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Grooming behavior in relation to varroa (*Varroa destructor*) infestation level of Carniolan honey bee colonies (*Apis mellifera carnica*)

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Abstract

Grooming behavior is one of the traits that enables *Apis cerana* resistance to ectoparasitic mite *Varroa destructor*. The study examines the proportion of damaged mites on 52 colonies (5 genotypes) of *A. m. carnica* in Croatia. The mites were collected on the sheets of the screen bottom boards, analyzed for the location of injury for each mite and correlated to infestation of colonies with *V. destructor* mites. In total, 2,425 *V. destructor* mites were analyzed, with an average of 46.6 per colony. The average (±SE) of 12.69±0.93% mites with injuries were recorded ranging from 0% to 32%. No significant difference (ANOVA, F (4, 47)=0.503, P=0.734) was found between different genotypes. The most common injuries of mites were located on the first pair of legs, while the lowest proportion of injuries were found on the idiosoma. No significant correlation was found between grooming behavior and colony infestation rate. Grooming behavior did not affect the colony infestation rate in tested colonies.

Keywords: *Apis mellifera carnica*, colony infestation, grooming, *Varroa destructor*

Introduction

With grooming, bees disperse pheromones and remove foreign particles or bodies from the body itself (such as dust, pollen or pests) (Boecking and Spivak, 1999). To a certain extent bees are able to remove *Varroa destructor* mite from the body with the legs or sometimes with the mandibles. Grooming behavior is highly expressed in the Asian honey bee, *Apis cerana*, which is the original host of *V. destructor.* For *A. cerana* grooming behavior is one of the main resistance traits against the mite (Peng et al., 1987). In western honey bee (*Apis mellifera*) grooming behavior is noted, however, it is not as pronounced as in *A. cerana* (Büchler et al., 1992; Fries et al., 1996). The grooming behavior can be evaluated by calculating the proportion of injured mites found on the sheet of the screen bottom board. Injury on the mite is noticed as a lack of the part of the leg, complete absence of one or few legs or injury

on the idiosoma. In the research on *A. m. intermissa*, Boecking and Ritter (1993) found an average of 19.3% of injured mites, while Moosbeckhofer (1992) states that the portion of injured dark mites in Carniolan bee colonies (*A. m. carnica*) is 9.5%. Rosenkranz et al. (1997), however, cited a share of as much as 45% of injured mites found. The aim of this research was to investigate for the first time to what extant grooming behavior is expressed in Carniolan bees in Croatia and how it affects the infestation rate of the colonies.

Material and methods

The study was carried out in Čeminac (N 45°40'12''; E 18°40'40"; 90 m altitude), County of Osječko-baranjska. Altogether 52 colonies in Langstroth hives with five different genotypes of Carniolan bees (10 or 11 per group) were included in the research. Groups were headed by daughters of five different mother queens and were half-sisters within the group. The groups 1 to 3 originate from mother queen lines that were in breeding program for last 5 generations. The group 4 originate from mother queen that was never under any artificial selection, while the group 5 originate from the mother queen bred at Bee Institute in Kirchhain (Germany). The rate of the grooming behaviour was obtained by calculating the proportion of damaged mites found on the sheet of the screened bottom board. Adult (dark) mites were collected from sheets every three days from 20.7.2016. until 10.8.2016. The sheets were coated with a layer of edible oil to prevent mite from escape or possible damage to mites by the predators. For each colony, a minimum of 30 adult mites were collected, allowing a high quality analysis (Bienefeld et al., 1999). The collected mites were examined under the 40x magnification and the existence and exact location of the damage was recorded. Percentage of damaged mites was calculated for each colony and group. After mite collection, number of bees and brood cells was determined with Liebefeld method (Imdorf et al., 1987). Colony infestation rate with *V. destructor* mites was examined after collecting the mite samples. Adult bee infestation was determined with the soapy water wash method, brood infestation rate was analyzed by checking at least 250 pupae in a stage of purple eyes or older. Natural daily mite fall was observed during collecting the samples.

Data for grooming behavior followed the normal distribution (Shapiro-Wilk test). One-way ANOVA followed by the Bonferroni post hoc test was used to determine the difference between groups and the type of injury. Descriptive phenotypic mean values with the standard error are given. Other data were not normally distributed and the difference between groups was evaluated with Kruskal-Wallis test. Spearman's correlation coefficient was used for correlation analysis. For statistical analysis, SPSS v20 (SPSS Inc., 2011) was used.

Results

On the 10th of August, the average adult bee infestation rate was 2.28±2.67% and no significant difference between groups was found (H (4)=0.684, P=0.953). The average brood infestation was 10.59 ±12.69% with no significant difference between groups (H (4)=1.826, P=0.768). The average natural daily mite fall was 27.24±65.12 and no significant difference between groups were found (H (4)=2.494, P=0.646). On

average the colonies had 12,170 bees, ranging from 9,769 (group 4) to 13,178 (group 1) with no significant difference between groups (H (4)=4.633, P=0.327). The average number of brood cells was 12,489, with a range from 11,484 in group 4 to 13,585 in group 5. No significant difference between groups were found (H (4)=2.613, P=0.624).

A total of 2,425 mites were analyzed, averaging 46.6 per colony. On average 12.69±0.933%of the mites had injuries, with high variability (from 0% to 32%). The highest proportion of injured mites was found in group 3 (14.73±2.076) and the lowest in group 2 (11.3±2.749) (Figure 1). However, no significant difference (ANOVA, F (4, 47)=0.503, P=0.734) was found between the groups. Also, no significant differences were found between the groups due to the type of damage on the mite (ANOVA, P>0.05). The most common type of injury on mites was located on the first pair of legs in all groups. The percentage of injuries on the second and the third pair of legs was basically the same in all groups, while the last pair of legs was rarest damaged. The lowest proportion of injuries were found on idiosoma. The relationship between grooming behavior and varroa mite infestation rate is shown in Table 1. There was no significant correlation found for grooming behavior with adult bee infestation, brood infestation or natural daily mite fall. The significant positive correlation was found between natural daily mite fall, adult bee and brood infestation.

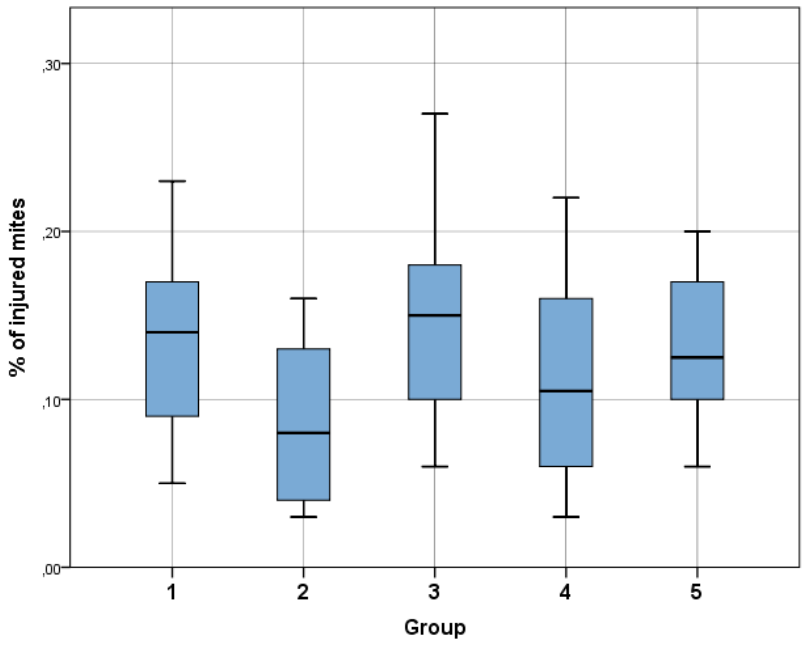

Figure 1. The average proportion of damaged mites per group

Table 1. Spearman's correlation index between grooming, bee infestation, brood infestation and natural mite fall

		Adult bee infestation	Natural daily mite fall	Brood infestation
Grooming	Correl. coeff.	0.014	-0.075	-0.003
Adult bee infestation	Correl. coeff.		0.706**	0.633**
Natural daily mite fall	Correl. coeff.			0.742**

**Correlation is significant at the 0.01 level (2-tailed).

Discussion

Damage on the collected mites can be a consequence of cleaning the infested brood cells (Rosenkranz et al., 1997) or predators' attacks (such as ants). To get the most accurate data, only dark brown adult mites for the analysis were used. A lot of research has shown the genetic variability of grooming behavior and that the injuries on mites are result of bee grooming activity (Büchler et al., 1992; Ruttner and Hänel, 1992; Guzman-Novoa et al., 2012). A similar rate of damaged mites was found in other studies (Moosbeckhofer, 1992; Fries et al., 1996; Guzman-Novoa et al., 2012; Invernizzi et al., 2015), while in Africanized bees in America was determined greater proportion of damage on mites (Rosenkranz et al., 1997). Locke and Fries (2011) state around 30% of the damaged mites in surviving population in Sweden. However, they report that grooming is not reason for resistance. Kruitwagen et al. (2017) came to the same conclusion in the Netherlands, same as Oddie et al. (2017) in Norway. All of these studies confirm that grooming behavior does not play a significant role in the survival of resistant populations in Europe. Lack of correlation between injuries on mites and varroa infestation rate of colonies in this study also confirm this. The most common injuries on mites were found on the first pair of legs and the least type of injuries were located on the idiosoma, which is consistent with other studies (Ruttner and Hänel, 1992; Rosenkranz et al., 1997; Bienefeld et al., 1999). In this research there was no correlation between proportion of injured mites and the colony infestation rate, as was found by Moosbeckhofer (1992). A few studies on Africanized bees have found correlation between mite injuries and mite population in the colonies. Arechavaleta-Velasco and Guzmán-Novoa (2001) found strong negative correlation between the grooming behavior and infestation rate of colonies, which was confirmed by Mondragon et al. (2005) who also found negative correlation.

Conclusions

The variability of grooming behavior on tested colonies ranged from 0% to 32% of damaged mites with an average of 12.69%. There was no significant difference found between groups. Most commonly, the injuries on the mite were located on the first pair of legs. No correlation was found between grooming behavior and infestation rate of colonies. These results lead to a conclusion that frequency of grooming

behavior in tested colonies in Croatia is similar to other studies on Carniolan bees. Grooming behavior did not have an effect on colony infestation rate in tested colonies.

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