Morphological characterization of the *Apis mellifera* drones in the Southern Urals

N R Gazizova¹, A G Mannapov², V N Sattarov³, V G Semenov⁴*, A I Skvortsov⁴ and I N Madeybekin⁴

¹Department of Therapeutics Occupational Pathology, Ufa Scientific Research Institute of Occupational Medicine and Human Ecology, 94 Stepana Kuvykina street, Ufa, 450106, Russia
²Department of Aquaculture and Beekeeping, Russian State Agrarian University – Moscow Agricultural Academy named after K.A. Timiryazev, 49 Timiryazevskaya street, Moscow, 127550, Russia
³Department of Physical Geography, Bashkir State Pedagogical University named after M. Akmulla, 3a October Revolution street, Ufa, 450008, Russia
⁴Department of Morphology, Obstetrics and Therapy, Chuvash State Agricultural Academy, 29 K Marx Street, Cheboksary, 428003, Russia

*E-mail: semenov_vg@edu.academy21.ru*

**Abstract.** The results of the morphological characterization of *Apis mellifera* drones are presented in order to identify the biopotential of the “clean” native population in bee yards in 12 regions of the Republic of Bashkortostan. The presence of I, I, O morphotypes – dark and gray in drones, is established. The O morphotype (dark) corresponds to the Central Russian subspecies, and the drones characterized by the O color (gray) can be related to the subspecies with a gray color of abdominal terga. The analysis of morphometric features in drones is identified as *Apis mellifera mellifera* allowed to distinguish the following characteristics: O (dark) morphotype with brown (coffee hue) or black hairs color on the abdomen; proboscis length (average) – 3.81 ± 0.08 ... 3.97 ± 0.03 mm, cubital index – 1.36 ± 0.07 ... 1.45 ± 0.09, wing length (front right) – 11.98 ± 0.49...12.08 ± 0.45 mm, wing width (front right) – 3.72 ± 0.21 ... 3.92 ± 0.23 mm, tergite length (third) – 2.90 ± 0.12 ... 3.51 ± 0.01 mm, tergite width (third) – 6.40 ± 0.02 ... 6.44 ± 0.04 mm, sternite length (third) – 2.64 ± 0.02 ... 2.66 ± 0.05 mm, sternite width (third) – 4.52 ± 0.10 ... 4.62 ± 0.07 mm, tarsal index – 49.59 ± 0.89 ... 52.15 ± 1.93%.

1. Introduction

The main limiting factors for existing subspecies and populations of honey bees (*Apis mellifera*, 1758) include: crossbreeding, which is associated with the introduction of various taxonomic groups [1-3]; extinction of native habitats and basic nectariferous and polliniferous plants [4, 5]; the environmental problems due to the pressure of exotoxicants and the air masses flow from industrial centers, spread of pathogens of various diseases [6, 7].

According to experts, in the Southern Ural, a serious problem for beekeeping is the gradual degradation of bees surviving subpopulation (Burzian wild hive) and the “purebred” honey bee disappearance - a unique Bashkir population of the Central Russian or dark European subspecies [8, 9].
It should be noted that as a result of anthropogenic interference, the change of the honey bees’ taxonomic status is also manifested in a number of other regions. For example, in Central Russia and Siberia only some of the $Apis mellifera$ populations have survived: Tatarsky, Vologdsky, Orlovsky, Novosibirsky, Chelyabinsky, Samarsky, etc. [10].

The current situation in the system of honey bee populations’ preservation requires the implementation of measures based on the modern and classical methodologies for assessing the $Apis mellifera$ taxonomic belonging based on a pure breeding system [11].

In this regard, scientific studies for assessing the morphological features of drones are of great theoretical and practical importance in order to identify the taxonomic belonging of honey bee colonies. A number of scientists note that the haploid nature of hereditary basis of drones allows getting an overview of the subspecific belonging of not only the drones themselves, but also the queen bee from which they come [12-13].

The purpose of this work is to conduct a comprehensive monitoring of morphological features of $Apis mellifera$ drones in the Southern Ural of the Republic of Bashkortostan to identify the population’s biopotential.

2. Experimental part

For research we took drones (8700 pcs.) from the bee yards in 12 regions of the Republic of Bashkortostan (Arkhangelsky, Abzelilovsky, Beloretsky, Baymaksky, Burzysansky, Ishimbaysky, Zianchurinsky, Gafuriysky, Zilairsky, Kugarchinsky, Khaibullinsky, Meleuzovsky), covering the territory of the Southern Ural. In all areas, except of Burzysansky region (7 bee yards), two bee yards were investigated. 300 drones were sampled from each apiary.

A comprehensive assessment of the morphological features of $Apis mellifera$ drones was carried out, which includes the morphometric assessment of drones, the assessment of chitinous covers on the abdomen (morphotypes) (figure 1), and the color of drones’ hairs (figure 2) [11].

Figure 1. Morphotypes’ classes of drones: O – dark; $I_1$ – small "islands" (in different positions); $I_s$ – wide saddle-backed stripe; $I_{ls}$ – small "islands" and a wide saddle-backed stripe; $I$ – large islands; $1R$ – 1 ring [11].

2.1. Determination of the coloration of chitin hairs of trunts on the abdomen

The scale of color according to Goetz (figure 2) reflects various shades of drones’ European breed hairs. "Gray" drones is characteristic to the Carpathian breed. Drones of the dark European breed have brown hair color. Sometimes in the same family, one can see drones with a rusty-brown and coffee-brown color. "Black" color can occasionally be found among the drones of the Caucasian breed. To determine the color of the chitin hairs, the drone was placed between two colored squares each of the four degrees of color, the abdomen to the left, to the light square, and the back to the right, to the dark one. If the background shines through the hairs, it means that their color coincided with the color of the field.

In the scale, the following designations of color’s degrees are distinguished: grey (gray – sand, gray – clayey), brown (brown-rusty – brown-coffee), black (black-smoky – black-soot), yellow (yellow-pease – yellow-quince).
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Figure 2. Color scale to determine the drones’ hairs color on the abdomen [11].

For measurements the Microscope biological Micromed 3-3-20M (Russia’s production) was used. For convenience, the ocular micrometer linear measurements were converted in mm, and the indices (tarsal index) were expressed in %.

The following features were studied: morphotype class, hairs color according to the Goetze color scale, proboscis length, right anterior wing length and width, third tergite and sternite, cubital and tarsal indices (figure 3-8). Literary sources have no complete data on the morphometric features of drones of the Central Russian subspecies, therefore, only 4 features were used to assess them, and the corresponding standards are available for them: O and I morphotype class, the color of hairs on the abdomen – brown or black, proboscis length – 3.6…4.0 mm, cubital index – 1.0…1.6 [11].

The killing of drones was carried out in a killing bottle (a jar for killing insects) “charged” with a 10% ammonia solution. A standard wide-mouthed jar (0.5 L) with a tight-fitting cap was used as a killing bottle, to prevent evaporation. Cotton wool was fixed under the cap, onto which the appropriate solution was applied. Drones were mercy killed for approximately 30 minutes. Subsequently, the insects were shaken from the killing bottle, pierced with an entomological pin No. 2 (ENTO SPHINX s.r.o., Czech Republic) in the thoracic section, according to the entomological requirements for insects preparation. Labeling was carried out for long-term storage of drones.

2.2. Methods for measuring morphometric features

To make measurements, the drone was taken with the thumb and index finger of the left hand, the head back, and the abdomen to the right. In this position, the proboscis is visible well (figure 3). During dissection, the sharp tweezers were taken, soft tissues were pierced between the brides, and the proboscis was tilted forward with a slight press. Then one of the two brides was captured and with it the entire proboscis was separated. On a light stand, all external parts, that is, the bridle, lower jaws and muscle remains, were separated with a second tweezer. The axis of the sucking apparatus with the mandibular root, the chin and tongue remained. The proboscis was placed between two glasses (subject and cover) to measure the proboscis. Then it was placed back, curved side up on the glass
plate, applied a drop of glycerin on the cover glass and gently turned over so that the drop hung down and slowly dropped onto the proboscis.

**Figure 3.** Proboscis structure:
1 – *Glossa*; 2 – *Subentum*; 3 – *Mentum*.

The length was measured from the highest point on its base to the opposite edge along the largest axis (a), and the width is perpendicular to this axis in its widest part (b) (figure 4).

The length (a) of the tergite and the sternite was measured along the axis of the bee's body, and the width (b) was defined as the distance between the protrusions (figures 5, 6).

**Figure 5.** The third tergite of the abdomen: a – length; b – width.

**Figure 6.** The third sternite of the belly of the bee: a – length; b – width.

To make measurements, the drone was placed on an object-plate and at the same time, holding the fingers of the left hand (head and back), the abdomen was disarticulated at the level of the third tergite. When dissecting, you need to take sharp tweezers or a dissecting needle, and first you need to count the third tergite and separate this section of the abdomen. Then it remains to divide tergite and sternite. After separating these parts of the body, they were laid out on a object-plate, glycerin was dropped, a cover glass was covered on top, and appropriate measurements were made.

The cubital index was determined by the ratio of the intercubital vein’s length "a" to the length of the intercubital vein "b" of the third cubital cell of the forewing (figure 7). The method of measuring this feature is shown in figure 8.

To carry out measurements, the bee was taken with the thumb and index finger of the left hand, head forward, abdomen back and tweezers located in the right hand separated the right hind leg from the body along with the thigh. Then the separated leg was placed on the object-plate and, holding it with tweezers on the left hand, the first segment of the leg was separated with the second tweezers on the right hand. For measurements, the leg segment was placed between two pieces of glass (subject and cover), while a drop of glycerin was applied and further measurements were made. The tarsal index was determined by the ratio of the length and width of the first segment of the hind leg.
3. Research results

Many scientists noted that the color variations of the insect cuticle are extremely diverse and this feature is fundamental in determining the types and geographic forms of the populations studied [11]. A yellow color on the terga’s cuticle of the abdomen is a characteristic feature of many southern honey bees subspecies. The appearance of this color in bees of the Central Russian subspecies in the forest area indicates the crossbreeding processes. In the course of work carried out in bee yards, four species of the following morphotype were revealed (according to the chitinous integuments color on the abdomen): Iₕ, I, O – dark and O – gray (figure 9). Drones with O (gray) color (figure 9d), unlike other morphotypes, were not found in all the studied regions, for example: in the Arkhangelsk, Gafuri and Kugarchinsk regions, these species were not recorded. The obtained data indicate the dominance of drones characterized by O morphotype (dark) – Central Russian subspecies (figure 9c). Analyzing the results of studies based on taxonomic assessment, it should be concluded that purebred Central Russian bees can much less be found than other taxa, because other groups in total exceed the “purity” indicator: Iₕ, I, and O (gray) (figure 9d) – 3852 (58.36%), O (dark) (figure 9c) – 2748 (41.64%).

In 2017, an increase in the number of drones of the Central Russian subspecies by 3.85% was established. Drones of the O (gray) morphotype in a minimal amount (0.83%) were registered in bee yards of the Kugarchinsky region, in comparison with 2016. There was also a decrease in the number of morphotypes of other drone groups: Iₕ, by 1.78% (figure 9a), I – 1.09 (figure 9b) and O (gray) – by 0.98%, respectively. Also, a decrease in the total number of morphotypes that are not typical for the
Central Russian subspecies was revealed. The analyzed indicator amounted to 3598 (54.51%), which was lower by 3.85% of the previous year (figure 10).

In 2018, a decrease in the number of drones of the Central Russian subspecies was revealed, compared to 2016 – by 7.35% and since 2017 – by 11.20%. Also, in comparison with the previous years, drones with O morphotype (gray) were recorded in bee yards of all regions. The number of I, I and O (gray) drones increased, compared to 2016, by 2.66, 4.47 and 0.22%, respectively. A similar situation was observed, in comparison with 2017, i.e. I – increased by 4.44, I – by 5.56, and O (gray) – by 1.2%. The increase in the total number of morphotypes, that are not typical for Central Russian bees, amounted to 7.35% compared to 2016 and 11.20% from 2017 (figure 10).

![Figure 10. The ratio morphotypes’ number O (dark) and O (gray), I, I (group of other morphotypes) on the territory of the southern Urals.](image)

Taking into account the registered morphotypes, their morphometric assessment was carried out. Based on a detailed assessment of metric data on morphotypes, it is revealed that I drone individuals are the largest in comparison with other groups. They surpass other groups according to seven features: proboscis length, wing (front right) length and width, cubital and tarsal indices, third tergite length and width, and third sternite width. For example, the proboscis length on average was characterized by a maximum indicator of 4.45±0.05 (table 1). The Lim upper boundary value also compared to other morphotypes had a maximum value (4.50 mm), in contrast to the lower boundary value (4.40 mm). The indicator specified was also registered in drones with I morphotype (3.90…4.40 mm, table 1). A similar pattern was observed along the width of the right front wing, length and width of the third tergite, as well as the width of the third sternite. In general, the analysis of obtained data indicates a decrease in drones’ body size in the following order: I, I, O (dark) and O (gray), despite some coincidence of measured features in the identified groups.

The analysis of morphometric features of drones identified as *Apis mellifera mellifera* in the South Ural allows to distinguish the following morphological characteristics: O morphotype (dark) with brown color (coffee hue) of abdominal hairs (according to the Prof. Goetze’s color scale or the F. Ruttner’s method (2006) this subspecies may also be characterized by black color); average proboscis length is from 3.81±0.08 mm to 3.97±0.03 mm, cubital index is from 1.36±0.07 to 1.45±0.09, right front wing length is from 11.98±0.49 to 12.08±0.45 mm, right front wing width is from 3.72±0.21 to 3.92±0.23 mm, third tergite length is 2.90±0.12 to 3.51±0.01 mm, third tergite width is from 6.40±0.02 to 6.44±0.04 mm, third sternite length is from 2.62±0.03 to 2.66±0.05 mm, third sternite width – from 4.52 ± 0.10 to 4.62±0.07 mm, tarsal index – from 49.59±0.89 to 52.15±1.93 % (table 1).
Table 1. Characteristics morphotypes’ classes of *Apis mellifera* drones on the territory of the Southern Urals by morphometric parameters.

| Parameter                        | Indicator | **I**             | **O (dark)** | **O (gray)** |
|----------------------------------|-----------|-------------------|--------------|--------------|
| Length of rostrum, mm            | Lim       | 4.40…4.50         | 3.90…4.40    | 3.75…4.30    | 3.60…4.00    |
|                                  | M ± m     | 4.45±0.05         | 4.18±0.25    | 4.10±0.27    | 3.91±0.17    |
|                                  | Cv, %     | 1.14              | 6.02         | 6.58         | 4.40         |
| Length of right fore wing hemelytron, mm | Lim       | 12.50…12.70       | 12.60…12.70  | 11.21…12.60  | 11.30…12.50  |
|                                  | M ± m     | 12.60±0.10        | 12.66±0.05   | 12.09±0.68   | 12.22±0.52   |
|                                  | Cv, %     | 0.81              | 0.40         | 5.63         | 4.22         |
| Width of right (fore wing), mm   | Lim       | 4.40…4.50         | 4.31…4.40    | 4.20…4.31    | 3.40…4.31    |
|                                  | M ± m     | 4.45±0.05         | 4.36±0.05    | 4.27±0.05    | 4.10±0.39    |
|                                  | Cv, %     | 1.14              | 1.04         | 1.26         | 9.55         |
| Cubital index                    | Lim       | 1.88…2.00         | 1.47…1.56    | 1.28…1.41    | 1.21…1.50    |
|                                  | M ± m     | 1.94±0.06         | 1.52±0.05    | 1.36±0.06    | 1.43±0.12    |
|                                  | Cv, %     | 3.15              | 2.98         | 4.68         | 8.71         |
| Length of the 4th (tergite), mm  | Lim       | 3.53…3.60         | 2.90…3.53    | 2.85…2.90    | 2.85…3.52    |
|                                  | M ± m     | 3.57±0.04         | 3.26±0.32    | 2.88±0.02    | 3.36±0.29    |
|                                  | Cv, %     | 1.00              | 9.75         | 0.85         | 8.57         |
| Width of the 4th (tergite), mm   | Lim       | 6.54…6.60         | 6.52…6.54    | 6.51…6.52    | 6.40…6.51    |
|                                  | M ± m     | 6.57±0.03         | 6.53±0.01    | 6.51±0.01    | 6.48±0.05    |
|                                  | Cv, %     | 0.46              | 0.15         | 0.08         | 0.73         |
| Length of the 4th (sternite), mm | Lim       | 2.72…2.74         | 2.72…2.74    | 2.55…2.72    | 2.60…2.71    |
|                                  | M ± m     | 2.73±0.01         | 2.73±0.01    | 2.66±0.08    | 2.68±0.05    |
|                                  | Cv, %     | 0.37              | 0.37         | 3.14         | 1.76         |
| Width of the 4th (sternite), mm  | Lim       | 4.70…4.72         | 4.66…4.70    | 4.41…4.66    | 4.41…4.66    |
|                                  | M ± m     | 4.71±0.01         | 4.68±0.02    | 4.57±0.12    | 4.60±0.11    |
|                                  | Cv, %     | 0.22              | 0.43         | 2.68         | 2.34         |
| Tarsal index, %                  | Lim       | 52.94…53.85       | 49.06…51.92  | 49.02…49.06  | 49.02…53.85  |
|                                  | M ± m     | 53.40±0.46        | 50.68±1.44   | 49.05±0.02   | 52.72±2.08   |
|                                  | Cv, %     | 0.87              | 2.84         | 0.04         | 3.94         |

The drone individuals which are identified as hybrid forms were characterized by I, I, O (gray) and O (dark) morphotypes. According to the prof. Goetze's scale, the hair color corresponded to gray (sand), brown (rusty and coffee) and black (smoky and soot colors) hues. The identification of morphometric features allowed establishing that the proboscis length *lim* lower limit in drones in all the studied regions corresponded to the requirements of the Central Russian subspecies standard (3.6-4.0 mm). However, in these samples, the proboscis length *lim* upper limit was exceeded. It was also found that the right front wing length and width indicators of drones of this group had higher values than the Central Russian subspecies. The cubic index indicators of drones were characterized by significant fluctuations in the regions, but they corresponded to Central Russian bees. According to the third tergite length indicators, drones in the bee yards of regions were also characterized by diversity. *Lim* of this feature ranged from 2.80 to 3.60 mm. The third tergite width *lim* lower boundary in drones was the same in all studied regions (6.40 mm, figure 5). The third sternite length *lim* lower boundary in drones in most of the studied regions was 2.60 mm. Only in two regions it was 2.55 mm (Abzelilovsky and Arkhangelsky regions). A similar tendency was observed along the upper boundary. The average value ranged from 2.63 to 2.66 mm. The third sternite width *lim* lower boundary in drones in most (eight) studied regions was 4.41 mm. In three regions (Abzelilovsky, Gafuri and Ishimbay) this indicator differed in value – 4.42, 4.40 and 4.39 mm, respectively (table 1). The tarsal index indicators in this group of drones did not go beyond the *lim* obtained from
individuals identified as the Central Russian subspecies. The analysis of features variation coefficients indicates the division of data set into separate small groups. Moreover, these features include: cubital index, proboscis length, right front wing length and width, third tergite length. In general, it can be noted that drone individuals classified as a hybrid group are characterized by both a variety of external color and morphometric features.

A monitoring of morphological features of drones in the Shulgan-Tash nature reserve, which is a reserve of bees of the Central Russian subspecies, allowed establishing that all individuals in the samples corresponded to the O class morphotype, i.e. Central Russian subspecies. According to the color scale, the drones were characterized by brown or coffee color of hairs, which also corresponds to the standard of this subspecies.

According to the obtained morphometric measurements, drones according to the average proboscis length corresponded to the standard of Central Russian subspecies (3.6…4.0 mm): 3.90±0.12 (Kashalya), 3.88±0.10 (TalliYalan), 3.93±0.07 (Baysalyan), 3.90±0.10 (Kapova cave), 3.91±0.09 (Bala-tukay), 3.89±0.14 (Gadelgareevo), 3.92±0.08 (Kush-Alga bash), with a similar situation with Lim sample indicators, respectively. According to the cubital index indicators, it was also found that the average values and Lim sample indicators were in compliance with the Apismelliferamellifera standard (1.0…1.6): 1.33±0.10 (Kashalya), 1.33±0.07 (TalliYalan), 1.34±0.07 (Baysalyan), 1.41±0.09 (Kapova cave), 1.34±0.06 (Bala-tukai), 1.32±0.06 (Gadelgareevo), 1.40±0.10 (Kush-Alga bash) (table 1). A similar situation was observed in the Lim cubic index indicators of drones in the samples. An analysis of the obtained data on additional morphometric features (right front wing, third tergite and sternite length and width, tarsal index) allows concluding that the differences between the average values and the extreme Lim variants in drones are insignificant and similar within the groups, which allowed noting the homogeneity of the studied sample.

In general, it can be noted that the studies showed presence of I, I, O morphotypes in honey-bee colonies – dark and gray. Taking into account existing standards, the O (dark) morphotype corresponds to the Central Russian subspecies, the drones characterized by the O (gray) color can be related to the gray mountain Caucasian (Apis mellifera caucasica), Carpathian (Apis mellifera carpatica) or Crainsky (Apis mellifera carnica). The presence of I drones indicates a genetic “impurity” of honey bees, which are characterized by yellow hues on the abdomen: Italian subspecies (Apis mellifera ligustica), yellow valley Caucasian (Apis mellifera remipes), etc. Nevertheless, the drones’ presence with the Central Russian subspecies morphotype shows some preservation (34.29%) of the native bees biopotential (figure 10). At the same time, the data on the assessment of morphotype classes is confirmed by the results of morphometric studies, where the analysis of the variation’s coefficients indicated that the data array was divided into the separate groups (caused by the influence of the various subspecies’ morphometric characteristics of the native bees or the introgression).

4. Conclusion
Morphological studies of drones conducted in the Southern Ural make it possible to note the presence of individuals with O morphotype — the dark Central Russian subspecies (34.29% of the total number of samples), which proves the native population biopotential preservation to some extent, the main factor of which, in our opinion, should be considered the natural mechanisms of drones diversity in the given territory. The dynamics of the number of drones with different morphotypes (I, I, and O – gray) and an increase in individuals with the standard of the Central Russian subspecies (O – dark) in 2017, as well as their slight decrease in 2018, are due to the lack of breeding measures for breeding bees. At the same time, one of the surviving populations of Apis mellifera mellifera in this territory is the Burzyansky, taxonomic “purity”, which is supported by selectivity and breeding based on the wild hive bees of the Shulgan-Tash nature reserve (Burzyansky region). The conducted studies allowed establishing the mono-group (within the Apis mellifera mellifera taxonomic standard) of drone samples according to the morphometric features discussed above, which characterizes the presence of “pure” queen bees and their subpopulation structure. Taking into account the fact that the number of "pure" bee populations is currently decreasing worldwide and there is a collapse of bee colonies
(Colony collapse disorder), it should be noted that the identified purebred families in the southern Urals will be the biogenetic material for the restoration and conservation of *Apis mellifera mellifera* populations in all countries. The quantitative data obtained for the first time on the drones’ morphological characteristics in this area is an information and analytical base for forming a global catalog.

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