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

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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 phoretic and 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 lay 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 Hoopinger, 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 as 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.
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 offspring 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). 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:

\[ Y_{ijk} = \mu + \text{Genotype}_i + \text{Colony}_{(Genotype_i)} + e_{ijk} \]

Where:
- \( Y_{ijk} \): record of \( k \)th observation in \( j \)th colony that belongs to \( i \)th genotypic group.
- \( \mu \): population means.
- \( \text{Genotype}_i \): \( i \)th genotypic groups \( (i=1 \ldots 7) \).
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).

Loke (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 F1 Carniolan 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.
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).

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Table 1

<table>
<thead>
<tr>
<th>Origin of drone pupae</th>
<th>Sample</th>
<th>Infested cells</th>
<th>Reproducing mites</th>
<th>Number of mites producing more than 3 offsprings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivor</td>
<td>563</td>
<td>93</td>
<td>65</td>
<td>24</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;Carniolan</td>
<td>397</td>
<td>186</td>
<td>132</td>
<td>39</td>
</tr>
<tr>
<td>H&lt;sub&gt;1&lt;/sub&gt; of survivor × Commercial</td>
<td>411</td>
<td>221</td>
<td>164</td>
<td>49</td>
</tr>
<tr>
<td>H&lt;sub&gt;1&lt;/sub&gt; of survivor × Carniolan</td>
<td>366</td>
<td>246</td>
<td>200</td>
<td>96</td>
</tr>
<tr>
<td>Maragheh</td>
<td>466</td>
<td>245</td>
<td>179</td>
<td>33</td>
</tr>
<tr>
<td>Bostan Abad</td>
<td>558</td>
<td>241</td>
<td>210</td>
<td>53</td>
</tr>
<tr>
<td>Varzeghan</td>
<td>507</td>
<td>199</td>
<td>155</td>
<td>79</td>
</tr>
<tr>
<td>Total</td>
<td>3268</td>
<td>1431</td>
<td>1105</td>
<td>373</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Source of bees</th>
<th>Infested cells (%)</th>
<th>Reproducing mites (%)</th>
<th>Fecundity</th>
<th>Mites producing more than 3 offsprings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivor</td>
<td>14.2±2.1&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>2.354±0.282&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>F&lt;sub&gt;1&lt;/sub&gt;Carniolan</td>
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<td>53.7±3.1&lt;sup&gt;T&lt;/sup&gt;</td>
</tr>
<tr>
<td>H&lt;sub&gt;1&lt;/sub&gt; of survivor × Commercial</td>
<td>42.5±2.0&lt;sup&gt;ea&lt;/sup&gt;</td>
<td>85.8±2.8&lt;sup&gt;ea&lt;/sup&gt;</td>
<td>2.254±0.138&lt;sup&gt;ea&lt;/sup&gt;</td>
<td>44.5±3.2&lt;sup&gt;ea&lt;/sup&gt;</td>
</tr>
<tr>
<td>H&lt;sub&gt;1&lt;/sub&gt; of survivor × Carniolan</td>
<td>40.3±2.1&lt;sup&gt;T&lt;/sup&gt;</td>
<td>78.5±3.0&lt;sup&gt;T&lt;/sup&gt;</td>
<td>2.929±0.146&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>1.870±0.138&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>1.505±0.131&lt;sup&gt;T&lt;/sup&gt;</td>
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<tr>
<td>Total</td>
<td>45.2±16.1</td>
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<td>2.215±464</td>
<td>40.69±12.27</td>
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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.

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