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The Influence of Mite *Varroa destructor* Infestattion Rate on Morphometric Indicators of *Apis mellifera* L. Workers

[1]Galina S. Mishukovskaya, [2]Varis R. Tuktarov, [1]Ruzil Kh. Avzalov, [3]Alfia V. Andreeva, [1]Fitrat G. Yumaguzhin, [2]Marat G. Giniyatullin, [6]Vener N. Sattarov, [3]Elsa R. Ismagilova, [5]Ivan V. Chudov and [2]Dmitriy V. Shelekhov
[1]Department of Animal Physiology, Biochemistry and Feeding,
[2]Department of Beekeeping, Private Animal Science and Breeding,
[3]Department of Infectious Diseases, Animal Hygiene and Veterinary Inspection,
[4]Department of Bioecology and Biological Education,
[5]Department of Morphology, Pathology, Pharmacy and Non-Contagious Diseases,
Federal State Budgetary Educational Institution of Higher Education,
Bashkir State Pedagogical University, 50 Letiya Oktyabrya Street 34, 450001 Ufa, Russia
[6]Department of Bioecology and Biological Education,
Federal State Budgetary Educational Institution of Higher Education,
Bashkir State Pedagogical University, Named after M. Akmulla,
3-a October Revolution Str., 450001 Ufa, Russia

Abstract: The effect of varroosis infestation rate on morphometric indicators of wild bees living in natural conditions in the mountain-forest zone of the Republic of Bashkortostan was studied. There were no significant differences in the wing size while the high infestation rate of bee colonies with *Varroa destructor* (22-24 %) resulted in smaller proboscis length by 12%. Significant differences were also, found for mean values of the length and the width of the 3rd abdomen sternite of worker bees from the colonies with varying degrees of infestation ($p>0.001$) and the length and the width of the wax mirror. No significant dissimilarities in the size of the 3rd tergite and the cubic index were found. The measurement of the fat body cells characteristics by the method of Tuktarov and Ishmeeva showed that the size of both fat cells and bees oenocytes for the colonies with higher infestation rate was significantly less ($p>0.001$) than that for the bees from lower infested colonies.

Key words: *Apis mellifera*, wild bees, varroosis, morphometry, fat body infestation rate, conditions

INTRODUCTION

Varroa destructor is the most common and one of the most dangerous *Apis mellifera* ectoparasites. It has been a serious threat to honeybees worldwide for decades. Currently, the varroosis is recognized as one of the most important factors increasing the risk of the "collapse of bee colonies". Although, the mite itself cannot be the cause of collapse, however, it is the carrier of pathogens of various viral infections being considered as the main causes of this phenomenon (Asha *et al*., 2013; Bernardi and Venturino, 2016; Santos *et al*., 2016).

The mite infests both the brood (larvae and pupae) and adult bees. The damage caused by the mite is manifested in lower weight of the beesemerging from the cell, reduced energy content in a hemolymph and higher number of deformed bees (Asha *et al*., 2013; Contzen *et al*., 2003).

The behavior of bees also changes their flight activity decreases, spatial orientation worsens. The lifespan of invasive bees is reduced, the death of colonies during the wintering increases (Van Dooremalen *et al*., 2012). On average, the colony affected by the invasion lives without appropriate treatment for no more than 3-5 years.

Corresponding Author: Galina S. Mishukovskaya,
Federal State Budgetary Educational Institution of Higher Professional Education,
Bashkir State Agrarian University, 50 Letiya Oktyabrya Street 450001 Ufa, Russia

Another negative effect of Varroa mite on bee colonies is manifested in the fact that as the result of its influence on the body size and the metabolism gene expression, Varroa invasion increases the sensitivity of bees to insecticides (Rinkevich *et al*., 2017).

It is difficult to overestimate the influence of *Varroa destructor* on the state of the modern beekeeping. No other parasite does so much harm to the world's apiaries. The main reason is that Varroa mite has passed to the parasitism on a honey bee recently and the balance of parasite-host relationships has not developed yet. In addition, it is spread too quickly through the apiaries of all continents. Anti-varroosis treatment increases the cost of the beekeeping production and the risk of the chemical contamination (Rosenkranz *et al*., 2010).

The problem of the varroosis is especially, acute in the regions with long and severe Winters, the Republic of Bashkortostan is one of them. Infested colonies are unable to survive in long Winters and death of bees during Wintering in the region can be considerable as the case was in the long Winter of 2016-2017.

Despite the fact that the current researches in the field of the parasite impact on the honey bee cover many aspects, there are still a number of unsolved issues concerning primarily morphological changes in the bodies of infested bees.

In addition, these studies are conducted on the bees kept in conditions of apiaries. There is very little information about the impact of the varroosis on the populations of wild bees that are not covered by modern beekeeping technologies.

In our research we studied the influence of varroosis infestation rate on morphometric indicators of the wild bees that live in the natural environment.

MATERIALS AND METHODS

The studies were conducted in the reserve "Shulgan-Tash", located in the mountain-forest zone of the Republic of Bashkortostan (Russia). The research target were the colonies of wild bees. These colonies lived in the natural environment and didn't get any anti-varroosis treatment. A total of 3 groups of colonies (n = 5) were selected, the degree of varroosis of which was 1-3 (group 1), 12-15 (group 2) and 21-23% (group 3). The mite was separated by shaking bees in warm soapy water at 120 rpm for 1 h on a desktop shaker, then counting bees and crumbled mites. The Varroa mite infestation rate as a percentage was determined by dividng the number of mites by the number of bees in the sample and by multiplying the resulting value by 100 (Zemene *et al*., 2015).

For morphometric measurements 100 bees were selected from every group. The selected bees were stored in 70% ethanol and then dissected.

The dissected body parts of worker bees were placed on a glass slide and covered with another glass. The dissections were scanned, processed with Photoshop Software and the necessary measurements were carried out (Abou-Shaara *et al*., 2011).

The following exterior features of bees were estimated: length of proboscis, length of fore wing, width of fore wing, length of a third tergite, width of a 3rd tergite, length of a third sternite, width of a 3rd sternite, length of a wax mirror, width of a wax mirror, cubital index of bees.

To determine the degree of the fat body development 10 working bees were selected from bee colonies of all three groups when there are only bees of autumn generation.

In the process of dissecting the bee head was separated then tweezers for the last abdomen segment removed the intestine. Scissors were introduced into the resulting hole in the abdomen and made two longitudinal incisions in the border region of sternites and tergites. Sternites were removed and saline was injected into the abdomen with a pipette. At the same time, the white skin of the chitin walls of the air sacs surfaced and it was withdrawn, freeing the place of the incision for inspection with the help of a microscope at a 24-fold increase, the degree of fat body development was estimated by Maurizio (1954).

In order to study the morphometric indicators of the fat body of bees the method of Tuktarov and Ishmeeva (2008) was used. For this purpose isolated cells of this organ were obtained and the area of the cell and nucleus was measured. The abdomen of worker bees was fixed in 12% solution of formalin 0.1 M phosphate buffer (pH = 7.4), the fat body was separated from the chitin of the abdomen and placed in a fresh fixing solution. After the incubation in solution KOH by shaking the fat body was divided into cells. The preparations were stained with histological dyes, measured diameter (width) (a), length (b) of the cell and nucleus. The cell and the nucleus area (μm^2) were calculated by the formula:

$$S = 0.7854 \times a \times b$$

All received data are subjected to the statistical processing with the use of the student t-test.

RESULTS AND DISCUSSION

Wild bees living on the territory of the reserve "Shulgan-Tash" represent the burzyan population of the Central Russian or dark forest breed of bees.

The dark forest bee *Apis mellifera* is a subspecies of the honey bee *Apis mellifera* which was adopted in the process of the evolution to the conditions of the

continental climate with long cold winters. By the end of the 20th century bees of this subspecies survived only in some isolates. The most extensive area has a population of dark forest bees in the Urals and the Volga Region.

The vast majority of bee colonies in the Southern Urals is contained in frame hives and only about 1% live in natural and artificial hollows in tree trunks in the mountain-forest zone of the Republic of Bashkortostan where the state reserve "Shulgan-Tash" is located.

To study the morphometric characteristics of worker bees, samples were collected from the forests on the territory of the reserve. As shown by the results of the measurements, the main morphometric indicators of the body of the burzyan bees populations are within the standard of Central Russian breed of bees: average cubital index was 60.3%, the width of the third tergum-4.8 mm, the length of the proboscis -6.1 mm. The variability of these characteristics was in the range of CV = 1.6-9.5%. The cubic index was very variable (CV = 9.5%), the length of the trunk (Cv = 1.6%) and the width of the third tergum (CV = 2.2%) differed with less variability.

The inverse relationship between the value of individual indicators of the external structure of the bee's body and the rate of infestation with *Varroa destructor* mite is established.

There were no significant differences in the wing size but a high degree of bee colonies infestation (with lesions of 22-24%) led to the decrease in the length of the worker bee's proboscis by 12% (Fig.1).

Significant differences were revealed also for average values of length and width of the 3rd sternite of the worker bees abdomen from the colonies with different infestation rate ($p>0.001$) of both the length and the width of a wax mirror (Fig. 2). No significant differences were noted in the size of the 3rd tergum and the cubic index.

In addition to morphometric indicators the condition of the bee's fat body was also evaluated. The tissue of the fat body of bees from the colonies with the low infestation rate was of milky color with individual folds. Fat cells are oval in some types there are inclusions. On the scale of Maurizio (1954) the fat body of these bees was estimated at an average of 3 points.

With the average degree of invasion (12-14%), the bee's fat body was translucent in the form of a thin layer of cells of bluish-white color. Oval fat cells contacted with each other in the cytoplasm small vacuoles and a significant grain were visible. Oenocites had the form of small cells with shifted to the periphery of the cytoplasm nucleus. With the high (21-23%) degree of invasion, the fat body was transparent, through it the chitin cover was translucent. In the last two cases, the evaluation of the fat body was 1-2 points.

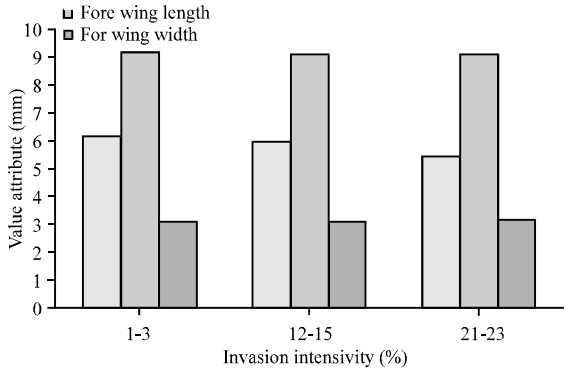

Fig. 1: The influence of varying degrees of infestation with *Varroa destructor* on the length of the proboscis and the size of the fore wing of worker bees

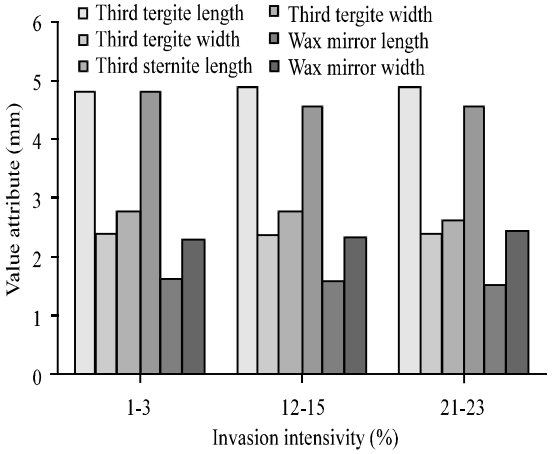

Fig. 2: The influence of different degrees of infestation by *Varroa destructor* on the parameters of the abdominal department of the worker bee's body

Table 1: The state of the fat body of worker bees at different degrees of infestation of Varroa destructor mite colonies

The intensity of infestation (%)	The area of the fat body cells of bees (μm²)		The bee fat body development, the scores (on a scale of Maurizio)
	Of the fats	Of the oenocytes	
1-3	2721±177.4	492±32.1	3.01±0.05
12-15	2165±159.1	401±28.7	2.11±0.03
21-23	1827±154.7	374±21.2	1.67±0.07

The measurement of fat body cells by the method of Tuktarov and Ishmeeva (2008) showed that the size of both the fat cells and bee's oenocytes from the colonies with the high infestation ratewas significantly $p<0.001$ in comparison with the bees from the colonies with the low infestation rate (Table 1)

Thus, the analysis of the obtained data demonstrates the relationship between several morphometric indicators

of the bee's body with the intensity of invasion. At the same time, it should take into account that these data reflect the impact of Varroaon wild bees living in the wildworld and are not subjected to the medical and preventive handling against the varroosis.

The study of the varroosis infestation in the wild honey bee colonies is of particular interest. There a few studies devoted to this problem (Locke, 2016; Lopienska-Biernat *et al*., 2017). Wild bees are exposed to the same infections and infestations that are characteristic for the colonies kept in frame hives. After *Varroa destructor* mite appeared in the apiaries of the burzyan district of the Republic of Bashkortostan in 1977-1979, it was soon discovered in wild bee colonies as well. It would be reasonable to assume that wild bees would die without regular treatment with acaricidal drugs. There is no need to discuss host adaptation to a new parasite in the process of the natural selection for such a short period though there is evidence that in some populations there is a decrease in the reproductive properties of the mite as a result of the host's adaptation to the invasion (Locke, 2016). However, burzyan wild bees survive in conditions of the varroosis invasion, since, 1977 without any special treatment. Moreover, the bee's infestation in the wild is by 20% lower than in apiaries where anti-varroosis treatment was carried out (Nikolenko *et al*., 2002; Pashayan *et al*., 2015). However, now compared to the pre-varroosis period bee colonies became weaker, they swar and die more. Our results indicate that Varroa infestation has an impact on the morphological indicators of the workers. The data about the size of individual parts of the body as a rule are used in bee systematics to determine their breed. The studied bees belong to the burzyan population of the central Russian bees and on the main exterior indicators they correspond to the standard of this breed. From other breeds it differs by larger body size, shorter proboscis and dark-gray color. Molecular genetic studies confirm the preservation of the purity of the gene pool of the burzyan wild dark forest bee and it belongs to the subspecies *A. m. mellifera* (Nikolenko, *et al*., 2002).

Morphometric features are also, used to assess the quality of individuals as they reflect the specifics of the development of economic and useful features. So, the wing length is associated with the potential ability of bees to collect food. The tergite size determines the degree of capacity of the bee abdomen including a honey crop. The wax mirrors area characterizes the activity of the builder bees (Contzen *et al*., 2013).

According to Rahimi *et al*. (2015) exterior features can be used to predict the productivity of bee families as a positive correlation between the length and width of the fore wing, the cubital index, the length and the width of the third and the fourth tergites and the sternite parameters and a negative correlation between the width of the hind wing and the productivity.

Thus, it is obvious that the morphological characteristics of honey bees are of great practical importance. However in the modern literature we have not met data about the impact of the varroosis invasion on the characteristics of the external structure of the worker bees. These features are laid in the metamorphosis process at the pupal stage and after the emergence of the bee from its cell throughout the life of the imago remain unchanged.

Varroa is an ectoparasite that feeds on the hemolymph of larvae, pupae and adult bees. Affecting mainly the bee's brood, it infests the formation processes of the imaginal structures during metamorphosis. In our studies statistically significant reduction of the sizes of imaginal structures in the worker bees under the influence of the mite is determined: the reduction of the length of proboscis, the length and the width of the 3rd sternite of the abdomen, the sizes of the wax mirrors. Moreover, the inverse relationship between the individual indicators value of the bee's exterior and the degree of colonies infestation with *Varroa destructor* was revealed. This is corresponded with the data of Bowen-Walker and Gunn (2001) who found that one-day bees in the infested colonies were characterized by the damp and dry weight as well as the content of water in the body of these bees. They also revealed the negative correlation between these indicators and the degree of bee colonies infestation.

The reduction under the influence of the mite Varroa of the volume of the hemolymph in the pupae body of worker bees in the later stages of the metamorphosis, the reduction of the protein concentration and free amino acids in the hemolymph affects the biosynthesis processes and consequently, the growth processes negatively as a result, perhaps, it was the reduction in the size of individual body parts identified in our research (Contzen *et al*., 2004).

The insect's fat body performs the function of the accumulation of reserve substances in the body and the release of the final metabolism products. The fat body development is one of the main indicators of the bee's physiological state as it depends on the winter survival, the lifespan of individuals, collecting and processing nectar.

In our studies the dependence of the worker bee's fat body on the degree of colonies invasion is determined. The development level and the fat cells size of the bee from high-invasive colonies were significantly lower compared to the bees from low-invasive colonies. Such

studies of Pashayan *et al*. (2015) on the example of the carpathian bees are kept in the conditions of apiaries, it was also shown that the increase of the invasion intensity leads to the delay in the fat body development.

The degree of fat body development reflects the state of metabolic processes in the bee's organism. Significant changes in a lipid profile, decrease of saturated fatty acids concentration and increase of unsaturated ones were revealed in the invasive prepupae of worker bees. Worker bees, being invasive at the pupal stage, do not accumulate proteins in a hemolymph including the main spare protein vitellogenin than the non-invasive bees (Roy-zokan *et al*., 2015; Zalewski *et al*., 2016). Vitellogenin synthesized in the fat cells of the body, not only performs the function of the reserve protein but it is also used for the synthesis of the larval feed, determines the division of a labor in the bee colony, it is a component of the immune system of bees, it contributes to the lengthening of the queen and over-wintering bees life. The lack of this vital component with a low fat body level under the varroosis influence leads to the bee's lifespan reduction, to the colony weakening and creates the threat of death during the wintering.

CONCLUSION

In the conditions of long and severe winters of mountain-forest zone of the Republic of Bashkortostan, this problem is particularly relevant. That is why the constant monitoring of the degree of colonies invasion with *Varroa destructor* is a compulsory condition of the unique population preservation of the burzyan wild bee.

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