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Hygienic behavior and fat body development in worker bees (*Apis mellifera* L.)

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Abstract. *Testing for the level of manifestation of the hygienic behavior of 32 bee colonies from the local honey bee (Apis mellifera L.) from three apiaries has been carried out. Depending on the level of manifestation of the cleansing instinct, two groups of bee colonies have been formed - hygienic (these clean over 95% of the cells with dead brood up to the 48th hour after killing the brood) and non-hygienic (these clean less than 95% of the cells with dead brood up to the 48th hour). Worker bee samples have been taken from the tested bee colonies to determine the level of fat body development. The fat body is important for the bee organism, since in addition to being a fat and carbohydrate storage depot, it is also considered to be the center of metabolism in insects. The analysis of the results from the present study revealed that the mean value of the level of fat body development in worker bees from the group of hygienic bee colonies was 2.76±0.038. In the group of non-hygienic colonies this value was 7.61% lower (2.55±0.079). This difference is statistically significant at p≤0.05. Significant differences have been found in the development of the fat body in worker bees from apiaries at a distance of 100 km without regard to hygienic behavior and according to hygienic behavior. The results obtained suggest that there is a correlation between the level of fat body development and the hygienic behavior in bees.*

Keywords: honey bees, hygienic behavior, fat body

Introduction

The main natural mechanism for combating pathogens in bees is hygienic behavior. It is expressed in a social immune response at the level of the bee colony, through collective actions of individual specimens (Wilson-Rich et al., 2009). As early as the 1930s, Tarr (1937) referred to the mechanism of manifestation of hygienic behavior, namely, the detection of sick or dead brood by worker bees and its removal from the hive. This limits the spread of the disease in the bee nest. Hygienic behavior is inherited in the offspring (Rothenbuhler, 1964; Taber, 1982; Lapidge et al., 2002) and is a desirable trait for bee resistance to diseases in the brood, as well as to the ectoparasite mite *Varroa destructor* (Spivak and Reuter, 1998; Boecking and Spivak, 1999; Harbo and Harris, 1999; Boecking et al., 2000; Palacio et al., 2000; Spivak and Reuter, 2001; Stanimirovic et al., 2001, 2002a). The manifestation of hygienic behavior in the bees, although genetically determined, is influenced by a number of factors, such as: strength of the bee colonies (Stanimirovic et al., 2002b); climate features (Mondragon et al., 2005); the release of nectar in nature (Trump et al., 1967; Momot and Rothenbuhler, 1971). In Bulgaria, the influence of the stimulating feeding of the bee colonies during the autumn period on the degree of development of the fat body of worker bees has been established (Shumkova et al., 2019).

Availability of pollen and honey is of great importance to the bee individuals and is particularly important for the development and functioning of many organs, including the fat body. According to Maurizio (1961), the fat body development is a major criterion for the physiological state and life expectancy of the insects. According to Toth and Robinson (2005), worker bees accumulate fat reserves after consuming pollen and honey, and in the last 1-2 weeks of their life these stocks decrease. The above changes are also related to the functions that worker bees perform in the bee colony. Young bees feeding the brood exhaust their protein and lipid stores accumulated in the fat body to produce royal jelly (Crailsheim, 1992). Lotmar

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(1939) observed differences in the degree of fat body development in summer and winter bees. According to Fluri and Bogdanov (1987), the fat body increases in size in the winter bees. It accumulates protein pellets in the fall that are used by the bee's body during wintering (Maurizio, 1961). According to researches carried out by numerous authors, it is actively involved in metabolic processes accumulating fat, carbohydrates and proteins that are related to the metabolism of other organs as well (Roma et al., 2010; Hoshizaki, 2013; Aljedani, 2018). According to Arrese and Soulages (2010), it can store energy and release it when the bee's body needs it. Vitellogenin is synthesized in the fat body, which is involved in the production of food for the brood (Seehuus et al., 2007) and in the regulation of food collection behavior (Nelson et al., 2007). According to a number of studies, vitellogenin acts as an antioxidant (Seehuus et al., 2006) and is involved in enhancing innate immunity (Amdam et al., 2004; Amdam et al., 2005).

The established link between the hygienic behavior of bees and the fat body, the survival rate and the resistance of the bee colonies to adverse conditions and diseases determined the purpose of this study.

The objective of the study is to investigate the relationship between the degree of hygienic behavior and the development of the fat body in worker bees (*Apis mellifera* L.).

Material and methods

Testing bee colonies for level of manifestation of hygienic behavior

The study was conducted in 2019 at the beginning of the active bee season (April – May).

Testing for the level of manifestation of hygienic behavior of 32 bee colonies from the local honey bee (*Apis mellifera* L.) from three apiaries in Bulgaria has been carried out:

- Apiary in the village of Madzherito, Municipality of Stara Zagora, altitude 152 m - 13 bee colonies.
- Training apiary of Trakia University - Faculty of Agriculture, Stara Zagora, altitude 160 m - 13 bee colonies.
- Apiary village of Okop, Tundzha municipality, altitude 114 m - 6 bee colonies.

The town of Stara Zagora and the village of Madzherito are located in the same region with a distance between them of 8 km, and the village of Okop is located 100 km from the town of Stara Zagora and the village of Madzherito.

In addition, the colonies involved in the experiment have been aligned by strength, amount of sealed worker brood and food supplies. Bee colonies are not fed additional pollen and sugar solution.

The testing for hygienic behavior of bee colonies was conducted in 2019 by the method of Newton and Ostasiewski (1986) - killing the brood by piercing with a thin pin. In the present study, a square frame 5x5 cm (100 cell) was used for testing (Gurgulova et al., 2003; Lazarov et al., 2016) – Figure 1.

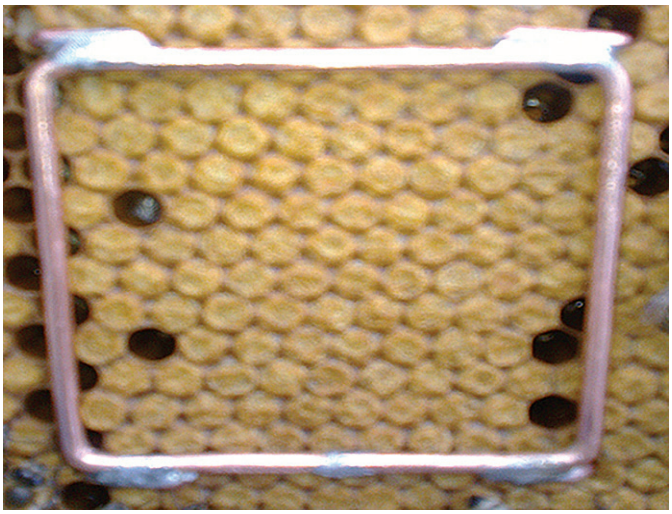


Figure 1. Marking the test field on the comb of brood with a square frame

From each bee colony, a bee comb with sealed worker brood has been selected. The frame is placed on a brood comb containing young pupae – 14th – 15th day of worker bee development (Büchler et al., 2013; Olszewski et al., 2013). The empty cells in the testing area, outlined by the template, are counted and are not taken into account during the analysis. The caps of all tested cells with brood are punctured with a thin entomological pin (size No 1, without destroying them), thus killing the pupae in them. According to the research of Spivak and Downey (1998), Marcangeli (2001) and the Bulgarian authors Gurgulova et al., (2003), Jeliazkova and Gurgulova (2003) the first reading of the uncapped and cleaned cells was performed on the 48th hour. Depending on the time required by the bees to detect and clean the cells with the dead brood, two groups of bee colonies have been formed: hygienic - colonies that clean more than 95% of the cells in the tested area at the 48th hour after killing the brood; non-hygienic - clean less than 95% of the cells at the 48th hour.

Determination of the level of development of the fat body in worker bees

After reporting the test results, young non-flying worker bees were taken to determine the degree of development of the fat body. Worker bee samples have been taken from all bee colonies - 60 worker bees from each colony.

The level of fat body development of 671 bees has been determined using the Maurizio (1954) method - 5-point scale.

The data obtained were processed variationally and

statistically on Descriptive statistic – Normal distribution with software STATISTICA 12, Copyright © Stat Soft Inc. 1984-2014 (StatSoft, 2014). It was used to establish the reliability of the obtained statistical differences with One – Way ANOVA analysis.

Results and discussion

Testing bee colonies for hygienic behavior

Table 1 summarizes the test results of bee colonies for level of manifestation of hygienic behavior.

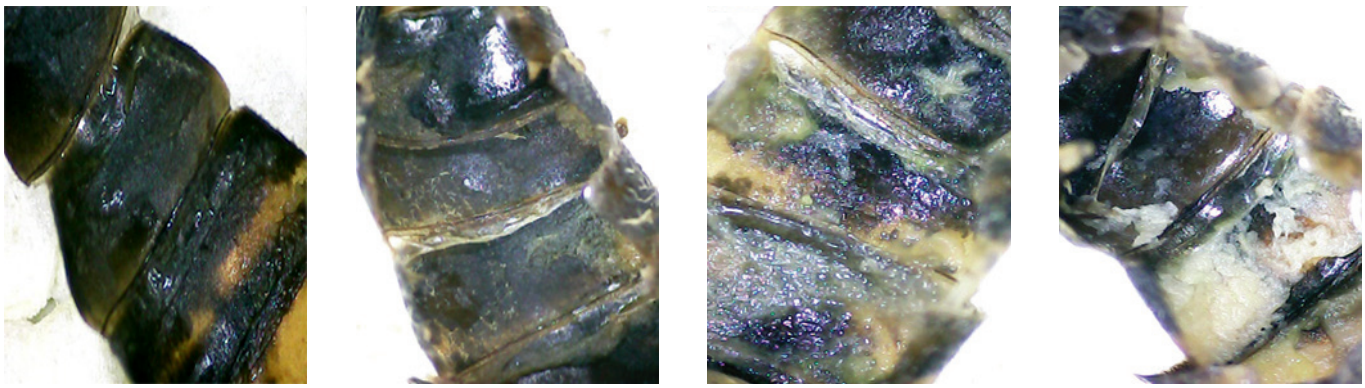
Table 1. Results for uncapped and cleaned cells in testing bee colonies for hygienic behavior

	Uncapped and cleaned cells (%), 48thhour				
	mean	SE	SD	min	max
Hygienic bee colonies (n=27)	99.49	0.185	0.960	96.590	100.000
Non-hygienic bee colonies (n=5)	87.16	4.463	9.980	71.280	94.790

A total of 27 colonies have been defined as hygienic and 5 colonies - as non-hygienic. In bee colonies with high level of manifestation of hygienic behavior, at the 48th hour the cleaned cells averaged 99.49±0.185% of the total number of cells with dead brood. The bee colonies from the second group (low hygiene level – non-hygienic) cleaned 87.16±4.463% of the tested cells on the 48th hour, leaving 12.84% uncleaned ones. A probable reason for the low percentage of detected and cleaned cells with dead brood by the worker bees in non-hygienic bee colonies is that they detect dead brood at a later stage. According to some researchers (Masterman et al., 2000, 2001; Gramacho and Spivak, 2003), hygienic bees have a more acute olfaction to the smell of sick body than bees with poor manifestation of hygienic behavior. This allows them to remove the dead brood before rotting processes have developed.

Determination of the level of development of the fat body in worker bees

Worker bees have been taken from the defined two groups of bee colonies - with high (hygienic) and low (non-hygienic) level of manifestation of hygienic behavior according to the level of development of the fat body. Figure 2 shows the degree of development of the fat body of worker bees included in our study.



2nd degree 3rd degree 4th degree 5th degree

Figure 2. Different degrees of development of the fat body

The obtained results are statistically processed and presented in Table 2.

Table 2. Degree of fat body development in worker bees from bee colonies with different levels of hygienic behavior - total for the three apiaries

Groups according to hygienic behavior	Degree of development of the fat body					p
	mean	SE	SD	min	max	
Hygienic bee colonies (n= 27) (n=544 bees)	2.76	0.038	0.895	1	5	0.017 *
Non-hygienic bee colonies (n= 5) (n=127 bees)	2.55	0.079	0.888	1	5	

* The reported differences between groups were significant at p≤0.05

It was found that in worker bees from the first group (hygienic colonies), the mean value of the studied trait was higher than that in bees from non-hygienic colonies. The fat body in bees from the hygienic bee colonies has a development degree of 2.76±0.038, which is 56% of the maximum indicated grade 5 – (Table 2). In worker

bees from the non-hygienic bee colonies this value is 2.55±0.079, which compared with the maximum degree of development of the fat body (5) by the Maurizio (1954) scale used, is 51%. The comparative analysis of the results shows that the average value of fat body development in worker bees from colonies with a high degree of hygienic behavior is 7.6% higher than the development of fat body in bees from nonhygienic colonies. The established difference between the mean values in the development of the fat body is statistically proven at significance level p≤0.05 – (Table 2). The significant differences found indicate better nutrient availability in worker bees from the colonies with high level of manifestation of hygienic behavior than those from the non-hygienic colonies.

Due to the greater distance (100 km) and some difference in the honey-bearing vegetation of an apiary in the village of Okop compared to the apiaries in Stara Zagora and Madzherito, a comparison was made between the condition of the fat body of worker bees without regard to hygienic behavior and according to hygienic behaviour. The data are presented in Tables 3 and 4.

In total for the apiaries in Stara Zagora and the village of Madzherito, the development of the fat body of the worker bees (regardless of hygienic behaviour) is 2.85 on average, which is 24.2% more than the average values for the studied bees in the village of Okop. The reported differences have a high degree of reliability (p≤0.001) – Table 3.

Table 3. Degree of fat body development in worker bees from bee colonies of apiary in the village of Madzherito and Training apiary of Trakia University compared to apiary in the village of Okop, regardless of hygienic behavior

Apiaries	Degree of development of the fat body					p
	mean	SE	SD	min	max	
Village of Madzherito and Training apiary of Trakia University (n=544 bees)	2.85	0.038	0.882	1	5	0.000 ***
Village of Okop (n=127 bees)	2.16	0.065	0.732	1	4	

*** The reported differences between groups were significant at p≤0.001

Regarding bee colonies with a high degree of hygienic behaviour, the data show statistically proven differences (p≤0.001) between the degree of development of the fat body of worker bees from the apiary in the village of Madzherito and Training apiary of Trakia University compared to the condition of the fat body of bees from the apiary in the village of Okop (Table 4). The established average value for the condition of the fat body of the bees from the hygienic bee colonies in the apiary in the village of Okop (2.11) is 27.7% lower compared to the fat body of the bees from the other two apiaries taken together (2.92).

Table 4. Degree of fat body development in worker bees from bee colonies of apiary in the village of Madzherito and Training apiary of Trakia University compared to apiary in the village of Okop, according to hygienic behavior

Apiaries	Degree of development of the fat body					p
	mean	SE	SD	min	max	
Hygienic bee colonies (n=544 bees)						
Village of Madzherito and Training apiary of Trakia University (n=439 bees)	2.92	0.041	0.868	1	5	p≤0.001 ***
Village of Okop (n=105 bees)	2.11	0.068	0.698	1	4	
Non-hygienic bee colonies (n=127 bees)						
Village of Madzherito and Training apiary of Trakia University (n=105 bees)	2.58	0.087	0.896	1	5	Unreliable differences
Village of Okop (n=22 bees)	2.41	0.182	0.854	1	4	

*** The reported differences between groups were significant at p≤0.001

For bee colonies with a low degree of hygienic behavior, small and statistically unproven differences in the condition of the fat body were found in the worker bees from the three apiaries.

It can be seen from Table 4 that for the worker bees from the apiary in the village of Okop, regardless of the hygienic behavior, no maximum degree of development of the fat body was reported (degree 5 according to Maurizio (1954) method - 5-point scale).

The results from this study suggest that there is a link between hygienic behavior and the condition of the worker bee fat body. Higher values have been found for the condition of the fat body in worker bees from colonies with high degree of manifestation of hygienic behavior compared

to those with low manifestation of hygienic behavior. This, in turn, is an indicator of better availability of stock nutrients, respectively better physiological status of bee specimens.

The information from other studies shows that the presence of reserves in the fat body of bees influences a number of physiological and behavioural processes. According to Mattila et al. (2001) and Shumkova et al. (2019) changes in the behavior and reserves in the fat body affect the life span of worker bees and support their successful wintering. On the other hand, Winston (1987) points out that the vitality of bees is related to their behavior and physiological condition and depends on the role of worker bees in the bee colony.

Considering the importance of the fat body as a body of stock nutrients for the bee organism involved in a number of biochemical processes, hygienic behavior, as a natural mechanism of resistance of bees to diseases, we believe that the results obtained supplement the scientific information on the importance of hygienic behavior on the viability, resistance to adverse climatic and environmental conditions and diseases of bee specimens and bee colonies in general.

Conclusion

Significantly higher values ($p \leq 0.05$) have been found for the development of the fat body in the worker bees from colonies with high degree of manifestation of hygienic behavior compared to those with low manifestation of hygienic behavior – 2.76±0.038 and 2.55±0.079, respectively.

The comparative analysis of the results for the development of the fat body in the worker bees from apiaries Madzherito and Trakia University, Stara Zagora compared to that in the bees from apiary in the village Okop, at a distance between the apiaries 100 km, shows the following:

- regardless of the manifestation of hygienic behavior, the values for the development of the fat body in the bees from apiaries Madzherito and Trakia University, Stara Zagora total are 24.2% higher than the development of the fat body in the bees from apiary in the village Okop and the reported differences are highly reliable;
- in bee colonies with a high degree of manifestation of hygienic behavior, the values of development of the fat body of worker bees from apiaries Madzherito and Trakia University, Stara Zagora in total, compared to the condition of the fat body of bees from apiary in the village Okop, are 27.7% higher - $p \leq 0.001$;
- for bee colonies with a low degree of hygienic behavior, small and statistically unproven differences in the condition of the fat body were found in the worker bees from the three apiaries.

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