

## Hygienic behaviour and dimensions of the chitin body parts in worker bees (*Apis mellifera* L.)

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### Abstract

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In the present study a total of 28 bee colonies from the local honey bee (*Apis mellifera* L.) have been tested for level of expression of hygienic behaviour. On the basis of the test, two groups of bee colonies have been identified - hygienic (these clean over 95% of the cells with dead brood up to the 48<sup>th</sup> hour after piercing) and non-hygienic (these clean less than 95% of the cells with dead brood up to the 48<sup>th</sup> hour). Morphometric measurements of the chitin body parts of the worker bees have been carried out on 12 features. Three of the studied morphological features (forelegs, upper mandibles, and proboscis) relate to the cleaning and polishing of the beecomb cells and maintaining hygiene in the bee nest. The upper mandible and the foreleg parts are new features not included in the measuring methods used in beekeeping. High degree of reliability ( $p \leq 0.001$ ) between hygienic and non-hygienic bee colonies has been determined for 7 morphological features - length of the fore wing and the first part of the cubital cell, length of femur and width of first tarsus of the foreleg, length of proboscis and length and width of the upper mandible. For the feature number of hooks on the hind wing a low degree of reliability ( $p \leq 0.05$ ) has been established. The results obtained in the present study concerning differences between morphometric signs of bee workers from hygienic and non-hygienic bee colonies complement the information available so far on the factors influencing the expression of hygienic behaviour in bees.

*Keywords:* *Apis mellifera*; hygienic behaviour; chitin body parts

### Introduction

Hygienic behaviour in honeybees is an important indicator that is inherited in offspring (Rothenbuhler, 1964; Taber, 1982; Moritz, 1988; Lapidge et al., 2002). Its expression is related to the identification and disposal of infected and dead larvae and bees outside the hive. Thus, the spread of the disease within the bee nest is limited (Nemkova, 2004; Palacio et al., 2010). Bee colonies that have high level of expression of hygienic behaviour are resistant to a number of brood and bee diseases: *American foulbrood*; *Ascopherosis*; *Varroosis* (Gilliam et al, 1988; Moretto, 1993; Hornitzky, 1995; Komissar, 1996; Spivak, 1996; Taber, 1996; Petrov, 1997; Boecking & Spivak, 1999; Boecking et al., 2000; Spivak & Reuter, 2001; Stanimirovic et al., 2001, 2002; Gurgulova et al., 2003; Ibrahim & Spivak, 2006; Harris, 2007; Darkazanli, 2008). Bee colonies with a high level of expression of hygienic behaviour require little or no treatment, there by reducing the risk of contamination of bee products with chemical substances (Spivak & Reuter, 2001). According to some authors, the level of expression of hygienic behaviour can be increased by using selective breeding (Harbo & Harris,

1999; Boecking et al., 2000; Lapidge et al., 2002; Büchler et al., 2010).

Studies by a number of authors (Trump et al. 1967; Momot & Rothenbuhler, 1971; Spivak & Gilliam, 1993; Stanimirovic et al., 2002; Mondragon et al., 2005) show that various factors influence the expression of hygienic behaviour: strength of the bee colonies, quantity of young bees in the colony, availability of protein and carbohydrate food; the nectariferous potential of the beekeeping area (abundant nectar production); some seasonal factors, etc. On the other hand, according to Spivak & Reuter (1998), the level of expression of hygienic behaviour does not affect the honey productivity of bee colonies.

There are numerous studies on the relationship between the dimensions chitin body parts of worker bees and productivity (honey, pollen, and wax productivity) of bee colonies. According to a number of authors, there is a correlation between the productivity of colonies and some morphological features of worker bees - proboscis length, fore and hind wing dimensions, dimensions of fore and hind leg, volume of the honey stomach, live mass of bees, etc. (Szabo & Lefkovich, 1988; Popravko, 1992; Krivtsov, 1995; Mostajeran et al., 2002). Knowing the anatomy and

physiology of the honey bee and the activities that bee workers perform in the bee colony, it can be seen that some chitin body parts (upper mandibles, proboscis, forelegs) are related to the cleaning of the bee nest.

In Bulgaria, studies on establishing the relationship between the hygienic behaviour of bee colonies and the dimensions of the chitin body parts of worker bees are scarce and controversial. According to Zhelyazkova & Gurgulova (2003), there is no reliable correlation between the dimensions of the chitin body parts of worker bees and the level of expression of hygienic behaviour. Measurements have been made using a binocular magnifying glass by the method of Alpatov (1948). On the other hand, Lazarov (2018) finds that in almost 50% of the measured morphological characteristics of worker bees from colonies with different levels of expression of hygienic behaviour, there are significant differences in the values of those body parts that are related to the productivity of bee colonies. The study makes use of a modern, fast and accurate computer method for determining the exterior traits of worker bees (Lazarov, 2016). Both studies measured 12 and 23 chitin body parts of the bee body, respectively, but a small proportion could be related to cleaning activities (respectively, hygienic behaviour). It would also be important to include other exterior features such as: dimensions of upper mandibles, dimensions of the different parts of the forelegs. The inclusion of the above-mentioned chitin body parts in morphological measurements of bees from colonies with various level of expression of hygienic behaviour will complement scientific information relating to factors influencing the expression of hygienic behaviour in bee colonies.

The objective of the present study is to investigate whether there is a relationship between the level of expression of hygienic behaviour in worker bees (*Apis mellifera* L.) and the dimensions of some chitin body parts related to cleaning activities of bees.

## Material and Methods

The present study uses bee colonies with bees from the Bulgarian honey bee (*Apis mellifera* L.). Testing for level of expression of hygienic behaviour and measuring the chitin body parts of worker bees were made during the active beekeeping season of 2018 and 2019. A total of 28 bee colonies from two apiaries have been tested:

- Training apiary of Trakia University - Faculty of Agriculture, Stara Zagora - 12 bee colonies.
- Apiary located in the village of Okop, Tundzha municipality - 16 bee colonies.

### Testing bee families for level of expression of hygienic behaviour

Bee colonies involved in the experiment have been aligned by strength, amount of sealed worker brood and food supplies in advance. To determine the level of expression of hygienic behaviour of bee colonies the method by

Gurgulova et al. (2003) has been used, similar to the method by Petrov (1997). From each colony combs with big area of sealed worker brood have been selected. A template 5 x 5 cm (100 work cells) was placed on the brood combs. The empty cells within the tested area have been counted and excluded from the analysis. The caps of cells with sealed worker brood have been punctured with a thin entomological pin (without destroying them), thus killing the pupae within the template area. At the 48<sup>th</sup> hour after puncturing the cells uncapped and cleaned by the bees have been counted. Depending on the time required by bees to detect dead brood and clean the cells outlined by the template, two groups of bee colonies have been formed: hygienic - cleaning more than 95% of the cells at the 48<sup>th</sup> hour after killing the brood; non-hygienic - cleaning less than 95% of the cells at the 48<sup>th</sup> hour.

### Morphological measurements of chitin body parts of worker bees

To determine the dimensions of some body parts in 19 of the tested bee colonies samples from non-flying worker bees (60 bees per sample) have been sampled and stored in ethyl alcohol. From the chitin body parts of 380 bees (20 bees from each bee colony), microscope slides have been prepared and the following 12 morphological traits have been measured: length and width of forewing; length of first and second part of the cubital cell; number of hooks on hind right wing; total proboscis length; length of femur and tibia on fore right leg; length and width of the first tarsus of the fore right tarsus; length and width of upper mandible.

Microscopic preparations have been prepared by the method of Alpatov (1948) and Abou-Shaara et al. (2013), while measurement of chitin parts has been performed by using an AutoCAD computer software (Mladenović et al., 2011; Lazarov, 2016).

$$\text{Tarsal index (\%)} = \frac{\text{width of first tarsus}}{\text{length of first tarsus}} * 100$$

On the basis of some of measurements, cubital index (Goetze, 1964) and tarsal index (Bizhev & Van, 1975) have been calculated using the following formulas:

$$\text{Cubital index} = a/b,$$

where: a - first part of the cubital cell;  
b - second part of the cubital cell;

The survey data have been processed variationally and statistically on a computer – Statistika software.

## Results

Table 1 summarizes the results from testing bee colonies for level of expression of hygiene behaviour. Of the tested 28 bee colonies, 22 have been identified as hygienic (78.6%) and 6 were non-hygienic (21.4%).

Of interest are the results in terms of the three traits included in the study - the fore leg with its individual parts (femur, tibia, first tarsus), upper mandible (length and width) and number of hooks on the hind wing.

For the separate apiaries, the following has been found: Training Apiary at Trakia University apiary - total number of tested colonies 12, of which 75% with high level of hygienic behaviour and 25% with low level of hygiene; apiary village of Okop - total number of tested colonies 16, of which 81.3% hygienic and 18.7% non-hygienic. It can be seen from Table 1 that for all bee colonies included in the testing, the percentage of uncapped

and cleaned cells in the colonies with high level of hygienic behaviour ranged from 97.87% to 100% and in those with low expression of hygiene from 83.33% to 94.79%.

Table 2 presents the summarized results (for both apiaries) from measuring the chitin body parts of worker bees from colonies with high and low levels of hygienic behaviour.

**Table 1. Results from testing bee colonies for level of hygiene by apiaries**

Apiary	Beehive No	Number of tested cells	Uncapping and cleaned cells, 48 <sup>th</sup> hour		Level of expression of hygienic behavior
			number	(%)	
1. Apiary -Trakia University, Stara Zagora (n = 12)	1	94	94	100	Hygienic
	2	99	99	100	Hygienic
	3	97	95	97.94	Hygienic
	5	96	91	94.79	Non-hygienic
	10	89	89	100	Hygienic
	12	97	90	92.78	Non-hygienic
	13	97	95	97.94	Hygienic
	16	95	93	97.89	Hygienic
	17	95	95	100	Hygienic
	18	94	94	100	Hygienic
	19	84	70	83.33	Non-hygienic
	22	94	92	97.87	Hygienic
2. Apiary (over 100 bee colonies) – village of Okop, Tundzha Municipality (n = 16)	132	92	92	100	Hygienic
	150	93	93	100	Hygienic
	063	94	94	100	Hygienic
	033	92	92	100	Hygienic
	129	89	89	100	Hygienic
	049	97	97	100	Hygienic
	064	94	92	97.87	Hygienic
	071	89	89	100	Hygienic
	054	94	88	93.62	Non-hygienic
	034	98	98	100	Hygienic
	138	100	92	92	Nonhygienic
	066	83	76	91.57	Nonhygienic
	126	99	99	100	Hygienic
	022	97	95	97.94	Hygienic
	041	100	98	98	Hygienic
	035	82	82	100	Hygienic

In the fore leg, significantly higher values for femur length ( $p \leq 0.01$ ) and width of first tarsus ( $p \leq 0.001$ ) have been found in bees from colonies with low levels of expression of hygienic behaviour (Table 2). Although in the length of the first tarsus the reported differences in dimensions in bees from hygienic and non-hygienic colonies are unreliable, the calculated tarsal index based on the length and width of this digit is highly reliable ( $p \leq 0.001$ ). Regarding the size of the upper mandible, the established values for length and width are higher in bees from colonies with low hygienic level - Table 2. The differences obtained for these traits between colonies with high and low levels of expression of hygienic behaviour are significant at  $p \leq 0.001$ . The mean value of the trait number of hooks on the hind wing is higher in bees from hygienic colonies

compared to bees from non-hygienic colonies, and the reported difference has low level of significance ( $p \leq 0.05$ ), (Table 2).

In the present study, bee samples for measuring chitin parts were taken at the end of the beekeeping season of 2018 (September), i.e. the effect of the season factor has been excluded. According to studies in our country (Bizhev et al., 1983), bees are of the highest quality during this period. The same authors point out that when analysing the results regarding the dimensions of the chitin body parts of worker bees, it is necessary to take into account the differences in the area of beekeeping (terrain, climate). In this regard, in Tables 3 and 4 data from measurements of chitin body parts in samples of worker bees from different apiaries have been presented.

**Table 2. Dimensions of chitin body parts in worker bees from colonies with high and low levels of hygienic behaviour - summarized data for both apiaries. Reliability of differences**

No	Morphological characteristics	Level of expression of hygienic behaviour				n=380
		Hygienic		Non-hygienic		p
		Mean±SE	Min/ Max	Mean±SE	Min/ Max	
1	Total length of fore wing, mm	8.95±0.012	8.41/9.46	9.03±0.015	8.56/9.43	0.000 ***
2	Width of fore wing, mm	3.14±0.006	2.89/3.34	3.16±0.007	2.96/3.33	0.115
3	Length of I <sup>st</sup> part of cubital cell, mm	0.60±0.003	0.43/0.69	0.58±0.004	0.42/0.70	0.000 ***
4	Length of II <sup>nd</sup> part of cubital cell, mm	0.24±0.002	0.17/0.31	0.25±0.003	0.17/0.32	0.077
5	Cubital index, (Goetze, 1964)	2.51±0.027	1.72/4.00	2.37±0.034	1.56/3.59	0.002 **
6	Length of femur of fore leg, mm	2.11±0.006	1.66/2.32	2.14±0.007	1.93/2.28	0.001 ***
7	Length of tibia of fore leg, mm	1.53±0.010	1.18/1.95	1.55±0.015	1.20/1.88	0.276
8	Length of I <sup>st</sup> tarsus of fore leg, mm	1.30±0.005	1.01/1.59	1.29±0.007	1.07/1.56	0.485
9	Width of I <sup>st</sup> tarsus of fore leg, mm	0.35±0.002	0.26/0.46	0.37±0.003	0.29/0.45	0.000 ***
10	Tarsal index, fore leg, %	27.33±0.195	20.44/35.92	28.64±0.271	22.79/37.84	0.000 ***
11	Length of proboscis, mm	5.64±0.035	4.77/6.83	5.87±0.057	4.43/6.88	0.000 ***
12	Length of upper mandibles, mm	1.35±0.003	1.10/1.47	1.38±0.005	1.22/1.60	0.000 ***
13	Width of upper mandibles, mm	0.32±0.002	0.25/0.41	0.34±0.003	0.26/0.43	0.000 ***
14	Number of hooks of hind wing	21.82±0.103	18/26	21.44±0.173	17/26	0.048 *

**Table 3. Dimensions of chitin body parts in worker bees from colonies with high and low levels of expression of hygienic behaviour - apiary Trakia University, Stara Zagora. Reliability of differences**

No	Morphological characteristics	Level of expression of hygienic behaviour				n=140
		Hygienic		Non-hygienic		p
		Mean±SE	Min/ Max	Mean±SE	Min/ Max	
1	Total length of fore wing, mm	9.06±0.015	8.81/9.46	9.10±0.016	8.83/9.43	0.048*
2	Width of fore wing, mm	3.21±0.008	3.00/3.33	3.18±0.007	3.03/3.31	0.032*
3	Length of I <sup>st</sup> part of cubital cell, mm	0.60±0.005	0.43/0.69	0.58±0.005	0.42/0.67	0.034*
4	Length of II <sup>nd</sup> part of cubital cell, mm	0.24±0.003	0.17/0.29	0.25±0.004	0.20/0.32	0.000***
5	Cubital index, (Goetze, 1964)	2.58±0.046	1.72/3.94	2.32±0.045	1.56/3.19	0.000***
6	Length of femur of fore leg, mm	2.16±0.010	1.78/2.32	2.17±0.008	2.01/2.28	0.568
7	Length of tibia of fore leg, mm	1.68±0.014	1.41/1.95	1.67±0.015	1.24/1.88	0.850
8	Length of I <sup>st</sup> tarsus of fore leg, mm	1.25±0.010	1.03/1.49	1.25±0.010	1.07/1.44	0.917
9	Width of I <sup>st</sup> tarsus of fore leg, mm	0.36±0.003	0.28/0.46	0.36±0.004	0.29/0.43	0.553
10	Tarsal index, fore leg, %	29.17±0.368	20.74/35.92	28.91±0.444	22.79/37.84	0.649
11	Length of proboscis, mm	5.96±0.071	4.83/6.83	6.37±0.043	5.31/6.88	0.000***
12	Length of upper mandibles, mm	1.34±0.006	1.10/1.47	1.36±0.005	1.25/1.44	0.015*
13	Width of upper mandibles, mm	0.32±0.003	0.25/0.40	0.33±0.003	0.26/0.39	0.080
14	Number of hooks of hind wing	22.45±0.174	19/26	21.45±0.286	17/26	0.002**

The data show that in 78.6% (9 traits and 2 indices) of the morphological characteristics included in the study, the mean values obtained were higher in bees from colonies with low level of hygienic behaviour compared to those from hygienic colonies. For 7 of the morphological characteristics (for 5 traits and 2 indices) significant differences in values of medium to high level ( $p \leq 0.01$ ) to high ( $p \leq 0.001$ ) have been reported (Table 2).

In bees from colonies with high level of hygiene, higher mean values have been found for the traits length of first part of the cubital cell ( $p \leq 0.001$ ), length of the first tarsus of the fore leg (unreliable difference) and number of hooks of the hind wing ( $p \leq 0.05$ ). The data from Table 2 show that the limits of variation in the dimensions

of the studied chitin body parts of worker bees are the greatest for the traits: total length of fore wing, the difference between the minimum and maximum values being 1.05 mm for colonies with high level of hygiene and 0.87 mm for non-hygienic bee colonies; proboscis length - difference of 2.06 mm and 2.45 mm, respectively. Difference between the minimum and maximum values in the range of 0.5 mm - 0.8 mm has been determined in the dimensions of the separate parts of the fore leg, namely: length of femur and first tarsus only in bees from colonies with high level of hygiene (0.66 mm and 0.58 mm, respectively); length of tibia in bees from both groups - hygienic and non-hygienic (0.77 mm and 0.68 mm).

**Table 4. Dimensions of chitin body parts in worker bees from colonies with high and low levels of expression of hygienic behaviour - apiary village of Okop, Tundzha municipality, county of Yambol. Reliability of differences**

No	Morphological characteristics	Level of expression of hygienic behaviour				n=240
		Hygienic		Non-hygienic		p
		Mean±SE	Min/ Max	Mean±SE	Min/ Max	
1	Total length of fore wing, mm	8.90±0.014	8.41/9.34	8.97±0.023	8.56/9.34	0.010**
2	Width of fore wing, mm	3.12±0.006	2.89/3.34	3.14±0.010	2.96/3.33	0.132
3	Length of I <sup>st</sup> part of cubital cell, mm	0.60±0.003	0.48/0.69	0.57±0.006	0.46/0.70	0.002**
4	Length of II <sup>nd</sup> part of cubital cell, mm	0.24±0.002	0.17/0.31	0.24±0.003	0.17/0.32	0.317
5	Cubital index, (Goetze, 1964)	2.48±0.033	1.76/4.00	2.42±0.049	1.75/3.59	0.356
6	Length of femur of fore leg, mm	2.08±0.007	1.66/2.28	2.11±0.009	1.93/2.25	0.038*
7	Length of tibia of fore leg, mm	1.46±0.009	1.18/1.79	1.42±0.013	1.20/1.68	0.020*
8	Length of I <sup>st</sup> tarsus of fore leg, mm	1.31±0.006	1.01/1.59	1.33±0.008	1.21/1.56	0.225
9	Width of I <sup>st</sup> tarsus of fore leg, mm	0.35±0.002	0.26/0.44	0.38±0.004	0.32/0.45	0.000***
10	Tarsal index, fore leg, %	26.51±0.201	20.44/33.87	28.36±0.309	22.92/35.71	0.000***
11	Length of probostics, mm	5.49±0.035	4.77/6.82	5.36±0.049	4.43/6.67	0.048*
12	Length of upper mandibles, mm	1.35±0.003	1.15/1.44	1.39±0.008	1.22/1.60	0.000***
13	Width of upper mandibles, mm	0.33±0.002	0.25/0.41	0.35±0.004	0.30/0.43	0.000***
14	Number of hooks of hind wing	21.54±0.122	18/26	21.43±0.199	18/24	0.645

It can be seen from the Tables 3 and 4 that at 50-58.3% (without indices) of the studied morphological traits higher values have been reported for the chitin body parts in worker bees from colonies with low level of expression of hygienic behaviour. In 66.7-71.4% of the above-mentioned traits, the reported differences between colonies with high level of hygiene are reliable.

The analysis of results from the two apiaries (Tables 3 and 4) shows that the recorded means values for the traits length and width of the upper mandible are higher in bees from colonies with low level of hygiene compared to those from hygienic colonies. The differences in length of upper mandible for the bees from the apiary at Trakia University

are reliable ( $p \leq 0.05$ ) as well as in length and width of the mandible for the bees from the apiary in the village of Okop ( $p \leq 0.001$ ). Concerning the dimensions of the separate parts of the fore leg in the bee samples from the different apiaries, the results obtained are different. For the traits length of tibia in the fore leg and number of hooks on hind wing, the values reported in the bee samples from both apiaries are higher in colonies with high level of expression of hygienic behaviour. The following differences have been statistically proven: number of hooks on hind wing for the bees from the apiary at Trakia University ( $p \leq 0.01$ ); length of tibia on the fore leg for the apiary in the village of Okop ( $p \leq 0.05$ ).

## Discussion

The results of this study with respect to all morphological traits studied indicate that the mean values obtained for the different apiaries (Tables 3 and 4), as well as the summarized data (Table 2), are within the range of variation established by other Bulgarian authors for the honey bee *A. mellifera* L. distributed in our country (Tsonev, 1964-1965; Mitev et al., 1972; Velichkov, 1976; Petrov, 1995; Petrov et al., 2001; Nenchev, 2007, 2008; Nenchev et al., 2007).

The results obtained in our study confirm the data from the available scientific literature that the variability of exterior traits (dimensions of chitin body parts) in honey bees varies within a very narrow range of 2-5% (Martynov, 1976; Bizhev et al., 1983; Sauthier et al., 2017). Therefore, these traits are genetically determined to a very large extent, unlike the economic and biological traits (winter hardiness, productivity, etc.), which are influenced by the environmental conditions and technology of rearing the bee colonies.

According to Couvillon et al. (2010), the average dimension of worker bees may be different for individual bee colonies, but it does not increase and decrease over the life span of bee specimens in a colony from hatching to death.

Respectively to Raine et al. (2006) and Raine & Chittka (2008), differences in body dimensions of worker bees result from the division of labor. The claims in this regard are contradictory. Studies by a number of authors suggest that larger worker bees are better at activities relating to provision of food supplies: carry more nectar in one flight (Goulson et al., 2002; Spaethe & Weidenmuller, 2002), have better developed vision, thermal, and odour receptors (Heinrich, 1979; Spaethe & Chittka, 2003; Spaethe et al., 2007); remember more quickly landmarks along the flight path and retain this information for a longer period of time

(Worden et al., 2005). On the other hand, there are studies according to which larger bees are better in nursing, an activity mainly performed by worker bees with smaller sizes of chitin body parts (Cnaani & Hefetz, 1994). The data provided contradict the hypothesis that differences in bee body size result from the division of labor.

According to some authors (Farris et al., 2001; Ben-Shahar et al., 2002; Whitfield et al., 2003; Fjerdingstad & Crozier, 2006) in the honey bee (*Apis mellifera* L.), worker bees in the same colony have similar values in terms of body shape and dimensions of its individual chitin parts, but differ in some age-related physiological traits that in turn lead to different behaviour. Invernizzi & Corbella (1999) and Arathi et al. (2000) found that the amount of young non-flying bees aged 11-15-17 days is of great importance for the expression of hygienic behaviour.

## Conclusions

In this study for measurement of young non-flying bees significantly higher values have been found for the dimensions of some chitin body parts (total fore wing length, femur length and width of the first tarsus on the fore leg, tarsal index, length of proboscis, length and width of upper mandibles) in worker bees from bee colonies with low level of expression of hygienic behaviour (non-hygienic). The morphological characteristics mentioned above are related to the cleaning activities of worker bees, respectively hygienic behaviour. Therefore, the data obtained in the study give reason to comment on the existence of a relationship between the dimensions of some chitin body parts of worker bees and hygienic behaviour, but no firm conclusion can be made. The results of this study are a first step in this direction and contribute to complementing the scientific information concerning factors affecting the level of expression of hygienic behaviour. In conclusion, we believe that this research needs to be expanded.

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