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Comparison of Hygienic and Grooming Behaviors of Indigenous and Exotic Honeybee (*Apis mellifera*) Races in Central Saudi Arabia

HASSAN M. BALHARETH, ABDULAZIZ S. ALQARNI[1]† AND AYMAN A. OWAYSS†
Ministry of Agriculture, Saudi Arabia, Riyadh 11195 P.O. Box 3520, Saudi Arabia
†Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, P.O. Box 2460, Saudi Arabia
[1]Corresponding author's: e-mail: alqarni@ksu.edu.sa , azizqarni@hotmail.com

ABSTRACT

Hygienic and grooming behaviors of native and exotic honeybee races reared in Riyadh, Saudi Arabia were investigated. Both races exhibited different rates of hygienic behavior. After 24 h the removal of dead brood in native race was significantly higher than that of exotic one being 85.28 and 43.84%, respectively. However, after 48 h the native race significantly removed 97.60% of brood, while exotic one removed 79.32%. After 72 h the removal was 99.78 and 93.29% for native and exotic races, respectively. Time consumed to detect and remove dead brood in colonies of the two races was variable *i.e.*, native race differentiated and removed dead brood faster than exotic one. Deformed *Varroa* mites, as a result of grooming behavior, were significantly higher in colonies of native race than those of exotic one being 31.13 and 11.27%, respectively. Leg-deformed mites in native and exotic colonies were 62.94 and 56.29%, and deformities in dorsal shield were 37.18 and 36.32% for the two races, respectively without significant differences. The remarkable defensive behavior exhibited by the native race, compared to that of exotic one, may be due to its different genetic structure as well as its compatibility with local environmental conditions.

Key Words: Hygienic; Grooming; *Varroa destructor*; Saudi Arabia

INTRODUCTION

The haemolymph-feeding mite, *Varroa destructor* (Anderson & Trueman), is the most serious pest threatening honeybees worldwide (Lodesani *et al*., 1992). This ectoparasitic mite was recorded in Saudi Arabia in 1987, and is found in all beekeeping areas causing great damages to bee colonies (Al-Ghamdi, 1990). Most beekeepers in Saudi Arabia often move their colonies to moderate areas and to flowering pastures (Alqarni, 1995). Therefore, infection with the varroa mite was continuously spreading. Besides, keeping bees in traditional log hives prevents beekeeper from efficient control of this mite. Different methods have been applied to manage this mite in modern hives including mechanical, chemical and natural procedures. Although chemical methods reduced the mite infestation and succeeded to a great extent, resistance of mite to most acaricides used for control was a significant problem. Also, chemical abuse leads to improper residues in hive products *e.g.*, honey and beeswax (Wallner, 1999). Depending on alternative techniques in controlling *Varroa* mite, natural materials *e.g.*, essential oils and botanical extracts were utilized and variable efficiencies were achieved (Imdorf *et al*., 1995; Sammataro *et al*., 1998; Omar *et al*., 2001) as well as integrated management of the mite (Owayss, 2002).

Naturally, honey bee developed some mechanisms to defense invaders. The term "hygienic behavior" was originally first mentioned by Rothenbuhler (1964) and means the ability of worker bees to identify the dead brood and remove it off the cell, thus reduce the infestation (Peng *et al*., 1987). The removal behavior of infected brood exhibited by workers of certain honeybee races is a focused approach to minimize the mite population (Spivak, 1996). Hygienic behavior is not only directed toward dead brood, but also towards the brood infected with bacteria or fungi (Boecking & Spivak, 1999), the greater wax moth (Villegas & Villa, 2006), the small hive beetle (Neuman & Härtels, 2004). The other defensive mechanism is "grooming behavior" where about 20% of adult workers of 15–20 days-old remove *Varroa* mites from their own bodies (auto-grooming) or from nestmates (allo-grooming). Distinct mite grooming has been shown in *A. cerana*, the original host of the mite, whereas *A. mellifera* exhibits this behavior to a lesser extent (Peng *et al*., 1987; Boecking & Spivak, 1999; Arathi *et al*., 2000; Arathi & Spivak, 2001).

These two defensive behaviors were studied in

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different honeybee races and hybrids *e.g., A. m. carnica, A. m. ligustica*, *A. m. caucasica*, *A. m. syriaca*, *A. m. lamarckii* and *A.m. meda*. Variable results related to racial performance, seasonal fluctuations and other factors were reported (Lodesani *et al*., 1996; Abdel-Wahab, 2001; Kamel *et al*., 2003; Omran, 2004; Hussein *et al*., 2005; Abou El-Enain, 2006; Amro, 2009; Zaitoun & Al-Ghzawi, 2009; Nagafgholian *et al*., 2011). Increasing *Varroa*-hygienic behavior by selective breeding enables controlling mite to a stable parasite-host relationship (Rosenkranz *et al*., 2010). Different *Varroa*-hygienic bee lines have been successfully bred (Harbo & Harris, 1999& 2005; Spivak & Reuter, 2001; Büchler *et al*., 2010).

Few researches concerning defensive behavior of the indigenous honeybee race was conducted (*e.g.*, Khanbash, 2001; Al-Ghamdi, 2002; Al-Medani, 2004). The present work aimed to evaluate the hygienic and grooming behaviors of the native bee race, *A.m. jemenitica*, compared to the most imported one, *A.m. carnica*, to Saudi Arabia. These two traits are important indicators for disease resistance in selection and breeding programs.

MATERIALS AND METHODS

Site of the experiment: This research was carried out during May, 2011 in a private apiary situated in Al-Hair, about 30 km south of Riyadh, Saudi Arabia.

Honeybee races and colonies: Two groups of equal strength honeybee colonies reared in wooden Langstroth hives were tested. The 1st group was headed by queens of native race, *A.m. jemenitica* and the second group with hybrid Carniolan, *A.m. carnica*, queens imported from Egypt. Ordinarily beekeeping practices including necessary supplementary feeding were conducted. The tested colonies were naturally infested with *Varroa* mites, and no *Varroa* control treatments were applied.

Assessment of hygienic behavior: Four honeybee colonies/ race were experimented for their hygienic behavior. An area (2.5 × 2.5 cm) of centered sealed worker brood (100 cell/1 comb/colony) was bordered and killed by piercing a fine wooden pin into each cell and then the comb was returned to its hive (Sammataro, 1996). The percentages of brood removal in each colony were recorded after 24, 48 and 72 h. Three experiments were conducted consequently with one week interval.

Assessment of grooming behavior: The former four honeybee colonies were also experimented three times with one week interval for their grooming behavior. A plastic sheet coated with Vaseline was placed onto each hive bottom board and separated with a screen mesh to prevent *Varroa* individuals from escape or climbing back to the brood frames. After 3 days, dropped mature female mites, depending on color, were separated, counted and examined for any deformity using a microscopic magnification at the Bee Research Unit, College of Food and Agriculture Sciences, King Saud University.

Statistical analysis: Experiments were randomized complete blocks designed under the same conditions during the study period. Data were analyzed and mean values were tested for significance following Snedecor and Cochran (1981) and SAS, 2008 software was applied.

RESULTS AND DISCUSSION

Hygienic behavior of native and exotic honeybees: The removal of artificially killed worker brood (%) was considered as an indicator of hygienic behavior. Data illustrated in (Table I) compares the hygienic behavior in the tested honeybee races; *A.m. jemenitica* (*AJ*) and *A.m. carnica* (*AC*) after 24, 48 and 72 of killing the brood. It was found that both races exhibited hygienic behavior, but with different rates. After 24 h, the % general mean of removal in *AJ* was significantly higher than that of *AC* being 85.28 and 43.84% for the two races, respectively. Also, variance between the two values was +94.55%. After 48 h, workers of *AJ* removed 97.60% of brood, while those of *AC* removed 79.32% with significant difference, and the variance was +23.61%. After 72 h, the removal rates were 99.78 and 93.29% for *AJ* and *AC*, respectively without significant difference. It is worth noting that time needed to detect and remove brood in colonies of the two races was variable *i.e.,* native race differentiated and removed dead brood faster than exotic one in the same period. Thus, this trait could be used as an indicator for selection of colonies with hygienic behavior. Al-Ghamdi (2002) found that *A.m. jemenitica* removed 100% of dead brood through 24 h, while *A.m. carnica* removed only 55% in the same period. Hygienic behavior is greatly different among and within bee populations and subspecies (Spivak & Gilliam, 1998 a& b). Kamel *et al*. (2003) mentioned that *A.m. lamarckii* removed 72.5% of dead brood, while it was 35.6% for *A.m. carnica*, but after 24 h they were 90.5 and 59.4% for the two races, respectively. Al-Medani (2004) reported that *A.m. jemenitica* removed 85.5% of dead brood during 48 h. Hygienic behavior is the first defense mechanism in honeybee against at least two diseases; American foulbrood and chalk brood. Hygienic bees differentiae and remove the infected brood before sporulation of the pathogen. Two variable genes were suggested to be responsible for this behavior; one gene for uncapping cells and the other one for removing diseased brood (Rothenbuhler, 1964). Mortiz (1988) reported that there are many genetic factors responsible for this complicated behavior which is expressed in a phenotype. On the other hand, Lapidge *et al*. (2002) proposed that there are seven genetic loci controlling hygienic behavior, which is very obvious in *A. cerana*. These bees remove *Varroa* mites of infected brood and stop reproductive cycle as well as killing immature stages (Fries *et al*., 1994). Africanized bees significantly removed infected pupae more than European ones at the same area in Brazil. Dead pupae were removed from plastic combs faster than those in wax ones (Boecking & Drescher, 1992).

Sensitivity to odors of diseased brood is increased in bees exhibit hygienic behavior. This mechanism is enhanced by the modulatory effect of octopamine, a noradrenaline-like neuromodulator (Goode *et al*., 2006). Thus, selection programs could be developed in native populations of *A.m. jemenitica* to increase its defensive behavior and other desirable traits.

Grooming behavior: Deformed mature *Varroa* female mites fallen onto hive bottom boards were taken as an indicator of grooming behavior in the two tested races. Data illustrated in (Table II) show the % of deformed mites. The % general mean of deformed mites was significantly higher in *AJ* colonies than that of *AC* ones being 31.13 and 11.27% for the two races, respectively. Al-Ghamdi (2002) reported that deformed mites were 35.54 and 33.14% in *A.m. jemenitica* and *A.m. carnica*, respectively. These close values may be due to variations among populations and/or low purity of the two races. Omran (2004) showed that deformed mites were 37.3 and 30.2% for *A.m. lamarkii*, and the most deformities in *Varroa* mites were in legs being 67.2 and 77.2% in two successive years for the same race. Al-Ghzawi *et al*. (2001) reported that deformed mites were 25.7, 13.5 and 11.2% in *A.m. syriaca*, *A.m. carnica* and *A.m. ligustica*, respectively. Also, Zaitoun and Al-Ghzawi (2009) found that these values were 19.75, 11.50 and 10.75% deformed mites for the same races, respectively.

The leg-deformed mites in *AC* colonies were higher than those of *AJ* ones *i.e*., general means were 62.94 and 56.29% for the two races, respectively (Table III), with no significant differences. This type of deformity is common, since the first three pairs of legs is the most impaired parts of the attacked mites by the bees (Abdel-Wahab, 2001; Al-Ghzawi *et al*., 2001). Collected mites from the debris of the colony were leg-deformed by 62.5% (Al-Medani, 2004).

On the other hand, values of dorsal shield-deformed mites were close in *AJ* and *AC* colonies whereas the general means were 37.18 and 36.32%, respectively (Table IV) without significant difference. Al-Medani (2004) reported that deformity in *A.m. jemenitica* was 13% of both legs and dorsal shield. The remarkable defensive behavior exhibited by the native race may be due to its genetic variability in mite-resistance compared to exotic one (Al-Otaibi, 2008) as well as adaptation with native environmental conditions (Alqarni, 2006; Ali, 2011).

Table I: Hygienic behavior expressed as % of mean removal of killed brood in native, *Apis mellifera jemenitica*, and exotic, *A.m. carnica*, honeybees in central Saudi Arabia

Races	General mean of 3 experiments (%)		
	24 h	**48 h**	**72 h**
A. m. jemenitica	85.28 b	97.60 a	99.78 a
A. m. carnica	43.84 c	79.32 b	93.29 b
% variance	+94.55	+23.61	+14.00

Means with different letters in same column are significantly different at 5% probability

Table II: Grooming behavior expressed as % mean of deformed *Varroa* female mites in native, *Apis mellifera jemenitica*, and exotic, *A.m. carnica*, honeybees in central Saudi Arabia

Experiments				
Races	**1st**	**2nd**	**3rd**	**General mean**
A. m. jemenitica	31.42 a	46.21 a	15.76 b	31.13 A
A. m. carnica	9.83 b	16.28 b	7.71 b	11.27 B

Means with different letters in same column are significantly different at 5% probability

Table III: Mean % of leg-deformed *Varroa* female mites in native, *Apis mellifera jemenitica,* and exotic, *A.m. carnica,* honeybees in central Saudi Arabia

Experiments				
Races	**1st**	**2nd**	**3rd**	**General mean**
A. m. jemenitica	73.21	46.07	49.58	56.29 n.s.*
A. m. carnica	56.51	63.55	68.75	62.94 n.s.

*means not significantly different at 5% probability

Table IV: Mean % of dorsal shield-deformed *Varroa* female mites in native, *Apis mellifera jemenitica* and exotic, *A.m. carnica,* honeybees in central Saudi Arabia

Experiments				
Races	**1st**	**2nd**	**3rd**	**General mean**
A. m. jemenitica	26.80	34.43	50.45	37.18 n.s.*
A. m. carnica	41.23	36.48	31.25	36.32 n.s.

*means not significantly different at 5% probability

CONCLUSION

Honey bee races, *A.m. jemenitica* and *A.m. carnica* hybrid, exhibited different rates of hygienic behavior. The removal of dead brood in native race was significantly higher than that of exotic one. The deformed mites as a result of grooming behavior were also significantly higher in colonies of native race than those of exotic one. The surpassing defensive behavior of the native race, compared to the exotic one, may be due to its different genetic structure as well as its compatibility with local environmental conditions. Further selective breeding programs can enhance the levels of hygienic and grooming behaviors in such colonies and provide significant resistance to diseases and parasites.

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