GENETIC AMELIORATION OF SOME POPULATIONS OF *APIS MELLIFERA CARPATICA* BEES FROM AREA OF FORESTS OF MOLDOVA

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Abstract

The aim of this paper was the creation, by scientific selection methods, of bee populations with increased morph-productive genetic potential, intended for reproduction of valuable genetic material for dissemination in small and medium apiaries. The research work was carried out on bee populations of Apis mellifera Carpatica race, from experimental apiaries of Institute of Zoology (IZ) of the Academy of Sciences of Moldova, which includes 50 bee colonies (considered as a middle apiary, after the number of families) and of the National Association of Beekeepers of Moldova (ANARM) with the number of 30 families (considered as small apiary), placed at stationary in forest areas of central part of Moldovan forests. Biological particularities, including external morph-metric features that characterize the race purity (tegument colour working bee, proboscis length, cubital index value, discoidal shift, specific (type) of capping of cells with honey, bee behaviour by opening the nest and examining the honeycomb), and morph-productive potential of bee families (over winter resistance, queen prolificacy, family strength, broods viability, resistance to disease and honey production) were evaluated during the years 2010-2014.

On the basis of assessment of morph-productive features, annually each family of bees has been evaluated according to the complex of characters, and the class of evaluation was attributed, that indicates the morph-productive genetic potential and its breeding value. Annually, from the total number of evaluated bee populations, the best families were selected and included in breeding batch, then used for semen collections for artificial growth of honeybee queens.

As a result of researches and selection at above apiaries, they were created and genetically consolidated 2nd population of bee colonies with high productivity and resistance to diseases. Bee populations correspond to the race standard by external morphological features, which certify the purity race, and possess high productivity at: productivity of honey (42.9–57.4 kg), increased resistance to diseases (91.6%) and over winter resistance (93.3–89.4%), the strong viability brood (92.3–92.4%), which, respectively, with 27.5%, 52.4%, 45.7-19.2% and 15.4-15.5% is higher than the race standards. Within the populations of colonies were created 2 batches of breeding with bees families genetically consolidated with high-level performance (proboscis length 6,60 – 6,62 mm, cubital index 44.6–45.3%, positive discoidal shift 84.5–93.0%, family strength 3.19–3.22 kg, queens prolificacy 1749–1873 eggs/24 h, over winter resistance 89.4–94.3%, brood viability – 92.4–92.5%, disease resistance 91.4–92.2%, honey production 49.3– 63.2 kg), correspond to the standards after morphological external features race purity, and far exceed the standard after morph-productive characteristics: queen prolificacy – with 9.3-17.1% ($P < 0.001$), family strength – with 36.9–39.2% ($P < 0.001$), over winter resistance – with 19.2–25.7% ($P < 0.001$), brood viability – with 15.5-15.6% ($P < 0.001$), disease resistance – with 52.3–54.7% ($P < 0.001$) and honey production – with 9.6–40.4% ($P < 0.001$).

Key words: genetic amelioration, *Apis mellifera Carpatica*, morph-productive characteristics

INTRODUCTION

Beekeeping in the Republic of Moldova, it is a branch of sector of zootechny, with particular social importance, due to the value and quality of products offered by it, job creation among vulnerable populations in rural areas and maintaining through pollination of homeostasis and the biodiversity natural ecosystems.

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Analysis of the situation in the country in recent years demonstrates that the productive potential of beekeeping sector is explored under level of possibility. Thus, from a bee's family only 16-18 kg of honey per year are obtained, compared to 30-45 kg - the potential of race [3]. Often in winter the death of bee families in significant proportion has been registered. In some apiaries the cases of "collapse of the bees" were registered - phenomenon of total disappearance of bees from the nest, caused by factors far unidentified precisely [8].

Retaining in the branch development is explained by the fact that the beekeeping does not apply modern methods and technologies [1], scientifically argumented. Traditional technology of improvement of livestock of bees in most apiaries are currently limited, at best, only to some phenotypic selection methods, without determining of racial purity and genotypic value of the bee families. The apiculture technology has a lack of efficient methods and proceeding for the selection of bee populations, such as: assessing of the genetic value of bee families and determination of the class of evaluation after the complex of characters and morph-productive features, directed progressive selection under independent limits of several characters, genetic consolidation of bee population by directed pairing of the queens with application of the method of instrumental insemination [7, 13], genotypic testing of queens bee after the qualities of descendants [6], by determining the heritability coefficient (h^2) and the effect of selections of main morph-productive characters [5, 9]. The selection of bee families is carried out without taking into account of some biological features of bee families, extremely important, such as: resistance to the specific diseases of bees, brood viability, behavioural indices, etc. [3].

According to our researches [15] in the bee populations of our country, was found the existence, at the same time, of some metis families of other races, especially the race *Apis mellifera Kaukasica* and *Apis mellifera ligustica*, dispersed unevenly in the territory, arising due to illegal importation of queens made by some beekeepers. Clandestine appearance of metises in apiculture can lead to negative consequences such as genetic segregation of race with appearance of unwanted metis, with unbearable aggressive traits, un-adapted to environmental conditions and various diseases, which cause difficulties in the exploration of bee families and lead to decreased of theirs productivity. Therefore, according to the National Program for conservation and genetic improvement of bees in Republic of Moldova [2], crossing of race *Apis mellifera Carpatica* with other races is not recommended. In this context, the populations of bee families expected for selection and reproduction requires prior morph-metric certification on subject of compliance to race purity, and an assessment of their biological particularities – morph-productive, which will then be used as a benchmark in selection and reproduction of genitor beekeeping material.

Because of the existence of scientific and innovative vacuum in genetic improvement of livestock of bees, in the country there is a big deficit of genitor material of bees for breeding (especially queens) with high genetic value. Officially the breeding apiaries produce only from 1.0 to 2.0 thousand breeding queens, while the necessity is 60000 queen bees per year, and their genetically and morph-productive quality, leaves much to be desired.

It is well known that genetic improvement of livestock of bees can be ensured only by families from pure race, healthy and strong, with high resistance to specific diseases, with a vigorous swing of the biological cycle of reproduction and growth of brood [10], with increased morph-productive skills, the aim of this paper was the creation, by scientific selection methods, of bee populations with increased morph-productive genetic potential, intended for reproduction of valuable genetic material for dissemination in small and medium apiaries.

MATERIAL AND METHODS

The research work was carried out on bee populations of *Apis mellifera Carpatica* race, from experimental apiaries of Institute of Zoology (IZ) of the Academy of Sciences of Moldova, which includes 50 bee colonies (considered as a *middle* apiary, after the number of families) and of the National Association of Beekeepers of Moldova (ANARM) with the number of 30 families (considered as *small* apiary), placed at stationary in forest areas of central part of Moldovan forests.

During 2010-2014, each apiculture season at the above apiaries were evaluated biological particularities, including morph-productive potential of bee families of respective generation. For estimation of this potential, the external morph-metric morphological features, which certifies the purity of race, were assessed (the tegument's colour of working bee, proboscis length, the value of cubital index of two rib segments which forming the obtuse angle on the basis of the third cubital cell of anterior right wing of the bee, discoidal shift of bifurcation point of inferior rib from radial cell to distal rib of cubital cell of anterior right wing of the bee, specific (type) of capping of cells with honey, bee behaviour by opening the nest and examining the honeycomb), by comparing the framing of their value within the race standard. At that stage, according to the calendar plan, was determined the level of morph-productive characters development of bee families, such as: over winter resistance, queen prolificacy, family strength, broods viability, resistance to disease and honey production.

Determination of external morph-metric features which characterizing the racial purity and the morph-productive features of bee families was performed according to the methodology developed by us [4] for Zootechnical norm for evaluation of bee families, growth and certification of apiarian genitor materia, approved by Government Decision no. 306 from 28.04.2011 (M.O. nr. 78-81 from 13.05.2011, art. 366) [11]. The honey production by bee families was determined by the method of Szabo T.I., 1989 [16].

On the basis of assessment of morph-productive features, annually each family of bees has been evaluated according to the complex of characters, and the class of evaluation was attributed, that indicates the morph-productive genetic potential and its breeding value. Annually, from the total number of evaluated bee populations, the best families were selected and included in breeding batch, then used for semen collections for artificial growth of honeybee queens. Thus, were identified and selected the colonies with strong instinct of accumulation of food reserves in nest, resistant to wintering and disease, with morph-productive features higher then race standards, which were used to reproducing the queens. To maximize the effect of selection and to get progeny with superior value next generations, annually the intensity and selection differential have been raised.

Based on the analysis of biological morph-productive particularities of bee families from breeding batches, they were divided after destination in - maternal and paternal families. The maternal families were used for collecting female genitor material (eggs) and paternal families - for collecting material genitor male (drone sperm). The selection of families was performed using the method of independent limits of the main morph-productive characters, whose indices, at least, correspond or exceed the minimum standard of the Carpathian race to the requirements for the class I of evaluation, which constituted:

- the tegument's colour – dark brown or medium;
- proboscis length - 6,4 mm;
- specific (type) of capping of cells with honey - mixed - predominantly dry, or dry;
- bee behaviour:

by opening the nest – gentle;

by examining the honeycomb – quiet and don't leaving the honeycomb;

- cubital index - 40%;
- pozitive discoidal shift – 70%;
- over winter resistance - 75%;
- family strength – 2.33 kg;
- queen prolificacy - 1600 eggs;
- broods viability - 80%;
- resistance to diseases - 60%;
- honey production - 45 kg;

In order to obtain the progeny of high value with enhanced genetic heredity at the experimental apiaries, the method of instrumental insemination of the queens with semen material of drones from valuable paternal families from breeding batches, was applied. The Queens were artificially reared by the methods F. Ruttner [12, 14] from genitor material of maternal families, selected in previous year. The directed pairing of the queens with males (semen) from paternal families was performed for genetic consolidation of progeny heredity, for increase the value of *heritability coefficient* (h^2) of the main morph-productive characters and the

efficiency of selection in the population. Instrumental insemination procedure was performed using Latshaw device under the trinocular microscope SM-2T, according to the method of F. Ruttner [13]. The queen's descendants of instrumentally inseminated mothers, were subjected to evaluation researches of biological and economic advantages and disadvantages, compared to their contemporaries.

The data obtained as a result of the research were processed statistically using computer software "STATISTICS-6" and appreciated their certainty, according to variational biometric statistics, after the methods of Плохинский Н. А. 1969 [17].

RESULTS AND DISCUSSIONS

As a result of systematic appreciation and research work in bee populations at above apiaries, annually were evaluated biological morph-productive particularities of bee families and the best breeding colonies were selected. To make a more objective assessment of biological particularities of bee families from the studied populations, we considered appropriate to show the dynamics of development morph characters, even in the early stages of selection works (2010). At the experimental apiary of the *Institute of Zoology* at the initial stage, the population of bee families was not only insufficient as livestock for selection (only 16 families), but enough mixed, as the level of development of external morph-metric traits that characterize the purity of race (Table 1).

Table 1 The dynamic of development level of external morph-metric characters and behaviour of bee families from experimental apiary of the Institute of Zoology in the years 2010-2014

External morph-metric characters and of behaviour traits	The level of development of characters, M ± m					Race standard	2014, % to standard
	2010 N=16	2011 N=50	2012 N=50	2013 N=50	2014 N=50		
1.Tegument's colour, dark brown + meddle, %	75.0±11.2	82.0±5.6	80.0±5.7	92.0±3.9	100 ± 0.0	100	100
2. Behaviour, %: - by opening the nest, gentle % - by examining the comb: not leaving,%	 87.5±8.5 87.5±8.5	 92,0±3.9 96.0±2.8	 96.0±2.8 94.0±3.4	 98.0±2.0 100 ± 0.0	 100 ± 0.0 100 ± 0.0	 100 100	 100 100
3. Type of capping of cells, mixt+dry, %	87.5±8.5	92.0±3.8	94.0±3.4	100 ± 0.0	100 ± 0.0	100	100
4. Proboscis length, mm	6.37±0.02	6.49±0.01	6.53±0.02	6.54±0.02	6.65±0.02	6.4	103.9
5. Cubital index, %	41.1±0.6	43.6±0.5	44.8±0.5	46.5±0.3	46.3±0.3	40	115.7
6. Positive discoidal shift, %	59.4±2.8	60.2±2.0	67.7±4.2	69.4±3.4	71.6±3.2	70	102.3

After the most of external morph-metric characters and behavioural features, the bee families don't meet the requirements of the standard of the race *Apis mellifera Carpatica*.

It was found that 25% of bee families did not correspond after tegument colour, standard criteria for the race. Among them, families of bees with yellowish pale or expressed yellow stripe on the abdomen, were found, not specific for Carpathian race colour. In more than 12% of families, the bees were aggressive at the opening of the honeycomb and were leaving the nest at the examination. The type of cell capping in nest of these families was wet type, unwanted for beekeepers and non-specific for the race.

The proboscis length of working bees was below the minimum standard of race, being 6.37 ± 0.02 mm only.

The value of cubital index of two rib segments which forming the obtuse angle on the basis of the third cubital cell of anterior right wing of the bee was at the minimal level of the race standard. Positive discoidal shift of bifurcation point of inferior rib from radial cell to distal rib of cubital cell of anterior right wing of the bee, as one of the most important external morph-metric characters, that characterizes racial purity, on average of studied bee population, was below the minimum standard, and was only 59.4 ± 2.8%, or 10.6% less, compared to the standard (t_d = 3.78; P <0.001).

These biological particularities of populations of bee families have imposed project research team to apply effective and innovative methods and proceeding to improve the morph-productive qualities of the families of selected populations. The first generation was obtained and implanted in 2011, the 2nd generation - in 2012, the 3rd generation - 2013 and 4th generation - in 2014. The systematic application of proceeding and scientific methods of selection and pairing of genitors has led to amelioration of morph-productive performance of bees' families and genetical consolidation of population from experimental apiaries.

Thus, during the years 2010-2014, the share of bee families with requested colour, dark brown and medium brown increased from 75.0% in 2010 to 100% in 2014. The share of bees families with gentle behaviour at nest opening, increased from 87.5% in 2010 to 100% in 2014. In a similar way increased the share of bees' families with peaceful behaviour, which do not leave the comb at the examination.

If in 2010, in the bee population 12.5% of the families had a wet type (unwanted) of capping of cell with honey, then during the 4 years of intensive selection, the mixed and dry type of cell capping of all families was obtained, which is specific for the race and are requested by the beekeepers.

The proboscis length, is a character with low variability and high heritability, it grew very slowly but successively from 6.37 ± 0.02 mm in 2010, up to 6.65 ± 0.02 mm in 2014, or 4.4% (t_d = 9.3; P <0.001).

Cubital index value of the two ribs obtuse angle forming the basis of the third cubital cell of the previous right wing working bee increased in this period also successively from 41.1 ± 0.6 in absolute units in 2010, to 46.3 ± 0.3 absolute units in 2014, or 12.7% (t_d = 7.8; P <0.001).

If by 2013 the share of bees with positive discoidal shift bifurcation point lower rib radial cell to cell distal cubital vein of the previous right wing, was below the race standard, then in 2014 this external morph-metric indicator reached the standard, increasing in the bee population in experimental apiary, from 59.4 ± 2.8 to 71.6 ± 3.2 percentage points or 20.5% (t_d = 2.9; P <0.01).

More obvious, the dynamics of value of external morph0-metric characters and behaviour in families of experimental apiary of IZ in the period 2010-2014 can be viewed in the histogram (fig. 1).

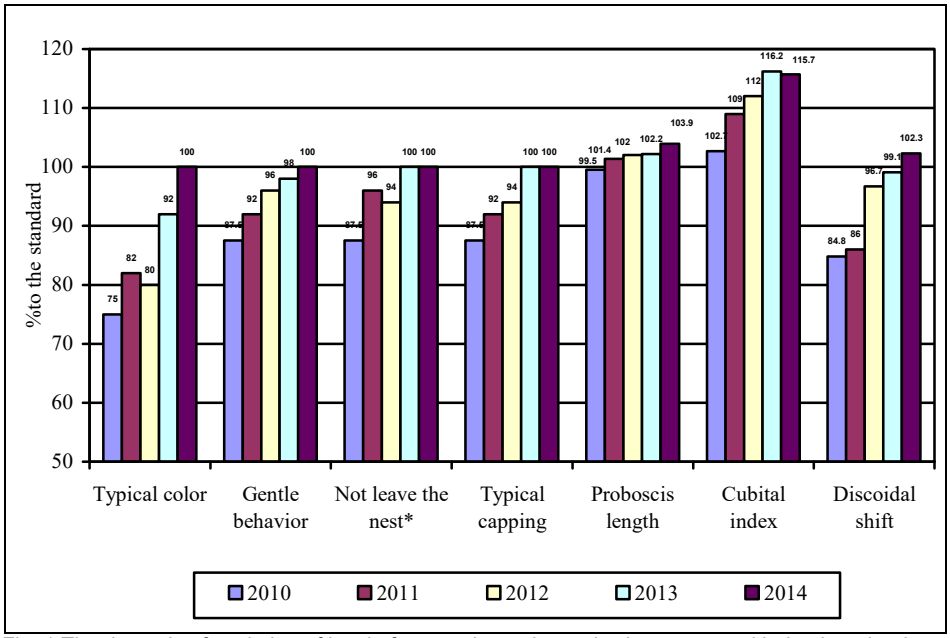

Fig. 1 The dynamic of evolution of level of external morph-metric characters and behavioural traits at the families from experimental apiary of IZ

The histogram shown that the level of development of external morph-metric characters and behaviour features of bees from the experimental families of apiary of IZ, compared to the race standard, increased successively each year, each selected character. If, initially in 2010, the level development of external morphological characters and behavioural features, such as the typical colour of the tegument, the share of bee families with gentle behaviour at nest opening, the share of bee families that do not leave the comb at its examination and the share of bee families with mixed predominantly dry and dry cell capping type and positive discoidal shift, were below the race standard, then subsequently in 2014, as a result of selection, these indices have reached the standard, even more than, some indices exceeded this level.

Also, initially, in 2010, the level indices morph-productive of bee families were below the race standard (Table 2).

Also, under standard level was the index of the prolificacy of the queens (98.9%) and, most important productive character - honey production was only at 86.2% compared to the race standard.

Table 2 The dynamic of development level of morph-productive characters of the bee families from experimental apiaries of IZ in 2010-2014 years

Morph-productive characters	The level of development of morph-productive characters, M ± m					Race stan-dard	2014, % to standard
	2010 N=16	2011 N=50	2012 N=50	2013 N=50	2014 N=50		
1. Queen prolificacy, egs/24 h	1583±34	1706±13	1740±16	1761±14	1781±17	1600	111.3
2. Family strenght, kg	2.83±0.07	2.97±0.02	2.37±0.02	3.03±0.02	3.13±0.02	2.33	134.3
3. Over winter resistance, %	80.1±2.5	82.5±0.9	86.2±0.7	91.1±0.4	93.3±0.4	75	124.4
4. Broods viability, %	85.1±0.3	87.1±0.3	88.6±0.4	91.0±0.2	92.3±0.2	80	115.4
5. Resistance to diseases, %	76.8±0.5	89.4±0.3	87.4±0.3	90.5±0.4	91.6±0.2	60	152.7
6.Honey production, kg	38.8±1.3	44.8±0.9	23.9±1.2	35.5±0.8	57.4±0.8	45	127.5
7.Class of evaluation,%: elite-record elite class I class II class III extra class	- 6.3±6.2 12.5±8.5 12.5±8.5 25.0±11.2 43.7±12.8	6.0±3.4 18.0±5.5 22.0±5.9 26.0±6.3 22.0±5.9 6.0±3.4	- - - 4.0±2.0 20.0±5.7 76.0±6.0	- - 2.0±2.0 18.0±5.5 56.0±7.1 24.0±6.1	42.0±7.0 48.0±7.1 8.0±3.8 2.0±2.0 - -	100	98

For genetic amelioration of morph-productive characters of bee population, the measures of directed progressive selection by all independent limits of selected characters, were taken.

As a result, prolificacy of the queen also increased annually, although slightly, but quite significantly from 1583 ± 34 eggs / 24 hours in 2010 up to 1781 ± 17 eggs / 24 hours in 2014 or with 12.5% (t_d = 5.2; P <0.001).

Although the family strength, was subject to a stagnation in 2012 due to unfavourable climatic and melliferous conditions (severe drought), however, increased significantly from 2.83 ± 0.07 kg in 2011 to 3.13 ± 0.02 kg in 2014 or with 10.6% (t_d = 4.3; P <0.001).

Due to the progressive directed selection and of measures fortification of the bees' immunity, by using in their food of bioactive supplements of new generation, we managed to improve substantially the resistance to disease and wintering of the bee families and brood viability.

Thus, the resistance to wintering of bee families increased significantly during this period, from 80.1 ± 2.5 percentage units in 2011, to 93.3 ± 0.4 percentage units in 2014 or with 16.5 % (t_d = 5.3; P <0.001), reaching a very high level, exceeding by 24.4% the race standard (t_d = 45.7; P <0.001).

Under the selection pressure the brood viability has grown steadily from 85.1 ± 0.3 percentage units in 2011, to 92.3 ± 0.2 percentage units in 2014 or with 8.5% (t_d = 20.0; P <0.001).

Quite obviously, this dynamic of increase the level of development of morph-productive characters of bees families from the population of IZ are shown in the histogram in Figure 2.

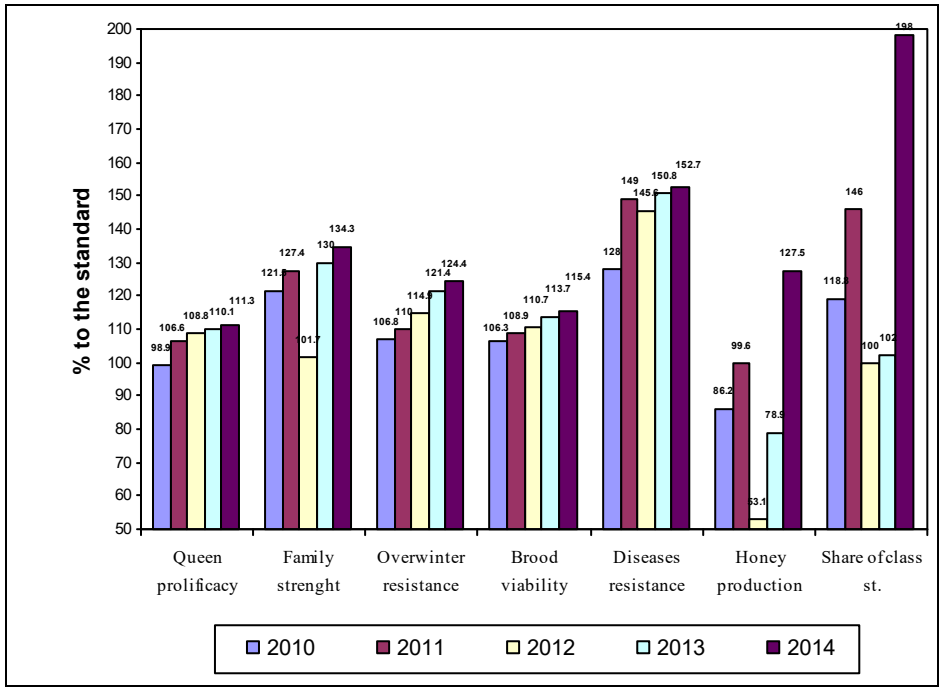

Fig. 2 Dynamics of evolution of the level of morph-productive characters of bee families at experimental apiary of IZ

Most important is the fact of substantial increase of bee families resistance to disease, from 76.8 ± 0.5 percentage units in 2011, to 91.6 ± 0.2 percentage units in 2014 or with 19.3% (t_d = 27.4; P <0.001).

The most difficult problem in the genetic amelioration of morph-productive characters of bee families was to realization honey production potential, which was strongly influenced by environmental factors. Thus, after the selection works, started in 2010, with the bee families with honey production (38.8 kg) under the race standard, in 2011 we succeeded in reproducing a new generation of bee families with honey production (44.8 kg) at limit the race standard.

Subsequently, followed two years of natural cataclysms (2012 - terrible drought, in 2013 - heavy rain and cold during spring harvest) that seriously affected the honey production in the bee families, reducing it to 53.1 and 78, 9% of the standard the race. For the full realization of the genetic potential of honey production of bee families, in 2014, measures of pastoral have been applied, thus, ensuring the necessary melliferous base of for harvest. These measures have given the desired result - a significant increase of honey production, reaching average a record level in selected population.

So, it was shown that under optimal harvesting conditions, honey production potential of bee families increased average per population from experimental apiary, from 38.8 ± 1.3 kg in 2010 to 57.4 ± 0 8 kg in 2014 or with 47.9% (t_d = 12.2; P <0.001).

The share of bee families of evaluation classes that corresponding to the standard level (class I + elite+ elite-record) after the complex of characters increased in population experimental apiary of IZ, from 18.8% in 2010 to 98% in 2014.

At the apiary of *National Association of Beekeepers,* the dynamics of development of the main morph-productive characters, increased, also, successively, according to the table 4.

Thus, the level of development of characters and features related to the racial purity characteristics, such as: the share of bees families with typical colour of the tegument, the share of bee families with

gentle behaviour at nest opening, the share of bee families that do not leave the comb at its examination, the share of bee families with mixed predominantly dry and dry cell capping type, the share of families with positive discoidal shift, the value of cubital index and proboscis length, constantly have increased during this period by 3,4– 10,6%.

Table 4 The dynamic of development level of morph-productive characters of the bee families from experimental apiaries of National Association of Beekeepers in 2010-2014 years

Morph-productive characters	The level of development of morph-productive characters, M ± m				Race standard	2014, % to standard
	2011 N=30	2012 N=30	2013 N=30	2014 N=30		
1.Tegument's colour, dark brown + meddle, %	83.3±6.9	83.3±6.9	86.7±6.3	86.7±6.3	100	86.7
2. Behaviour, %: - by opening the nest, gentle % - by examining the comb: not leaving,%	 93.3±4.6 86.7±6.3	 93.3±4.6 90.0±5.5	 96.7±3.3 93.3±4.6	 96.7±3.3 93.3±4.6	 100 100	 96.7 93.3
3. Type of capping of cells, mixt+dry, %	96.7±3.3	100±0.0	100±0.0	100±0.0	100	100
4. Proboscis length, mm	6.40±0.02	6.52±0.02	6.54±0.02	6.65±0.02	6.40	103.9
5. Cubital index, %	42.1±0.6	44.1±0.7	43.0±0.8	45.8±0.4	40	114.5
6. Positive discoidal shift, %	72.7±3.6	73.9±3.6	77.4±3.7	80.4±2.7	70	114.8
7. Queen prolificacy, egs/24 h	1648±18	1665±23	1680±19	1687±28	1600	105.4
8. Family strength, kg	2.61±0.07	2.32±0.03	3.13±0.03	3.26±0.02	2.33	139.9
9. Over winter resistance, %	82.5±0.9	87.7±0.9	89.5±1.2	89.4±0.5	75	119.2
10. Broods viability, %	79.1±0.6	88.4±0.4	91.5±0.2	92.4±0.3	80	115.5
11. Resistance to diseases, %	83.9±1.1	87.9±0.4	91.3±0.5	91.6±0.2	60	152.6
12.Honey production, kg	40.6±1.1	31.1±0.9	40.6±0.6	42.9±0.9	45	95.3
13.Class of evaluation,%: elite class I class II class III extra class	 3.3±3.3 40.0±9.0 16.7±6.9 30.0±8.5 10.0±5.5	 - 3.3±3.3 10.0±5.5 33.3±8.7 53.4±9.3	 3.3±3.3 40.0±9.0 13.3±6.3 33.3±8.7 10.0±5.5	 3.3±3.3 46.7±9.3 16.7±6.9 26.7±8.2 6.6±4.6	 100	 50.0

Among the morph-productive characters, the biggest rate of increase has had the level of development of family strength, which increased during the reference period, the averaged over population of the apiary ANARM, from 2.61 ± 0.07 kg in 2011 up to 3.26 ± 0.02 kg in 2014 or with 24.9% (t_d = 8.9; P <0.001).

Quite fast has evolved the dynamics of level of development of characters that characterizes the resistance and viability of bee families.

Thus, the resistance to wintering of families from apiary ANARM increased between 2011-2014, from 82.5 ± 0.9 percentage points in 2011, up to 89.4 ± 0.5 percentage points in 2014 or with 8.4% (t_d = 6.7; P <0.001).

The brood viability in the nest of bee families has increased substantially during this period, the averaged over reference apiary, from 79.1 ± 0.6 percentage points in 2011 up to 92.4 ± 0.3 percentage points in 2014 or with 16.8% (t_d = 19.8; P <0.001).

Resistance to diseases of bee families in the apiary has also increased, from 83.9 ± 1.1 percentage points in 2011, up to 91.6 ± 0.2 percentage points in 2014 or with 9.2% (t_d = 6.9; P <0.001).

And, the most important productive character of bee families, such as honey production slightly has not reached (2.1 kg) the race standard at the ANARM apiary, but increased from 40.6 ± 1.1 kg 2011, up to 42.9 ± 0.9 kg in 2014 or with 5.7% (t_d = 1.6; P <0.1).

As a result of increasing of the level of development of all the researched morph-productive characters, in the population of bees families of ANARM apiary, increased the share of bee families of evaluation classes that

correspond to the standard (class I and elite) from 43.3 percentage points in year 2011 up to 50.0 percentage points or with 15.4%.

Therefore, due to the systemic directed progressive selection of bee families after independent limit of the development level of external morph-metric characters and the morph-productive, and behavioural features, in the above mentioned apiaries were created populations with bees enough productive, with enhanced resistance to diseases that present valuable substrate for collecting genitor beekeeping material intended for breeding.

Annually, from the populations of bee families assessed by the complex of characters, were selected batches of the most valuable breeding families, after external morphological features which corresponded hundred percent to race standard requirements, and after morph-productive characters significantly exceeded these requirements.

At the *experimental apiary of IZ,* analysis of the dynamics of development level of morph-productive characters of bee families from breeding batches, demonstrates that their genetic value has risen successively permanent, and currently, is enough high (Table 5).

Table 5 The dynamic of development level of morph-productive characters of the bee families from *breeding batches* of experimental apiary of IZ, in 2010-2014 years

Morph-productive characters	The level of development of morph-productive characters, M ± m				Race stan-dard	2014, % to standard
	2011 N=8	2012 N=8	2013 N=9	2014 N=14		
1. Tegument's colour, dark brown + meddle, %	100±0.0	100±0.0	100±0.0	100±0.0	100	100
2. Behaviour, %: - by opening the nest, gentle % - by examining the comb: not leaving,%	 100±0.0 100±0.0	 100±0.0 100±0.0	 100±0.0 100±0.0	 100±0.0 100±0.0	 100 100	 100 100
3. Type of capping of cells, mixt+dry, %	100±0.0	100±0.0	100±0.0	100±0.0	100	100
4. Proboscis length, mm	6.56±0.02	6.57±0.02	6.56±0.02	6.62±0.02	6.40	103.4
5. Cubital index, %	44.3±0.8	45.2±0.8	45.4±0.5	45.3±0.6	40	113.2
6. Positive discoidal shift, %	84.5±2.1	82.5±2.8	84.1±4.8	84.5±2.9	70	120.7
7. Queen prolificacy, egs/24 h	1725±45	1756±36	1750±22	1873±21	1600	117,1
8. Family strength, kg	3.11±0,08	3.13±0.07	3.13±0.03	3.19±0.03	2.33	136.9
9. Over winter resistance, %	90.1±0,6	91.0±0.4	93.5±0.6	94.3±0,4	75	125.7
10. Broods viability, %	84.0±0.8	90,6±0.9	91.6±0.5	92.5±0.4	80	115.6
11. Resistance to diseases, %	92.1±0.8	92.6±0.7	92.6±0.4	92.2±0.4	60	153.7
12. Honey production, kg	54.3±0.9	54.6±0.7	56.1±0.4	63.2±0.9	45	140.0
13. Class of evaluation, %: elite record elite class I	 37.5±18.3 62.5±18.3 -	 37.5±18.3 62.5±18.3 -	 88.9±11.1 11.1±11.1 -	 100±0.0 - -	 100	 100 100

After the average development level of morph-productive characters, the bee families of the breeding batch from experimental apiary, significantly exceeded the race standard: the proboscis length - with 3.4% (t_d = 11.0; P < 0.001); the cubital index – with 13.2% (t_d = 27.2; P < 0.001); the share of families with positive discoidal shift– with 20.7% (t_d = 5.0; P < 0.001); the queen prolificacy – with 17.1% (t_d = 13.0; P<0.001); the family strength– with 36.9% (t_d = 28.7; P < 0.001); over winter resistance – with 25.7% (t_d = 48.2; P < 0.001); brood viability – with 15.6% (t_d = 31.2; P < 0.001).

But the highest genetic value of bee families of this breeding batch is high honey productivity and resistance to disease.

Thus, the bees families of breeding batch from experimental apiary, significantly has exceeded the standard of the race after honey production - 40.0% (t_d = 20.2; P <0.001) after disease resistance - 53.7 % (t_d = 134.2, P<0.001). The genetic breeding value of bee families of those batches, evaluated after the complex of morph-productive characters, is expressed in the highest class of evaluation. Annually, the share of bee families of the

superior evaluation class - elite-record, increased from 37.5% in 2011 up to 100% in 2014. Therefore, at present all the bee families in the breeding batch from the apiary are of the highest class of evaluation, which inspires confidence that the genitor material, taken and raised in families of this batch, has a genetic value and a high production potential.

At the ANARM apiary, also a breeding batch was selected for, genetically enhanced with highly productive bee colonies (Tab. 6).

Table 6 The dynamic of development level of morph-productive characters of the bee families from *breeding batches* of experimental apiary of ANARM, in 2010-2014 years

Morph-productive characters	The level of development of morph-productive characters, M ± m				Race stan-dard	2014, % to standard
	2011 N=6	2012 N=7	2013 N=6	2014 N=5		
1. Tegument's colour, dark brown + meddle, %	100±0.0	100±0.0	100±0.0	100±0.0	100	100
2. Behaviour, %:						
- by opening the nest, gentle %	100±0.0	100±0.0	100±0.0	100±0.0	100	100
- by examining the comb: not leaving,%	100±0.0	100±0.0	100±0.0	100±0.0	100	100
3. Type of capping of cells, mixt+dry, %	100±0.0	100±0.0	100±0.0	100±0.0	100	100
4. Proboscis length, mm	6.52±0.06	6.52±0.04	6.57±0.04	6.60±0.04	6.40	103.1
5. Cubital index, %	42.7±1.0	44.4±0.9	44.6±1.0	44.6±1.2	40	111.5
6. Positive discoidal shift, %	81.7±4.6	85.4±3.3	83.0±3.0	93.0±3.4	70	132.8
7. Queen prolificacy, egs/24 h	1722±15	1734±15	1741±25	1749±33	1600	109.3
8. Family strength, kg	2.88±0.04	2.91±0.05	3.17±0.04	3.22±0.03	2.33	138.2
9. Over winter resistance, %	83.1±0.9	83.3±0.7	82.0±0.7	89.4±1.2	75	119.2
10. Broods viability, %	91.8±0.4	91.9±0.2	91.9±0.3	92.4±0.6	80	115.5
11. Resistance to diseases, %	88.4±2.1	91.3±0.4	93.0±0.7	91.4±0.4	60	152.3
12. Honey production, kg	54.5±1.1	57.1±1.1	54.0±1.0	49.3±0.9	45	109.6
13. Class of evaluation, %:						
elite record	20.0±17.9	100±0.0	50.0±22.3	-		-
elite	80.0±17.9	-	50.0±22.3	20.0±20.0		100
class I	-	-	-	80.0±20.0	100	100

From the presented on the table data, it is seen that from the breeding batch of ANARM apiary annually bee families who corresponded hundred percent to the race standard they were selected, after external morph-metric characteristics, such as: the typical colour of tegument (dark brown or middle brown), gentle behaviour when opening the nest and peaceful at the examination honeycomb (and not leaving it), requested type of capping of cell with honey - mixed - predominantly dry and dry, which confer to honeycomb not only a pleasant competitive commercial aspect, but also a higher technological effect on honey extraction.

The level of development of morph-productive characters in this breeding batch of bee families always was growing successively in the period 2011-2014, except for some characters, in unfavourable, from the melliferous base viewpoint, years. Following a rigorous selection of bee families after the complex of morph-productive characters, in 2014, we succeeded in creation of a breeding batch, genetically consolidated, with highly productive families and increased resistance to disease. Thus, after average level of development of the morph-productive characters, bee families in the breeding batch of ANARM apiary, substantially exceed the race standard: at proboscis length – with 3.1% (t_d = 5.0; P<0.001); at cubital index – with 11.5% (t_d = 3.8; P < 0.001); the share of families with positive discoidal shift – with 20.7% (t_d = 6.8; P < 0.001); the queen prolificacy – with 9.3% (t_d = 4.5; P < 0.001); the family

strength– with 38.2% (t_d = 29.7; P < 0.001); over winter resistance – with 19.2% (t_d = 12.0; P < 0.001); brood viability – with 15.5% (t_d = 20.7; P < 0.001), resistance to disease – with 52.3% (t_d = 20.7; P < 0.001). Honey production in the medium per breeding batch families, was lower compared to previous years (due to weaker melliferous conditions in the location zone of apiary) but still it higher than the race standard by 9.6% (t_d = 4.8; P < 0.001).

Therefore, as a result of the application during the years 2011-2014 of directed progressive selection of bee families, taking into account their biological particularities, the breeding batches and genetically consolidated populations of high productive families with increased resistance to disease, were created at the experimental apiaries of the Institute of Zoology and ANARM

CONCLUSIONS

1. As a result of researches and selection at experimental apiaries of Institute of Zoology of the Academy of Sciences of Moldova, (a middle apiary, with 50 bee colonies) and of the National Association of Beekeepers of Moldova (small apiary, with 30 families), they were created and genetically consolidated 2 populations of bee colonies of *Apis mellifera Carpatica* with high productivity and resistance to diseases.

2. Bee populations correspond to the race standard by external morphological features, which certify the purity race, and possess high productivity at: productivity of honey (42.9–57.4 kg), increased resistance to diseases (91.6%) and over winter resistance (93.3–89.4%), the strong viability brood (92.3–92.4%), which, respectively, with 27.5%, 52.4%, 45.7-19.2% and 15.4-15.5% is higher than the race standards.

3. Within the populations of colonies were created 2 breeding batches with high productive bees families, with an livestock of 14 families at IZ apiary and 5 families at ANARM apiary, with genetically consolidated morph-productive performance at high level (proboscis length 6.60 – 6.62 mm, cubital index 44.6–45.3%, positive discoidal shift 84.5–93.0%, family strength 3.19–3.22 kg, queens prolificacy 1749–1873 eggs/24 h, over winter resistance 89.4–94.3%, brood viability – 92.4–92.5%, disease resistance 91.4–92.2%, honey production 49.3– 63.2 kg).

4. All the bees families from the breeding batches correspond to the request of race standards *Apis mellifera Carpatica*, after external morphological features, which characterise the race purity and has exceed the race standard after morph-productive characters: at queen prolificacy – with 9.3-17.1% (P < 0.001), at family strength – with 36.9 – 39.2% (P < 0.001), at over winter resistance – with 19.2–25.7% (P < 0.001), at brood viability – with 15.5-15.6% (P<0.001), disease resistance – with 52.3–54.7% (P < 0.001) and honey production – with 9.6–40.4% (P < 0.001).

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REFERENCES

[1] Bura M., Pătruică Silvia, Bura V.A. Tehnologie apicolă. Editura "Solness", Timişoara, Romania, 2005, p. 408

[2] Cebotari Valentina. Program de conservare şi ameliorare a albinelor din Republica Moldova. Realizări şi perspective în creşterea animalelor. Simpozion ştiinţific internaţional, Chişinău, 2006, p. 328-330.

[3] Cebotari Valentina, Buzu I., Toderici V. Morph-productive performance of bee colonies in breeding apiaries. University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania. International Scientific Symposium: „Tradition, performance and efficiency in animal husbandry" – 60 years of animal science higher education in Moldova. Lucrări Ştiinţifice, Cotaţie CNCSIB B$^+$, vol. 55, Seria Zootehnie, Iaşi, 2011, p. 63-67.

[4] Cebotari Valentina, Buzu Ion. Zootechnical norms regarding the honeybee colonies evaluation, breeding and certification of genetic material in beekeeping. Contemporary Science Association. Proceedings of the 1st International Animal Health Science Conference: The Beekeeping Conference.

Addleton Academic Publishers, New York, (Bucureşti), 2010, Library of Congress Control Number, p. 26-30.
[5] Cebotari Valentina, Buzu Ion. Study on the heritability of some morpho-productive characters of *Apis mellifera Carpatica* bee families populated in the Zone of Center of the Republic of Moldova. University of Agronomic Sciences and Veterinary Medicine of Bucharest. Scientific Papers, Series D. Animal Science, Vol. LVII, Bucharest, 2014, p. 35–42.
[6] Cebotari Valentina, Buzu Ion, Postolachi Olga et al. Testarea mătcilor de albine după calităţile descendenţei. University of Agronomic Sciences and Veterinary Medicine of Bucharest Faculty of Animal Science. Scientific Papers, Series D, Animal Science, Volume LVII, Bucharest, 2015
[7] Dariusz Gerula, Malgorzata Bienkowsca. Performance of artificially inseminated Honeybee queens that have bodily injuries. Journal of Apicultural Science. Poloni, Warschowie. Vol. 52, nr. 1, 2008, p. 13-19.
[8] De Jong David. Colony collapse disease in Brazil. APIMONDIA, Congresul stiinţific al 41-a, Franţa, Montpellier, 2009, p. 40.
[9] Fotii N. Data on heritability of the queen supersedure characteristic in the Carpathian bee (Apis mellifera Carpatica). Twenty-seventh International Congres of Apiculture. APIMONDIA, Athenes, Greece, 1980, p. 14-20.
[10] Marie-Pierre Chauzat, Sarah Zeggane. Case studies on mortalities of honey bee colonies (Apis mellifera) in France during the 2005-2006 winter. APIMONDIA, Congresul stiintific al 41-a, Franţa, Montpellier, 2009. p. 40.
[11] Normă zootehnică privind bonitarea familiilor de albine, creşterea şi certificarea materialului genitor apicol, aprobată prin Hotărârea Guvernului nr. 306 din 28.04.2011 (M.O. nr. 78-81, 2011, art. 366).
[12] Ruttner F. Creşterea mătcilor. Baze biologice şi indicaţii tehnice. Ediţia Apimondia, Bucureşti, 1980, p.166
[13] Ruttner F. Însămînţarea instrumentală a mătcii. Ed. Apimondia, Bucureşti, 1976, p.126
[14] Ruttner Friedrich. Creşterea mătcilor (traducere din germană de Erica Dumitrescu şi Elke Martini). Editura „APIMONDIA", Bucureşti, 1980, p. 331
[15] Siceanu A., Radoi Cecilia, Cebotari Valentina. Studiu comparativ al populaţiilor de albine din Romania – Podişul Moldovei şi R.Moldova, şi stabilirea unor tehnologii performante şi eficiente economic de ameliorare şi conservare a acestora aplicabile în stupine mari şi mijlocii. Conferinţa ştiinţifico-practică „Apicultura suport durabil pentru gimnaziile-internat". Chişinău, ORDSE, 2002, p. 35-39.
[16] Szabo T.I., Lefkovinch L.P. Effect of brood production and population size on honey production of honeybee colonies in Alberta, Canada. Apidologie, 1989, 20, p. 157-163.
[17] Плохинский Н.А. Руководство по биометрии для зоотехников. Изд. «Колос», Москва, 1969, 256 с.