

## **Morpho-ethological and biochemical-genetic characteristics of the local Bulgarian honey bee *Apis mellifera rodopica***

Plamen Petrov<sup>1</sup> and Evgeniya Ivanova<sup>2</sup>

<sup>1</sup>Agricultural University – Plovdiv, 12 Mendeleev Str. Plovdiv 4000, Bulgaria, Tel: +359 32 654 280, E-mail: [info@nrp-bg.org](mailto:info@nrp-bg.org)

<sup>2</sup>University of Plovdiv “Paisii Hilendarski”, 24, Tzar Assen Str. Plovdiv 4000 Bulgaria, Fax: +359 32 261 566, E-mail: [geneiv@uni-plovdiv.bg](mailto:geneiv@uni-plovdiv.bg)

### Introduction

The local Bulgarian honeybees have been studied morphologically since the 30s of the last century (Lazarov 1935, 1936, Tzonev 1960, Velichkov 1970).

Since 1999 it has been worked on the implementation of a new National Programme for Breeding and Improving Work with Bees. Its purpose is the conservation of the gene pool of the local Bulgarian honey bee.

With this regard, a morpho-ethological analysis by specific characteristics has been carried out in the country in order to determine the race standard (Petrov 1990, 1993, 1995, 1997). Biochemical-genetic researches of polymorphism in some protein and isoenzyme systems have been carried out also ever since 1991 (Ivanova et al. 1991, Ivanova et al. 1994, Ivanova et al., 1995, Ivanova and Popov 1997, Ivanova et al. 1996, Ivanova 1998, Ivanova et al. 2004, Ivanova et al. 2007). In the last years it has been purposefully worked for reuniting different approaches - morphometrical, ethological, isoenzymical and DNA analysis. The objective is to clarify the race standard of the local honey bee, which is a part of the European genetic resource of *Apis mellifera*. Due to the proved biological and productive advantages and its adaptation to the conditions specific for the country, the local honey bee should be preserved and its selection should be carried out on the basis of a serious scientifically grounded activity together with the common efforts of many scientists by using the possibilities of experience exchange with the European countries.

The objective of this study is morpho-ethological and Biochemical-genetic characterization of the local Bulgarian honey bee *Apis mellifera rodopica*. The results would be useful for selection and conservation of local Bulgarian honey bees.

### Materials and methods

Different regions from all over the country were studied. Totally over 15 700 samples were collected and 921 590 measurements under microscope were made (according to Alpatov, 1945; 1948). Parameters measured in this study were: mass of a non-inseminated and an inseminated queen; length of proboscis; forewing length; forewing width; cubital index; discoidal shifting; tarsal index; diameter of the spermatheca; total sum of the lengths of 3-rd and 4-rd abdominal tergum; coloration of the abdominal tergums; colour of the drone thorax pappi; wax-mirror back border concave; length of the pappi of the 5-th abdominal tergum; hair index of the 4-th abdominal tergum; length of the hind leg; colour of the pappi of the 2-nd abdominal tergum; colour of the pappi of the 3-nd to 6-th abdominal tergums.

More than 3000 individual honey bee samples from different queen reproductive bases in Bulgaria were studied using isoenzymic analysis of six enzymic systems (MDH, ME, EST, PGM, HK and ALP) corresponding to 6 loci. Worker bees were collected from every population and samples of bees were transported to the laboratory alive where they were stored at -20<sup>0</sup>C until their usage for electrophoretic analysis of allozymes. The thorax homogenization and electrophoresis in 7.5% polyacrylamide gel were done according to Ivanova (1996).

### Results and discussion

Summarized data about morphological and morphometric characteristics for queens, drones and worker bees of local Bulgarian honey bee are presented in Tables I to III. Until now, the race structure of the bees was examined in the regions of Strandzha, the Central and Western Rhodopes, the Middle Balkan Mountains, the Under-Balkan Valleys, the Struma River region and part of the Western Balkan Mountains. The results received give possibilities to compare local Bulgarian honey bees with other European subspecies and ecotypes.

Tabl.I. Morphological and morphometric characteristics for queens of *A. m. rodopica*.

Characteristics	Values
Live weight of an unfertilized queen (mg)	190 – 220
Live weight of a fertilized queen (mg)	230 - 320
Length of proboscis (mm)	4.00 – 4.25
Forewing length (mm)	9.6 – 10.4
Forewing width (mm)	3.25 – 3.45
Cubital index	2.5 – 5.6 typical >3,0
Discoidal shifting (%)	Positive
Tarsal index (%)	> 46.0 %

Tabl.II. Morphological and morphometric characteristics for drones of *A. m. rodopica*.

Characteristics	Values
Live weight (mg)	190 - 260
Length of proboscis (mm)	3.9 – 4.3
Forewing length (mm)	11.7 – 12.4
Forewing width (mm)	4.1 – 4.4
Cubital index	1.7 – 2.5
Discoidal shifting (%)	>80 % positive
Tarsal index (%)	
Total sum of the lengths of 3-rd and 4-rd abdominal tergum (mm)	5.5 – 5.6
Coloration of the abdominal tergums	Dark, with small yellow spots
Colour of the thorax pappi	Light brown

Tabl.III. Morphological and morphometric characteristics for worker bees of *A. m. rodopica*.

Characteristics	Values
Live weight (mg)	80 - 90
Length of proboscis (mm)	6.4 – .6.6
Forewing length (mm)	8.8 – 9.6 (9.1)
Forewing width (mm)	3.1 – 3.3 (3.2)
Cubital index	2.6 – 3.0
Discoidal shifting (%)	90–100% positive
Tarsal index (%)	53.8 – 59.1 (56.1)
Total sum of the lengths of 3-rd and 4-rd abdominal tergum (mm)	4.3 – 4.7 (4.6)
Wax-mirror back border (%)	90 – 100% concave
Length of the pappi of the 5-th abdominal tergum (mm)	<0.3
Hair index of the 4-th abdominal tergum	>1.5
Length of the hind leg (mm)	7.8 – 8.3 (8.1)
Coloration of the abdominal tergums	Dark, with small yellow spots
Colour of the pappi of the 2-nd abdominal tergum	Yellow-brown
Colour of the pappi of the 3-nd to 6-th abdominal tergums	Loght brown

Some differences between Bulgarian local bees and other subspecies of *Apis mellifera* were reported previously by Petrov (1996) and Petrov and Petkova (1996, 1997). Comparing data

about length of the hind leg, tarsal index, forewing length, forewing width and total sum of the lengths of 3-rd and 4<sup>th</sup> abdominal tergum of Bulgarian worker bees the authors conclude that local honeybees *A. m. rodopica* are reliably different ( $P \leq 0.05$ ; 0.01; 0.001) from *A. m. carnica*, *A. m. carpatica*, *A. m. ligustica*, *A. m. mellifera*, *A. m. caucasica* and *A. m. anatoliaca*.

Summarized data about allozyme characteristics of local Bulgarian bees (*A. m. rodopica*) are presented in Table IV.

Table IV. Summarized data about allozyme characteristics of local Bulgarian bees

Polymorphic locus	Alleles found	The most frequent allele for <i>A.m. rodopica</i>
Mdh 1	Mdh 65 Mdh 100	Mdh 100 > 0.590
Me	Me 100 Me 106	Me 100 > 0.730
Est 3	Est 80 Est 88 Est 100 Est 105 Est 118	Est 100 > 0.900
Pgm	Pgm 75 Pgm 100	Pgm 75 > 0.900
Hk	Hk 87 Hk 100 Hk 110	Hk 100 > 0.900
Alp	Alp 80 Alp 100	Alp 80 > 0.500

All enzyme systems (MDH-1, ME, EST-3, PGM, HK and ALP), were polymorphic in most of the populations studied and Est-3 100, Pgm75 and Hk100 were fixed in some of populations. (Table II). Two alleles were detected at Mdh-1 locus (Mdh 65 and Mdh 100), two alleles at Me locus (Me 100 and Me 106), five alleles - at Est-3 locus (Est 80, Est 88, Est 100, Est 105 and

Est 118), two alleles at Pgm locus (Pgm 75 and Pgm 100), three alleles at Hk locus (Hk 87, Hk 100 and Hk 110) and two alleles - at Alp locus (Alp 80 and Alp 100). Allele frequencies of all loci were calculated in order to characterize the local Bulgarian honeybee. According to Ruttner's morphometric analysis (1988), *A. m. macedonica* subspecies occurs in Bulgaria. Another point of view is the existence in Bulgaria of a native local honeybee *A. m. rodopica* (Petrov, 1995).

Dedej et al., (1996) reported no polymorphism in the EST-3 and ME loci for *A. m. Macedonica*. According to Bouga et al. (2005) - in Macedonian region of Greece there are three alleles (MDH<sup>100</sup>, MDH<sup>80</sup> and MDH<sup>65</sup>) in Mdh-1 locus and the most frequent of them is MDH<sup>80</sup>. At the same time, the frequency of ALP<sup>80</sup> for Macedonian bees is calculated as 0.846 which is higher than frequencies found for Bulgarian honey bees. Kandemir et al. (2005) found that the frequency of the MDH<sup>65</sup> allele is quite high - 0.879 in the Austrian colonies which were known to be *A. m. carnica*.

Comparing the enzyme polymorphism studied and information given by Dedej et al. (1996), Bouga et al. (2005); Kandemir et al. (2005) we could notice that there are differences between Bulgarian honey bees (*A. m. rodopica*), *A. m. macedonica* and *A. m. carnica*,

**ACKNOWLEDGMENTS:** *This study was supported by National Fund for Scientific Researches of Bulgarian Ministry of Education and Science by a contract DO 02-63/2008 and by Fund for Scientific Researches of University of Plovdiv "Paisii Hilendarski" by a contract BF-40/2009*

## References

- Alpatov V. V. (1945) Honeybee breeds. Scientific Works of Moscow University. 56 pp.
- Alpatov V. V. (1948) Honeybee breeds. MOIP. 183 pp.
- Bouga M., Kiliadis G., Harizanis P.C, Papatotiropoulos V., Alahiotis S. (2005) Allozyme variability and phylogenetic relationships in honey bee (Hymenoptera: Apidae: *A. mellifera*) populations from Greece and Cyprus. *Biochem. Genet.* 43, 471-484.
- Dedej S., Basiolo A., Piva R. (1996) Morphometric and alloenzymatic characterisation in the Albanian honeybee population *Apis mellifera* L. *Apidologie* 27(3), 121-131.
- Ivanova E., Dobrovolov I., Popov P. (1991)- Studies on the electrophoretic patterns of the esterase isozymes of honey bees *Apis mellifera* L. (Hymenoptera: Apidae) in ontogenesis. *Universite de Plovdiv "Paissi Hilendarski", Travaux Scientifiques*, 29(6): 243-245.
- Ivanova E., Popov P., Bogkova M. (1994)- Electrophoretic studies of the NAD-dependent MDH during the larvae stage from the ontogenesis of *Apis mellifera* L.(Hymenoptera; Apidae). *Biologie, Animalia*, 30(6): 57-60.
- Ivanova E., Popov P., Stoikova T. (1995)- Electrophoretic studies of polymorphism on soluble proteins and some isoenzymes of *Apis mellifera* L. in Bulgaria. *Animalia*, 31 (6): 51-56.
- Ivanova E. (1996) Variability of *Apis mellifera* in Bulgaria – ontogenetic and population-genetic aspects. PhD Dissertation University of Sofia, Bulgaria.
- Ivanova E., Popov P., Dobrovolov I., Tersieva P. (1996)- Polymorphismus der MDH-loci bei Imagines von *Apis mellifera* L. aus Bulgarien. *Animalia*, 32(6): 43-51.
- Ivanova E., Popov P. (1996-1997)- Electrophoretic studies of non-specific esterases during the ontogenetic course of domestic bee *Apis mellifera* L.(Hymenoptera; Apidae) in Bulgaria. *Genetic and Breeding*, 28(1-2): 13-16.
- Ivanova E. (1998) Electrophoretic studies on NAD P-dependent malate dehydrogenases (ME) during ontogenesis of honey bees *Apis mellifera* L. in Bulgaria. *Acta zoologica bulgarica*, 50 (2/3): 141-146.

- Ivanova E., Ivgin R., Kence M., Kenche A. (2004)- Genetic variability in honeybee populations from Bulgaria and Turkey. *First European Conference of Apidology*, 19-23 September 2004 Udine, Italy, 45.
- Ivanova E., Staykova T., Bouga M. (2007)- Allozyme variability in honey bee populations from some mountainous regions in southwest of Bulgaria. *Journal of Apicultural Research*, 46(I): 3-8.
- Kandemir I., Kence M., Kence A. (2005) Morphometric and electrophoretic variation in different honeybee (*Apis mellifera* L.) populations. *Turk. J. Vet. Anim. Sci.* 29, 885-890.
- Lazarov A. (1935)- Length of the honey bee proboscis, importance and approaches for its measuring: *Bee*, 6:156-158.
- Petrov P. (1990)- Characteristic and taxonomy of Bulgarian honey bees. *PhD Dissertation University of Moskow, USSR*, 133pp.
- Petrov P. (1993a)- Morphological characteristic of *A. mellifera* in Bulgaria: III.Colour. *J. of Animal Science*, 4: 73-79.
- Petrov P. (1993b)- About the morpho-ethological race standard of Bulgarian *A. Mellifera rodopica* in Bulgaria: *J. of Animal Science*, 4: 80-84.
- Petrov P. (1995)- Bulgarian honey bee *Apis mellifica rodopica* and it race standard. *Agrarian University of Plovdiv, Scientific works*, XL(3): 317-319.
- Petrov P., Petkova O. (1996)- Possibilities for using some quantitative characteristics in the taxonomy of Bulgarian honey bees *Apis mellifera rodopica*. Size of the abdomen. *J. of Animal Science*,4: 78-79.
- Petrov P. (1997a)- On the origin of the of Bulgarian honey bees (*A. mellifera rodopica*). *J. of Animal Science*, 1-2: 88-93.
- Petrov P. (1997b)- Study on the of Bulgarian *A. mellifera rodopica* from the west reserve of Bulgaria: *J. of Animal Science*, 5-6: 132-133.
- Petrov P. (1997c)- Morpho-ethological characterization of honeybee from Strandja region of Bulgaria: *J. of Animal Science*,7-8: 137-140.
- Petrov P., Petkova O. (1997)- Possibilities for using of the hind leg length and the tersetal index in the taxonomy of Bulgarian honey bees *Apis mellifera rodopica*. *J. of Animal Science*,1-2: 86-87.
- Ruttner F. (1988)- Biogeography and Taxonomy of Honeybees: *Springer – Verlag*, Berlin.