EVALUATION OF ETHOLOGICAL AND PRODUCTION TRAITS IN VARIOUS GENOTYPES OF MACEDONIAN HONEY BEE SUBSPECIES

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Abstract

The honey bee colonony is a complex society which has a wide range of behaviors. The most important are swarming, colony defense and hygienic behavior. These ethological traits have an impact on the honey yields and are of significant interests of the honey bee breeders. Therefore the ethological characteristics are recognized in selection and breeding programs. According to the breeding programs selection is directed to reduction of swarming and colony defense behavior and increased expression of hygienic behavior. The aim of this research was to evaluate these characteristics in 100 honey bee colonies from four genotypes (A, B, C and D) of the autochthonous honey bee population (*Apis mellifera macedonica)* in one of the registered honey bee queen breeding stations, located in Ohrid region, during 2016.The research included: scoring of the defensive and the swarming behavior of honey bee colonies according to four point system, testing hygienic behavior using Pin-test method and scoring according five point system as well as determining honey yield by weighing of extracted honey in kg per honey bee colony and scoring the honey yield according to the four point system. The results did not show statistically significant differences concerning swarming behavior and honey yield between colonies from the four genotypes. The results have shown statistical significant differences in the average cleaning success (hygienic behavior) after 24 hours, between A and B (p=0.0096) genotypes, and in the defensive behavior, between A and D (p=0.0166) and between C and D (p=0.0333) genotypes.

Keywords: *Apis mellifera macedonica*, swarming behavior, defensive behavior, hygienic behaviorur, honey yield.

Introduction

Honey bee breeding is a common goal of honey bee breeders and honey bee researches during the last 150 years, but it does not improve as rapidly as the breeding of other live stock. In honey bees *(Apis mellifera L*) the breeding activity is based on the performance evaluation at the level of each honey bee colony (Cauia *et al.* 2010). The evaluation consists, generally, of the appreciation of economic and ethological traits in order to be clasified and make selection of the best parrents. Most significant ethological traits which have an impact on the productivity of honey bee colonies (honey yield) are swarming, defense and hygienic behavior and thus have been recognized in selection and breeding programs (Ruttner, 1972).

Although swarming is a natural way of reproducing honey bee colonies, beekeepers believe it is a negative characteristic since it decreases the strength of the colony during honey extraction, which reflects the honey yields and other honey bee products. Beekeepers enacted breeding strategies to reduce expression of swarming behavior, in opposition to natural selection (Ruttner, 1972; Möbus, 1983; Poklukar, 1999; Moritz and Southwick, 1992).

Another well-known type of behavior in honey bees is colony defense consisting of recognizing of predators, alerting nest mates and enacting anti-predator behavior (Collins *et al*., 1980; Moritz *et al.,* 1987; Breed *et al.,* 2004). Improvement of this characteristic is particularly the goal of many selection programs; however, care should be taken and optimal value need to be found between the wish of the beekeepers to work with calmer bees and the danger, calm bees to become pray of

natural enemies (wasps, birds or mammals). Hygienic behavior in honey bees is a heritable trait of individual honey bee workers that confers colony-level resistance against various brood diseases. Hygienic honey bee workers detect and remove dead or diseased brood from sealed cells. However, this behavior is quite rare, with only c. 10 % of unselected colonies showing high levels of hygiene. Beekeepers can potentially increase this by screening colonies for hygiene and breeding from the best. However, the level of hygiene expressed by a colony is variable, which poses a challenge to colony selection (Bigio *et al.* 2013).

The aim of our research was to evaluate these characteristics in the Macedonian honey bee (*Apis mellifera macedonica*) which is an autochthonous subspecies (race) in Republic of Macedonia (Ruttner, 1988b; Kiprijanovska *et al.,* 2012; Uzunov *et al*., 2009, 2014).

Material and methods

The investigation was carried out during 2016 in one of the registered honey bee queen breeding stations located in Ohrid region (village Vapila). One hundred honey bee colonies divided in four genotypes (A, B, C and D) of the autochthonous honey bee colonies (*A. m. macedonica)* were evaluated for the following characteristics: swarming behavior, defense behavior, hygienic behavior and honey yield.

The swarming behavior was evaluated during the swarming period (May-June), according to the four-point system (Ruttner, 1972): 4 points = the colony has shown no swarming behavior for the entire season (has not constructed any queen cells); 3 points = queen cells were found in aroutine control. After the necessary expansion (additional supers) and the breaking up of the queen cells, no more queen cells were constructed; 2 points = queen cells were repeatedly found in routine control and swarming was difficult to control; 1 point = the colony swarmed or swarming could be prevented only by extensive intervention (e.g. nucleus).

The defensive behavior was tested every time the colonies in the apiary were visited and an average score was calculated. This trait was also evaluated according to the four-point system (Ruttner, 1972): 4 points = no protection and no smoke necessary to avoid stings; 3 points = no protection and only a little smoke necessary, no stings; 2 points = much smoke and protection (veil, gloves) necessary to avoid stings and in order to be able to work unimpeded; 1 point = working without a lot of smoke, face protection and gloves is not possible; stings occur even at a great distance from the apiary.

The hygienic behavior was tested using the pin-test (Gramacho and Gonçalves, 2003): 100 cells containing white or pink-eyed pupae pierced through the cell capping with an entomological needle size n° 2 (diameter = 0.45 mm). The removal of the killed pupae by the adult honey bees was estimated after a time interval of 24 hours. Colonies were scored according to the proportion of cleaned brood comb cells 24 hours after killing the pupae on the following five point system (Gregorc and Locar 2010): 5 > 95 %; 4 = 90 – 95 %; 3 = 80 – 89 %; 2 = 70 – 79 % and 1 < 70 %.

The honey yield was determined by weighing the extracted honey in kg per honey bee colony and was scored according to a fourpoint system (Gregorc and Locar 2010): 1 and 2 - below apiary average honey yield and 3 and 4 - above apiary average honey yield.

The data were analyzed using MS Excel program to carry out descriptive statistics on evaluated traits and analysis of variance (ANOVA) and HSD Post-Hoc test to analyze the differences between genotypes.

Results and discussion

The data for the evaluation of the tested traits are given in Table 1 and the differences in the tested traits between genotypes are given in Table 2.

Swarming behavior – the data in Table 1 show that related to swarming behavior the average scoring values are ranged from 3.87 to 4.0. All honey bee colonies from genotypes A and C have not constructed any queen cells for the entire season. The queen cells were found in three honey bee colonies from genotype B and in one honey bee colony from genotype C, but after the breaking up

of the queen cells no more queen cells were constructed. The results did not show statistically significant differences concerning swarming behavior between colonies from the four genotypes (Table 2) whereas swarming behavior in all 4 genotypes was positively evaluated.

Table 1. Colony scores for observed traits

Trait	Genotype A			Genotype B			Genotype C			Genotype D		
	$\bar{X}$	Sd	min-max	$\bar{X}$	Sd	min-max	$\bar{X}$	Sd	min-max	$\bar{X}$	Sd	min-max
Swarming behavior (1-4)	4	0	4-4	3,87	0,3378	3-4	4	0	4-4	3,96	0,2	3-4
Defense behavior (1-4)	3,88	0,3316	3-4	3,48	0,7141	2-4	3,84	0,3741	3-4	3,40	0,7071	2-4
Hygienic behavior (1-5)	2,20	0,7637	1-4	3,16	1,1060	1-5	2,44	1,1575	1-5	2,64	1,1503	1-5
Honey yield (1-4)	2,72	0,7371	1-4	2,60	0,7637	1-4	2,56	0,5830	2-4	2,48	0,6531	1-4

Our results correspond to the report of Adam (1968) and Ruttner (1988 a) who reported that a low swarming tendency is one of the main values of the Macedonian honey bees. The low swarming tendency of *A. m. macedonica* was established by Antevski (2015), who reported that the average scoring values in his research is 3.85. Uzunov *et al*. (2014) found variation in swarming tendency of the Macedonian honey bees depending on the origin-low swarming tendency in the Bulgarian population (3.30), higher in the Greek one (2.62) and intermediate in the Macedonian population (3.27) and they explain that this probably reflects the wide range of the *A. m. macedonica* origin which was covered.

Defense behavior - the results have shown statistical significant differences in the defensive behavior, between A and D and between C and D genotypes (Table 2). The results in Table 1 show that genotype D had the lowest average grade (3.40) and three honey bee colonies were evaluate with 2 within this genotype. In genotype B, three honey bee colonies were evaluate with 2 as well, but the average grade was higher (3.48). The honey bee colonies from the genotypes B and D may be characterized as moderately defensive and much smoke and protection is necessary to avoid stings and in order to be able to work unimpeded. Genotype A was the highest scored (3.88), while genotype C had an average grade of 3.84. In these two genotypes, no honey bee colonies were evaluated with a score of less than 3, and they may be characterized as calm.

Our results correspond with the researches made by Uzunov (2013) and Antevski (2015). Uzunov (2013) reported that two genotypes of *A. m. macedonica* were characterized as calm related to defense behavior with an average grade of 3.5. Antevski (2015) also characterized the honey bee colonies from the two genotypes of *A. m. macedonica* as calm, with grades 3.70 and 3.63, respectively.

Hygienic behavior - significant statistical difference between evaluated honey bee colonies was indicated in regard to the hygienic behavior (Table 2). Genotype B was scored as a line with most expressive hygienic behavior whereas genotype A as a line with the least expressive hygienic behavior (Table1). Average percentage of dead pupae removed by the adult honey bee was 83.92 % in genotype B and 75.84 % in genotype A. In genotype B four honey bee colonies were evaluated with 5 (the percentage of dead pupae removed was > 95 %), in genotype C one honey bee colony was evaluate with 5, in genotype D two, and in genotype A no honey bee colonies were evaluate with 5.

According to Spivak and Gilliam (1998 a), colonies that either begin to or completely remove 95 % or more of pupae within 24 hours of death are considered to be "rapid hygienic" and in populations of honey bees that have not been selected specifically for rapid hygienic behaviour, approximately 10% of the colonies will carry this trait. Our results show that on apiary level 8 % of honey bee colonies meet the requirement for rapid hygienic behaviour which gives opportunity for choosing colonies for selection purposes.

Table 2. Analysis of variance of colony scores for observed traits

Trait	One- ANOVA way analysis					
Swarming behavior	Source of variance	Sum of squares	Df	MS	F	P
	Between Groups:	0.2605	3	0.0868	2.2592	0.0865
	Within Groups:	3.6894	96	0.0384		
	Total:	3.9499	99			
Defense behavior	Source of variance	Sum of squares	Df	MS	F	P
	Between Groups:	4.5100	3	1.5033	4.7726	0.0038*
	Within Groups:	30.2395	96	0.3150		
	Total:	34.7495	99			

Tukey HSD Post-hoc Test			
Genotype	Mean Difference	95% Confidence Interval	P
		Lower Bound / Upper Bound	
A vs D	-0.4800	-0.8951 / -0.0649	0.0166*
C vs D	-0.4400	-0.8551 / -0.0249	0.0333*

Hygienic behavior	Source of variance	Sum of squares	Df	MS	F	P
	Between Groups:	12.5100	3	4.1700	3.7315	0.0138*
	Within Groups:	107.2800	96	1.1175		
	Total:	119.7900	99			

Tukey HSD Post-hoc Test			
Genotype	Mean Difference	95% Confidence Interval	P
		Lower Bound / Upper Bound	
A vs B	0.9600	0.1782 / 1.7418	0.0096*

Honey yield	Source of variances	Sum of squares	Df	MS	F	p
	Between Groups:	0.7500	3	0.2500	0.5282	0.6640
	Within Groups:	45.4400	96	0.4733		
	Total:	46.1900	99			

Honey yield - the average honey yield per colony in the researched genetic lines was very close and ranges between 9.1 kg (genotype A) and 10.5 kg per honey bee colony (genotype C). The minimum

honey yield per bee colony (6.8 kg) was found in genotype A and the highest of 12.4 in genotype C, which is very close to the national average in the last ten years (www.stat.gov.mk, 2016).
There was no significant statistical difference concerning this characteristic (Table 2) which corresponds with the results from the researches made by Uzunov (2013), and Antevski (2015), which also found no differences between the genotypes of *A. m. macedonica* that they investigated in terms of honey yield.
The average scores for this characteristics were also very close and range from 2.48 to 2.72 (Table 1). Low estimates show there are very few honey bee colonies in all four genotypes that differ significantly in yield relative to the average, which limits the possibility of selecting honey bee colonies for selection purposes in relation to this trait.

Conclusions

Swarming behavior in all 4 genotypes was positively scored , because they showed a very low swarming tendency. Statistically significant differences were not identified between the genotypes.
Defensive behavior in all 4 genotypes was positively scored too, where statistically significant differences were identified between the genotypes. The honey bee colonies from the genotypes B and D may be characterized as moderately defensive and the honey bee colonies from the genotypes A and C may be characterized as calm. Statistically significant differences between the genotypes give opportunity for choosing honey bee colonies for selection purposes related to this characteristic.
The influence of the genotype was determined for the hygienic behavior, where statistically significant differences were identified between genotype A and genotype B. The honey bee colonies which meet the requirement for rapid hygienic behaviour can be selected for producing honey bee queens to achieve high hygienic standards rather quickly.
There is no statistically significant difference between the genotypes in the honey yield which limits the possibility for choosing honey bee colonies for selection purposes in relation to this trait, but with the improvement of other characteristics, an increase in the honey yield can be expected, as many studies have identified the positive correlation between the ethological and production treids of honey bee subspecies

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