IJAS Iranian Journal of Applied Animal Science

Expression of Suppression of Mite Reproduction in Drone Brood Cells of Honey Bees of Different Genotypic Groups in East Azarbaijan Province of Iran

Research Article

M. Elmi[1], S.A. Rafat[1*], S. Alijani[1], G. Tahmasbi[2] and A. Javanmard[1]

[1] Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[2] Department of Honeybee, Animal Science Research Institute of Iran (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

Received on: 2 Jan 2019
Revised on: 10 Mar 2019
Accepted on: 16 Mar 2019
Online Published on: Mar 2021

*Correspondence E-mail: rafata@tabrizu.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Varroa destructor is an ectoparasitic mite of the honey bee and is a primary cause of colony loss of apiculture in the world. The aim of this study was to determine infestation levels and the suppression of mite reproduction in drone brood cells of independent colonies. A number of East Azarbaijan native honey bee colonies were isolated for three years without treating against varroa. Then, seven genotypic groups were prepared with three colonies in each group: native survivors, F1 generation of Carniolan colonies, H1 generation of survivor × commercial, H1 generation of Carniolan × survivor and commercial native colonies originated from three different regions of Maragheh, Bostan-Abad, and Varzeghan in the East Azarbaijan province. A total of 3268 fifteen to eighteen-days old drone pupae from twenty-one colonies were assessed for infestation and suppression of mite reproduction. The mean of drone pupae infestation percentage, reproducing mite percentage, mites reproducing more than three offsprings percentage, and fecundity number were found to be 45.20%, 76.13%, 40.69%, and 2.215, respectively. Significant differences were observed in the four studied traits between the understudy groups. The survivor group had the lowest mite infestation level and fertility (14.2% and 68.6%, respectively). Native commercials originated from the above three mentioned regions in the East Azarbaijan province had lower fecundity and lower number of mites reproducing more than three offsprings than other groups. Our results suggest that establishing varroa surviving colonies from native colonies can reduce varroa infestation and enhance the levels of suppression of mite reproduction (SMR). Moreover, a significant variation was observed within and between understudy groups in the colonies. Therefore, it can be concluded that selective breeding programs can enhance the levels of SMR.

KEY WORDS drone, East Azarbaijan province, honey bee, infestation, reproduction, suppression of mite reproduction, *Varroa destructor*.

INTRODUCTION

Ectoparasitic mite (*Varroa destructor*) and associated viruses are dangerous to honey bees, *Apis mellifera*, (Rosenkranz *et al*. 2010). Rearing honey bee colonies usually requires repeated chemical treatment against *Varroa destructor*, except in African breeds (Rosenkranz *et al*. 2010; Bocking and Genersch, 2008).

The life cycle of the mite is comprised of phoreticand reproductive phases. In the phoretic phase, the mite is connected to the adult honey bee body to feed and transportation. For reproduction, mother mite has entered into brood cell just prior to capping of cells. Mite reproduction is identified as ability of mother mite to produce at least one viable and mated offsprings before emerging pupae as an adult bee from the infested cell (Dietemann *et al*. 2013). A

number of mechanisms have been shown to interfere with mite reproductive success (Rosenkranz *et al*. 2010). One of these mechanisms is the host larvae factors. Results of some experiments show the mite gets some volatiles from the cuticles through feeding on larvae hemolymph. These chemicals of the larval cuticle can cause ovary activation (Garrido and Rosenkranz, 2004). Honey bee larva volatile with its effect on egg-laying of the mite can, somehow, cause failure of mite reproduction in some honey bee races or populations.

Majority of mother mites perform unsuccessful reproduction in honey bee larvae cells. Some mites do not lie at all (Rosenkranz *et al.* 2010; Garrido and Rosenkranz, 2004), some may lay only male or female eggs, and some delay laying eggs (Donzé *et al.* 1996; Martin *et al.* 1997; Locke and Fries, 2011). Inhibition of mite reproduction by honey bee larvae factors is one of the primary mechanisms of suppression of mite reproduction (SMR) (Behrens *et al.* 2011). SMR is the reduction in reproduction of female mites within brood cells. SMR is of particular interest because of its association with low mite infestations, e.g., in African honey bee subspecies (Garrido and Rosenkranz, 2004). Mite infertility by thirty percent and above can have a negative effect on population growth of varroa (Harbo and Hoopingarner, 1997).

Several mechanisms acting simultaneously can produce SMR phenotype. In this study, we measured SMR through 3 variables. SMR can vary due to factors such as brood and colony genotype. The percentage of mites that cannot reproduce varies depending on the species and host breed (Fries *et al.* 1994; Martin, 1998; Rosenkranz, 1999). In European bee breeds, 5-20% of mites will become infertile after entering into worker or drone cells (Kavinseksan *et al.* 2016). The infertility rate of mites is reported to be more than 50% in Africanized honey bees in Brazil (Rosenkranz, 1999).

This trait has been widely spoken of in the context of breeding programs because of its apparent effectiveness and its heritability (Harbo and Harris, 2004). Although SMR has been identified in many populations of honey bees in the world, no attempts have yet been made to examine this trait in populations of the Iranian native or the imported breeds. In recent years, Iranian beekeepers have started to use imported Carniolan breed in their apiaries as well a native breed (*Apis mellifera meda*). Some reports are considered the Carniolan breed susceptible to varroa (Oddie *et al.* 2018).

The goal of this research was to test the various honey bee genetic groups, including mite surviving colonies and colonies obtained from crosses with native and Carniolan breeds, as well as some native commercial colonies of various regions of East Azarbaijan province in Iran; with the aim of maintaining varroa resistance through the incorporation of SMR trait. We evaluated drone brood cells of colonies for SMR variables, including mite infertility, mite fecundity, and mites produced more than three offsprings. Drone broods are well known as varroa host from the epidemiological, genetic and physical point of view (Behrens *et al.* 2011).

MATERIALS AND METHODS

Establishment of the test colonies

The first step in this study was to select mite surviving colonies. We isolated fifty native honey bee (*Apis mellifera meda*) colonies of East Azarbaijan in the apiary of Research and Education Center for Agriculture and Natural Resources of East Azarbaijan (RECANR) located in Saeed Abad, Tabriz. All chemical treatments against varroa were withheld in 2013 and a survival test was initiated and continued for three years. Typically, 30 to 35 colonies survived each year in the apiary depending upon the climatic conditions of the year. As established colonies died out, they were replaced by new ones in the following year. Daughter queens reared from the most superior survivors were free mated and introduced into newly established colonies.

A pure Carniolan (*Apis mellifera carnica*) queen (ID: B125) purchased from Alvand Queen Rearing Company in 2015 and properly introduced into an established foster colony. Therefore, there were three different lines: Carniolan, mite-susceptible, (Locke, 2016; Oddie *et al.* 2018); survivor colonies, naturally mite-resistant and commercial native honey bees.

The following seven genotypic groups were obtained with three colonies in each group in 2015 and 2016: native survivor colonies (Figure 1 D), F1Carniolan colonies with the free mated queen (Figure 1 E), H1 cross of survivor queen × commercial unselected drones (Figure 1 H), H1 cross of Carniolan queen × survivor drones (Figure 1 H), and commercial native colonies originated from three different regions of Maragheh, Bostan Abad, and Varzeghan in the East Azarbaijan province.

A unique mating method was utilized to control mating. Drone producer colonies were moved to the southern region of the country (more temperate area) in the winter. One to two drones comb were placed in each of them for early drones rearing. One week prior to the emergence of the drone's pupae, the drone colonies were transferred to a winter quarter located in the East Azarbaijan region. At this time of year, this region was almost empty of other colonies and if there were still local colonies, they had not initiated drones producing because of the seasonal limitations.

All of the established colonies were managed in a similar way in RECANR during the experiment period.

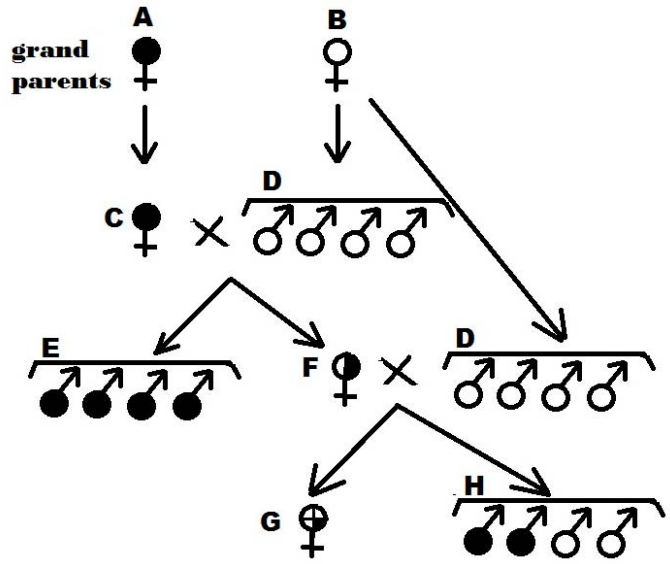

Figure 1 Crosses performed to gain drones for record register
A: pure grandmother colony (Carniolan or survivor depending on the text); B: drone producing grandfather colony (survivor or commercial depending on the text); C: virgin daughter queen of A; D: drones produced by B; E: drones produced by Queen C that mated to D drones, due to haploidic they inherit only their mother genes; F: daughter of C Queen and D drones (F1); G: F2 virgin queen and H: drones produced by F1 colony or H1 drones

To obtain good mite infestation levels, these test colonies did not get treatments against varroa for one year.

Measurements and calculations

During drones producing season in June 2016, a drone comb was placed in each infested test colony to rear drone pupae. After 15 to 18 days; when drone pupa had purple eyes and brown body color, the capping of the cell was removed carefully with a needle. The contents of each cell were evacuated on cardboard. With the help of forceps and magnifying glasses, the number of mothers and viable offspring mites in the cell and on the pupae were counted and recorded (Lee *et al.* 2010). At this stage of drone pupae, the mother mites (foundress) are dark brown and offsprings at the deutonymph stage are white or light brown color. Therefore, they are easily identified and counted. A minimum of 100 drone pupae obtained from each colony was examined. A total of 3268 from 21 test colonies were observed and recorded. To measure the infestation rate of drone pupae to the varroa mites, the number of infected cells with at least one mite, divided into the number of examining cells (Dietemann *et al.* 2013). The number of mother mites in each infected cell was counted and recorded. Pupae cells containing at least one mother mite and one viable offspring were considered as a reproductive mite.

By dividing the number of mites produced at least one viable offspring on the number of cells containing at least one mite, the number of reproducing mites (fertility) obtained (Dietemann *et al.* 2013).

Another parameter which was of considerable importance and measured in this study was the rate of mite reproduction. Mites that had more than three offspring were also recorded separately (Behrens *et al.* 2011). Also, by dividing the number of offspring into the number of mother mites in each infected cell, the number of offspring per mother (fecundity) was calculated.

Data analysis

Data were analyzed using SAS (2003) statistical software and mixed model procedure. In this analysis, the region was considered as a fixed effect and the colony within each region was considered to be a random effect. The statistical model was:

$$Y_{ijk} = \mu + Genotype_i + Colony_j(Genotype_i) + e_{ijk}$$

Where:
Y_{ijk}: record of k^{th} observation in j^{th} colony that belongs to i^{th} genotypic group.
μ: population means.
$Genotype_i$: i^{th} genotypic groups (i=1…7).

$Colony_j$ ($Genotype_i$): effect of the j^{th} colony that has nested in i^{th} genotypic group (j=1…3).
e_{ijk}: residual effect.

The mean comparison of the colonies in different genotypic groups was based on the LSM test and at the significance level of 0.05. The correlation coefficients of the Pearson method were used to examine the relationship between the traits.

RESULTS AND DISCUSSION

None of the sampled colonies were free of varroa mites. There were significant differences (P<0.01) in infestation levels, a number of reproducing mites and number of mites producing more than three offsprings among and between genotype groups (Table 1).

An infestation of drone pupae

The mean infestation percent of drone pupae to *varroa* mite in all understudy colonies was 45.16 (Table 2). This level of infestation was well above the economic threshold identified by Delaplane and Hood (1999) and previous report of Elmi *et al.* (2015) in the region. Means comparisons showed that the infestation percentage of drone pupae to varroa mite is the lowest in survivor colonies (14.32%) and the highest in colonies of the F1 Carniolan (66.33%) (Table 2). Any crosses made from survivor colonies (survivor×Carniolan and survivor×commercial) possessed a relatively low level of infestation, too. Low level of infestation of survivor colonies to mite has been reported despite the fact that these colonies have been untreated against varroa for three years; indicating the comparative resistance of these colonies to varroa mites.

Reproducing mites

The average percentage of reproducing mites in drone brood cells of all colonies was 76.13%. The lowest reproductive rate was observed in drones brood cells of survivor colonies (68.6%), but the highest in H1 drones of survivor × commercial hybrid colonies (85.8%) (Table 2). Locke (2016) estimated the success of reproductive mites in the worker brood cells of the different genotypes from 43 to 85 percent and concluded that this trait is heritable. Alattal *et al.* (2017) estimated mite fertility in worker pupae in colonies of *A. m. jemenetica* and A. m. Carniolan is 87.5% and 89.4%, respectively; concluding that these breeds are an appropriate host for the varroa mite, and infertility of mites cannot be considered as an appropriate trait for breeding resistant colonies against *varroa* in condition of Saudi Arabia, and other resistance mechanisms should be considered.

Calderon *et al.* (2012) reported that mite reproduction in drone cells was significantly higher (64.8%) than worker cells (37.6%) in Africanized honey bees (AHB).

The mite reproduction in drone brood cells in this study was similar to that reported in other studies with worker brood cells. Because of the relatively longer post capping period of drones, larger cell size and amount of available food, mites can produce many offsprings in drone brood cells (Harris, 2007). This clearly demonstrates that mite reproduction in drone brood cells of genotypic groups in this study is lower compared to results of other similar studies except in AHB that is naturally mite resistant. Furthermore, survivor colonies had very low level of mite reproduction, which is consistent with the results of Calderon *et al.* (2012) with AHB.

Mite fecundity

The mean number of offspring produced by one reproductive varroa mite (or fecundity) in all colonies was 2.215 (Table 2). Kavinseksan *et al.* (2016) reported that the mean number of offsprings per reproductive varroa mite in worker brood cells infested by single foundress of the Primorsky colonies and Thai commercial colonies are 1.3 and 2.2 progeny per foundress, respectively. De Guzman *et al.* (2008) reported that reproductive varroa mites in *A. mellifera* worker brood cells produced 1 to 1.7 progeny per female mite. Martin (1994), Martin (1995a), Martin (1995b) calculated the effective reproduction rate (i.e. the number of vials/mature daughters per invading mother) as 1.3 to 1.45 in a single infested worker brood, while for drone brood it was 2.2 to 2.6. Results of mean comparisons in this study showed that the number of progenies per reproductive varroa mite in drone brood cells infested by single foundress of the Varzaghan region colonies with the mean of 1.505 was significantly lower than that of other genotypic groups (P<0.05) (Table 2). Colonies of the cross survivor × Carniolan with an average of 2.929 had the highest fecundity of mites in drone brood cells. Also mite fecundity in drone broods of F_1Carniolan colonies is second. Therefore, it can be confirmed that Carniolan breed is susceptible to *varroa* mite from the viewpoint of fecundity.

Mites producing more than three offsprings

The mean percent of mites producing more than three offsprings in all the studied colonies was 40.69 (Table 2). Survivor × Carniolan cross colonies had the highest percentage of the mites producing more than three offsprings (%54.3, mean), and Carniolan group was second highest. Surprisingly, commercial colonies originated from Varzegan, Bostan Abad and Maragheh regions had inferior mites, which produced more than three offsprings. This trait is critically valuable.

Table 1 Numbers of inspected drone brood cells, infected cells, reproduced mites and mites reproduced more than three offspring in different genotypic groups

Origin of drone pupae	Sample	Infested cells	Reproducing mites	Number of mites producing more than 3 offsprings
Survivor	563	93	65	24
F_1 Carniolan	397	186	132	39
H_1 of survivor × Commercial	411	221	164	49
H_1 of survivor × Carniolan	366	246	200	96
Maragheh	466	245	179	33
Bostan Abad	558	241	210	53
Varzeghan	507	199	155	79
Total	3268	1431	1105	373

Table 2 Differences of least squares means of studied traits in different regions

Source of bees	Infested cells (%)	Reproducing mites (%)	Fecundity	Mites producing more than 3 offsprings (%)
Survivor	14.2 ± 2.1^{f}	68.6 ± 5.8^{b}	2.354 ± 0.282^{ab}	46.9 ± 6.5^{ab}
F_1Carniolan	66.3 ± 2.5^{a}	82.4 ± 2.7^{a}	2.548 ± 0.133^{a}	53.7 ± 3.1^{a}
H_1 of survivor × Commercial	42.5 ± 2.0^{de}	85.8 ± 2.8^{a}	2.254 ± 0.138^{b}	44.5 ± 3.2^{b}
H_1 of survivor × Carniolan	40.3 ± 2.1^{e}	78.5 ± 3.0^{ab}	2.929 ± 0.146^{a}	54.3 ± 3.4^{a}
Maragheh	46.9 ± 2.4^{cd}	72.4 ± 3.0^{b}	2.048 ± 0.149^{b}	31.4 ± 3.5^{c}
Bostan Abad	53.7 ± 2.3^{b}	72.7 ± 2.8^{b}	1.870 ± 0.138^{bc}	31.2 ± 3.2^{c}
Varzeghan	52.2 ± 2.2^{bc}	72.5 ± 2.7^{b}	1.505 ± 0.131^{c}	22.8 ± 3.0^{c}
Total	45.2±16.1	76.13±6.24	2.215±.464	40.69±12.27

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 3 Pearson correlation coefficients between traits (N=1434, Prob> |r| under H0:Rho=0)

			Number of		
			Mother mite	Reproductive mite	Mites producing more than 3 offsprings
Number of	Reproductive mite	Pearson correlation	-0.016		
		Sig. (2-tailed)	0.538		
	Mites producing more than 3 off-springs	Pearson correlation	-0.013	0.444*	
		Sig. (2-tailed)	0.627	< 0.0001	
	Offsprings per mite	Pearson correlation	-0.234*	0.573*	0.779*
		Sig. (2-tailed)	< 0.0001	< 0.0001	< 0.0001

Research has shown that mites that cannot produce more than three offsprings do not possess a crucial role in increasing the population of the mite in a colony. Because in such a situation, none of the newborns (protonymph) will have enough time to mature and mate (Villa *et al.* 2009). In this study over the 50% of the mites could not produce more than 3 offsprings. Therefore, about half of the mites can't be effective in increasing the mite population through this mechanism. Heritability of this trait is high (reviewed by Elmi and Rafat, 2011). The results of the present study show that this parameter of SMR can play an important role in creating a mite-resistant population.

Correlation between study traits

Pearson correlations were not significant between the number of reproductive mites, fecundity and the number of mites producing more than three offsprings (Table 3).

This incidence was somehow consistent with previous reports from several researchers that the number of progenies produced by reproductive varroa mites was independent of the frequency of non-reproductive mites in a colony (Rosenkranz and Engels, 1994; Martin, 1995b; Kavinseksan *et al.* 2016).

However, there was a significant negative correlation between the number of mother mites in drone pupae and the number of offsprings per mite (n=1432 and r=-0.2343), which was consistent with other previous reports (Huang, 2012).

The per capita fecundity decreases as the number of mother mites per cell increases. In addition, mites invading brood cells in the old combs produce fewer offsprings. This led researchers to speculate that mites, themselves, might produce a chemical (a pheromone) to inhibit each other's reproduction (Huang, 2012).

CONCLUSION

Our results confirm that there is a significant variation in mite infestation levels and SMR variables between breeding populations of the East Azarbaijan province. Therefore, there is potential to establish varroa resistance in the Iranian *Apis mellifera* populations. Survivor colonies, after over three years without mite treatment, had lower levels of mite infestation and fertility. This survivor population provides valuable insight and can be a suitable selection method to be used in field selection programs. However, since the fecundity and reproduction rate of mites were low in commercial colonies, there is a need for more research to better relate SMR trait to mite surviving colonies.

ACKNOWLEDGEMENT

We are grateful to the Research and Education Center for Agriculture and Natural Resources of East Azarbaijan (RECANR) for financial support. We also thank the beekeepers of East Azarbaijan province, who helped us in preparing test colonies.

REFERENCES

Alattal Y., Alghamdi A., Single A., Ansari M.J. and Alkathiri H. (2017). Fertility and reproductive rate of varroa mite, *Varroa destructor*, in native and exotic honey bee, *Apis mellifera*, colonies under Saudi Arabia conditions. *Saudi J. Biol. Sci*. **24,** 992-995.

Behrens D., Huang Q., Geßner C., Rosenkranz P., Frey E., Locke B., Moritz R.F.A. and Kraus F.B. (2011). Three QTL in the honey bee, *Apis mellifera* suppress reproduction of the parasitic mite *Varroa destructor*. *Ecol. Eevol*. **1(4),** 451-458.

Bocking O. and Genersch E. (2008). Varroosis—the ongoing crisis in beekeeping. *J. Verbraucher. Lebensmittel*. **3,** 221-228.

Calderon R.A., Urena S. and van Veen J.W. (2012). Reproduction of *Varroa destructor* and off-springs mortality in worker and drone brood cells of Africanized honey bees. *Exp. Appl. Acarol*. **56,** 297-307.

De Guzman L.I., Rinderer T.E. and Frake A.M. (2008). Comparative reproduction of *Varroa destructor* in different types of Russian and Italian honey bee combs. *Exp. Appl. Acarol*. **44,** 227-238.

Delaplane K.S. and Hood W.M. (1999). Economic threshold for *Varroa jacobsoni* Oud in the southeastern USA. *Apidologie*. **30,** 383-395.

Dietemann V., Nazzi F., Martin S.J., Anderson D.L., Locke B., Delaplane K.S., Wauquiez Q., Tannahil C., Frey E., Ziegelmann B., Rosenkranz P. and Ellis J.D. (2013). Standard methods for varroa research. *J. Apic. Res*. **52(1),** 1-47.

Donzé G., Herrmann M., Bachofen B. and Guerin P.M. (1996). Effect of mating frequency and brood cell infestation rate on the reproductive success of the honeybee parasite *Varroa jacobsoni*. *Ecol. Entomol*. **21,** 17-26.

Elmi M. and Rafat S.A. (2011). Varroa sensitive hygienic honey bees. *Iranian Honey Bee Sci. Technol*. **3,** 34-44.

Elmi M., Baybordi S., Bahreyni R. and Asghar Rezayi A. (2015). Survey on Honey Bee Pests and Predators in East Azarbayjan. Research Report of Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

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

Garrido C. and Rosenkranz P. (2004). Volatiles of the honey bee larva initiate oogenesis in the parasitic mite *Varroa destructor*. *Chemoecology*. **14,** 193-197.

Harbo J.R. and Harris J.W. (2004). SMR: This Honey of a Trait Protects Bees from Deadly Mites. Agricultural Research. Available at: https://agresearchmag.ars.usda.gov/ar/archive/2004/may/bees0504.pdf.

Harbo J.R. and Hoopingarner R.A. (1997). Honey bees (Hymenoptera: Apidae) in the United States that express resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). *J. Econ. omic Entomol*. **90,** 893-898.

Harris J.W. (2007). Bees with varroa sensitive hygiene preferentially remove mite infested pupae aged five days post capping. *J. Apic. Res*. **46,** 134-139.

Huang Z. (2012). Varroa mite reproductive biology. *American Bee J*. **152,** 981-985.

Kavinseksan B., Wongsiri S. and Chotkitnusorn A. (2016). Reproduction of the bee mite, *Varroa destructor* Anderson and Trueman (Acari: Varroidae), in worker brood cells of Primorsky and Thai commercial honey bees (*Apis mellifera*). *Basic Res. J. Agric. Sci. Rev*. **5(2),** 37-46.

Lee K.V., Moon R.D., Burkness E.C., Hutchison W.D. and Spivak M. (2010). Practical sampling plans for *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) colonies and apiaries. *J. Econ. Entomol*. **103(4),** 1039-1050.

Locke B. (2016). Inheritance of reduced varroa mite reproductive success in reciprocal crosses of mite-resistant and mite-susceptible honey bees (*Apis mellifera*). *Apidologie*. **47,** 583-588.

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

Martin S.J. (1994). Ontogenesis of the mite *Varroa jacobsoni*Oud in worker brood of the honeybee *Apis mellifera* under natural conditions. *Exp. Appl. Acarol*. **18,** 87-100.

Martin S.J. (1995a). Ontogenesis of the mite *Varroa jacobsoni*-Oud in drone brood of the honeybee *Apis mellifera* under natural conditions. *Exp. Appl. Acarol*. **19,** 199-210.

Martin S.J. (1995b). Reproduction of *Varroa jacobsoni*in cells of *Apis mellifera* containing one or more mother mites and the distribution of these cells. *J. Apic. Res*. **34,** 187-196.

Martin S.J. (1998). A population model for the ectoparasitic mite *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. *Ecol. Mod*. **109,** 267-281.

Martin S.J., Holland K. and Murray M. (1997). Non-reproduction in the honeybee mite *Varroa jacobsoni*. *Exp. Appl. Acarol*. **21,** 539-549.

Oddie M.A.Y., Dahle B. and Neumann P. (2018). Reduced post-capping Period in Honey Bees Surviving *Varroa destructor* by means of natural selection. *Insects*. **9(4),** 149-155.

Rosenkranz P. (1999). Honey bee (*Apis mellifera*) tolerance to *Varroa jacobsoni* Oud in South America. *Apidologie*. **30,** 159-172.

Rosenkranz P. and Engels W. (1994). Infertility of *Varroa jacobsoni* females after invasion into *Apis mellifera* worker brood as a tolerance factor against varroatosis. *Apidologie*. **25,** 402-411.

Rosenkranz P., Aumeier P. and Ziegelmann B. (2010). Biology and control of *Varroa destructor*. *J. Invertebr. Pathol*. **103,** 96-119.

SAS Institute. (2003). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Villa J.D., Danka R.G. and Harris J.W. (2009). Simplified methods of evaluating colonies for levels of varroa sensitive hygiene (VSH). *J. Apic. Res*. **48(3),** 162-167.