. colonies in most parts of the world. On its natural host, *Apis cerana* F., the Varroa mite population is generally under the damage threshold because the mite parasitizes primarily drone brood (Koeniger *et al.,* 1981; De Jong, 1988, Tewarson *et al.,* 1992 and Rosenkranz *et al.,* 1993) and an intensive self, nestamate and group cleaning and grooming behavior was observed as a reaction by *Apis cerana* workers to mite infestation has developed methods to protect itself such as a more efficient grooming behavior (Peng *et al.,* 1987; Rath and Drescher, 1990; Boecking, 1992; Rosenkranz *et al.,* 1993 and Thakur *et al.,* 1996).

The mite behaves differently in A *mellifera* than it does in *Apis cerana* colonies. It regularly enters both worker and drone brood, but has a higher fertility and fecundity rate in the drone brood. 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 drone or worker, and the number of invading mother mites per cell. The number of mites entering a brood cell is inversely proportional to the number of offspring produced per female mite.

There is a wide variation in the fertility rate of mites reported among different countries (Ritter and De Jong, 1984; Ifantidis, 1984; Thrybom and Fries, 1991; Camazme, 1986; Sulimanovic *et al,* 1982 and Rezenkran, 1994). In addition, there is also variation in the fecundity rate (Shulz, 1984; Fuchs and Langenbach, 1989; Ifantidis, 1984, 1990;Engels *etal,* 1986 and Martin, 1994, 1995).

Without control, the mite populations can breakdown a colony in Michigan in one to one and a half years. In Europe the breakdown is much slower, occurring within three to four years (Rosenkranz and Engles, 1985).

The objective of this study was to investigate the reproduction, fecundity and fertility of Varroa mite in Michigan, USA.

Materials and Methods

Ten colonies of *Apis mellifera* L. infested with *Varroa jacobsoni* Oud. Were used in this study in East Lansing, Michigan USA. None of the colonies examined had previously been exposed to acaricides.

A total of 959 worker and 344 drone sealed brood cells

were examined on four different occasions from June 30 to October 15, 1995. Two brood combs from each colony were removed on each sampling day and the contents of at least 30 infested sealed brood cells were examined. Some of the colonies died during the study and were replaced. In order to estimate the average fecundity of the mites, both worker and drone sealed brood cells were examined. When possible, only those cells containing adult bees that were near emerging were used. If this was not feasible, pupae with dark eyes and light brown thorax were counted (>230 and 322 h post-capping for workers and drones, respectively). Slightly younger pupae were used for the fertility estimation.

The procedures used for examining the sealed brood cells (worker and drones) and recording the data were according to Ifantidis (1990) and are summarized as follows: worker and drone sealed brood cells were examined in cells that contained emerging adults while they were in the process of emerging, the number of adult Varroa females, as well as the number of skins of the last molt of young females, were counted. In addition, the number of adult male(s) and developing mites were also recorded (to use in case there was a question concerning the number of skins present). The number of original mother mites found in the cell was determined to be the number of female adults present minus the number of skins from female deutochrysalis. By dividing the number of skins by the number of original mother mites one finds the average number of offspring produced per mite in the cell.

Reproduction was calculated using single mite infested the tested brood cells. The presence of *Varroa jacobsoni* eggs, proto- or deutonymphs, confirmed that the female mites were reproducing. The total number of offspring per mother was calculated using two methods. The first one included mother mites that did not produce offspring while the second included only mother mites that produced viable offspring. The following characteristics of reproduction were recorded and analyzed: (1) total number of offspring per producing mother mite., (2) the fraction of mother mites without offspring., (3) the fraction of mother mites with only male offspring and (4) the fraction of mother mites that were dead in the cells.

Results

During the study 959-sealed worker brood cells were examined from 20-brood combs where 353- cells were found infested with 697-mites. The rate of infestation during the respective dates of inspection was recorded 40% in June, 50% in July, 52% in August and 67% in October. Whereas the average reproduction remained 86.8% in worker brood cells when including the mother mites producing male only and 82% when excluding the mother mites producing male only. The average percentage of worker brood cells that contained only male offspring were recorded 5.1 % as presented in Table 1.

The average number of offspring per mother mite in sealed worker brood cells was recorded 1.41 when including the mother mites producing only males and dead mites and excluding those producing only immatures, using single infested cells. Whereas, the average number of offspring per mother mite was increased to 1.82 when excluding the mother mites producing only males, dead mites and immatures, using single infested cells. The average number of offspring reduced to 1.09 when including the mother mites producing only males and dead mites and excluding those producing only immatures, using multiple infested cells. Whereas, the average number of offspring per mother mite was increased to 1.26 when excluding the mother mites producing only males, dead mites and immatures, using multiple infested cells as presented in Table 2.

192-sealed drone brood cells were examined from 11 -brood combs contained 498-mother mites. The rate of infestation during the respective dates of inspection was recorded 45% in June, 71% in August and 90% in September. Whereas the average reproduction remained 93% in drone brood cells when including the mother mites producing male only and 89.9% when excluding the mother mites producing male only. The average percentage of drone brood cells that contained only male offspring were recorded 2.3% as presented in Table 3.

The average number of offspring per mother mite in sealed drone brood cells was recorded 2.47 when including the mother mites producing only males and dead mites and excluding those producing only immatures, using single infested cells. Whereas, the average number of offspring per mother mite was increased to 2.79 when excluding the mother mites producing only males, dead mites and immatures, using single infested cells. The average number of offspring reduced to 1.87 when including the mother mites producing only males and dead mites and excluding those producing only immatures, using multiple infested cells. Whereas, the average number of offspring per mother mite was increased to 2.03 when excluding the mother mites producing only males, dead mites and immatures, using multiple infested cells as presented in Table 4.

Comparing worker and drone fertility and fecundity data, it was found that drone fertility was higher than worker (93 to 86.8%, respectively). There were more brood cells containing only males in worker cells as compared to drone cells (5.1 to 2.3%, respectively). The mite fecundity rate was also higher in drone cells than in worker cells

Table 1: ***Varroa jacobsom*** reproduction ***in Apis*** woiker cells under Michigan conditions

		Reproduction (Males present)		Reproduction (Excluding males)		No Reproduction		Male Only		Dead	
Date	N	n	%	n	%	n	%	n	%	n	%
6/30/95	53	46	86.8	44	83.0	7	13.2	2	3.8	0	0.0
7/22/95	76	67	88.2	63	82.9	7	9.2	4	5.3	2	2.6
9/15/95	75	66	88.0	60	80.0	8	10.7	6	8.0	2	2.5
10/15/95	51	43	84.3	42	82.4	6	11.8	1	2.0	2	3.9
Average			86.8		82.0		11.0		5.1		2.3

Table 2: Number of daughter mites produced per ***Varroa jac•obsom*** mother mites in worker brood under Michigan conditions

	All mother mites (including those producing only males and dead mites, excluding, those producing only immatures)				Mother mites that produce female offspring (excluding those producing; only males, dead mites and immatures)			
	Sinj>le infested cells		Total mite population		Single infested cells		Total mite population	
Date	n	offspring	n	offspring	n	offspring	n	offspring
6/30/95	46	1.46	105	1.04	37	1.81	93	1.11
7/22/95	68	1.47	130	1.16	55	1.82	112	1.31
9/15/95	69	1.41	128	1.22	53	1.83	106	1.38
10/15/95	31	1.29	68	0.93	22	1.81	53	1.25
Average		1.41		1.09		1.82		1.26

Table 3: ***Varroa jacobsom*** reproduction ***mAJpis mellifera*** drone cells under Michigan conditions

		Reproduction (Males present)		Reproduction (Ekcludinig males)		No Reproduction		Male Only		Dead	
Date	N	n	%	n	%	n	%	n	%	n	%
6/30/95	46	43	93.5	41	\59.1	4	8.7	2	4.4	0	0.0
7/22/95	38	35	92.1	34	\59.5	2	5.3	1	2.6	1	2.7
9/15/95	15	14	93.3	14	93.3	1	6.7	0	0.0	0	0.0
Average			93.0		\59.9		7.1		2.3		0.9

Table 4: Number of daughter mites produced per ***Varroa jac•obsom*** mother mites in drone brood under Michigan conditions.

	All mother mites (including those prodiucing only males and dead mites, excluding those prodiucing only immatures)				Mother mites that produce female offspring*1* (excluding those producing only males, dead mites and immatures)			
	Sinj>le infested cells		Total mite population		Single infested cells		Total mite population	
Date	n	offspring	n	offspring	n	offspring	n	offspring
6/30/95	41	2.39	48	2.29	35	2.80	41	2.66
7/22/95	76	2.45	117	1.66	27	2.81	109	1.74
9/15/95	14	2.57	44	1.66	13	2.77	42	1.70
Average		2.47		1.87		2.79		2.03

Table 5: Average number of offspring per mother mite taking number of mother mites per cell into consideration

	Mites per cell				
Tvpe of cell	1	2	3	4	Author
Workers	1.50	1.40	1.20	0.96	Moosbeckhofere/a/., 1988
	1.50	1.58	1.13	0.83	Blum, 1989
	1.40	1.09	1.16	0.91	Fuchs and Langenbach, 1989
	1.60	1.50	1.70		Martin, 1994
	1.41	1.30	1.00	0.62	This study
Drones	2.21	1.90	1.52	1.51	Fuchs and Langenbach, 1989
	2.10	1.90	1.60	1.40	Martin, 1995
	2.47	2.30	1.99	1.67	This study
A. cerana drones	2.30	1.70	1.20		Rath, 1991

(2.03 to 1.26 average number of offspring when the whole mite mother mite population was included, respectively). The rate of decrease in mite fecundity is illustrated in Fig. 1 for both worker and drone cells. The graph was generated from the offspring data that excluded male only, dead mites and immatures).

The logarithmic equations are as follows:

$Y = -1.423*\log(x) + 2.523$ for mites infesting worker cells

$Y = -1.196*\log(x) + 1.486$ for mites infesting drone cells

where:

Y = expected offspring

x = number of mother mites in brood cell

The worker and drone models had r^2 values of 0.751 and 0.836, respectively. The data generating the model

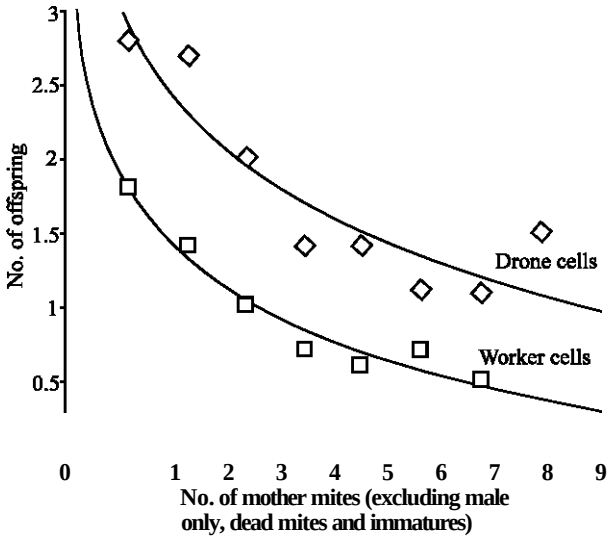

Fig. 1: Average number of adult females produced by mother mites

demonstrated the negative correlation between number of infesting mothers and average offspring per mother. On average, worker cells with one mother mite produce 1.41 offspring and cells with four mothers produce 0.62 offspring in worker cells, when including male only and dead mothers (Table 5). If excluding these mother mites, the fecundity rate in worker cells would be higher: 1.82 for one-mite infestations, 1.4 for two mites, 1.1 for three mites and 0.68 for four mites. In drone cells, the increased fecundity rates are: 2.8 for single mite infestations, 2.63 for two mites, 2.23 for three mites and 1.7 for four mites.

Discussion

There is a wide variation in both fertility and fecundity rates recorded in the literature. This is at least partly due to the methodology used in the study (especially in fertility rates) and the definition of fecundity. For example, Fries *et al.* (1994) demonstrated that the variation in the results of various studies resulted from the different methods of evaluating the data.

Comparing reproduction rates obtained in this study (Tables 1 and 3) with other studies, it is found that the mite reproduction rate of 86.8% in worker and 93% for drone broods was very representative of what other researchers have found. Blum (1989) reported mite reproduction rates at 88.7%, Fuchs and Langenbach (1989) recorded 92.7% and Buchler and Dresher (1990) found 86.6% in worker brood cells. In drone cells, Fuchs and Langenbach (1989) also recorded 92.2% fertility rates in drone cell.

The percent of mites that did not reproduce (11.0% in workers and 7.1% in drones) also was similar to figures presented by other researchers. In worker cells, Sulimanovic *etal.* (1982), Schultz (1984), Moosbeckhofer *et al.* (1988), Fuchs and Langenbach (1989), Ifantidis (1990) and Boot *et al.* (1995) reported infertility rates in worker cells of 13, 16, 7, 7, 14.1 and 8-12%, respectively. Fuchs and Langenbach (1989), Ifantidis (1984) and Schultz (1984) found lower levels of infertility in drone cells also. They reported 8, 4 and 5%, respectively.

Some of the mother mites produced only male offspring. This is probably because they had not mated, since haploid eggs of Varroa mites develop into males (De Ruijter andPappas, 1983). Martin (1995 a, b) attributes this partly to the death of the male before he is able to fertilize his sisters. He found in earlier studies, that 20% of the males died before they mated in worker brood (1994) and 10% in drone cells (1995).

In this study, it was found that 5.1% of the offspring were male only in worker cells (Table 1). This was similar to other studies. Boot *et al.* (1995), Schultz (1984), Moosbeckhofer *et al.* (1988) and Fuchs and Langebach (1989) reported a rate of 8-10, 6, 3 and 3%, respectively. For drone cells, a rate of 3% was observed (Table 2). Fuchs and Langenbach (1989) reported a rate of 1%.

The other parameter that was measured was the percent of mother mites that died in brood cells. It was that 2.3% died in worker cells (Table 1) and 2.7% in drone cells (Table 3). Only one other researcher reported this statistic, Martin (1994). He found a higher percentage in drones (7.7%) and a similar rate in workers (2%). He found that 32% of the deaths in drone cells were caused by failure of the mite to emerge from the brood food and found they were trapped in the cell wall. The percentage rose to 50% in worker cells.

Fecundity was measured in a variety of ways in the literature both because of different experimental techniques used and because of differing definitions of fecundity. For example, there were differences in the way that researchers extrapolated estimates where cells had to be opened before development was completed (Boots *et al,* 1995). In addition, some studies included all mothers that infested brood cells, which included those that died, did not reproduce and reproduced males only. These methods, in general, had less offspring per mother than those that excluded these groups from the study. This study compiled fecundity rates utilizing both methods in single infested cells and multiple infested cells calculated separately (Tables 2 and 4). Varroa females are biologically capable of laying up to six eggs in worker

cells and seven in drone cells (Ifantidis, 1984., Martin, 1994, 1995a, b). The fact that fecundity rates are much lower than this is attributed to offspring mortality, which occurs primarily in the deutonymph stage (Martin, 1994, 1995a, b).

When excluding problematic mothers from the analysis, it was found that mites averaged 1.82 offspring in single cell infestations of worker brood (Table 2). In drone cells, this figure increased to 2.79 offspring (Table 4). This is fairly consistent with other researchers. Fuchs and Schultz (1984), Ifantidis (1984), Fuchs and Langenbach (1989) and Martin (1994, 1995a, b) reported 1.82 and 2.69, 1.33 and 2.77, 1.69 and 2.76 and 1.45 and 2.2 in worker and drone cells, respectively.

When including all the female mites found in single infested cells these figures decreased. In Michigan, average offspring for worker brood decreased to 1.41 (Table 2) and to 2.47 for drones (Table 4). These statistics differed widely among other researchers. Schultz (1984) found 1.3 in worker cells and 2.6 in drones. Ifantidis (1984) reported 0.71 in worker and 1.7 in drone cells. Fuchs and Langebach (1989) found 1.4 in worker and 2.21 in drone. When the whole mother mite population is taken into consideration (single and multiple infested cells), the average number of offspring decreases for both calculation methods (Tables 2 and 4). The negative correlation of average offspring per mite and number of mother mites infesting the cell is well documented in the literature and is supported by the study reported here (Fig.l, Table 5). The Michigan data includes both dead mothers and only male reproduction. Fuchs and Langenbach (1989) attribute this lower fecundity rate to suppressed reproduction while Martin (1995a, b) shows that it is due largely to increased mortality of the offspring. Donze and Guerin (1994) showed that mites normally feed at a single feeding site. Martin (1995a, b) explained that with increased number of eggs, there is more competition for the feeding site and stronger deutonymphs and adults will out compete younger offspring.

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