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DNA-certification of breeds of bees of Ukraine in the system of preservation and development of their gene pool

Goal. To develop a method of DNA passporting and to establish gene pool standards for purebredness in domestic beekeeping by molecular genetic fingerprint markers with multiple localization in the genome (RAPD, ISSR). Methods. Molecular genetic, statistical, morphometric, ethological. Results The results of molecular-genetic monitoring of bees of Ukrainian, Carpathian and gray mountain Caucasian breeds for using different technologies of detection of polymorphism of genetic loci are presented. Genetic passports of rocks were developed based on identified identification alleles. The absolute marker of the gray mountainous Caucasian breed G800, which was present in all its representatives, is a reliable tool for identifying families of indigenous origin of bees of Ukrainian aboriginal breeds. Conclusions Establishing genetically determined genetic differences, confirmed by genetic-population indicators, makes it possible to create natural reserves of pure-breed bees as a compulsory component in the system of preservation of their gene pool and breeding improvement.

Key words: identification marker, genetic passport, bees breed, purebred criterion, gene pool.

One of the key problems in beekeeping is interbreeding hybridization, the search and preservation of reserves of pure-bred arrays of aboriginal bees in Ukraine. The decrease in the productivity of bee families of landed origin (winter resistance, resistance to infectious diseases, family strength, fertility of the uterus, etc.) leads to the need to find objective criteria for assessing the degree of pure-breeding, fenogenetic consolidation of bees' tribal beings families taking into account the inherited and economically useful features of each complex of breed [4, 14].

Currently, genetic passporting is an urgent task of modern breeding of farm animals, including honey bees. The spontaneous, uncontrolled inter-species mediation that took place over the past years and the selection of multifactorial features, mainly associated with the manifestation of honey productivity, have led to a violation of the unique gene complexes adapted to certain conditions for the existence of bee families that traditionally have developed over a long period. This in turn led to unpredictable combinations genetic material, reduction of non-specific resistance of bees as a result of imbalance of immune defense systems and adaptability to the dynamics of ecological and geographical factors.

Along with the classical morphometric methods of assessing the subspecies and ecotypic suitability of bees in order to determine the degree of their genetic homogeneity, consolidation and pure-bloodedness, informational markers traditionally use mitochondrial DNA locuses [11] and microsatellite genome sequences [16]. At the same time, the effectiveness of the use of these almost traditional molecular genetic markers remains low due to insufficient equipment of genetic control laboratories for modern equipment, low funding and limited number of loci available for simultaneous genotyping of individuals. This issue is particularly acute in bee keeping, as an international project to explore the gene pool and mapping the honey bee genes continues [10]. Therefore, it is necessary to find new approaches for the simultaneous marking of many genetic loci to determine the specificity of the genome of each individual under investigation in order to objectively assess the originality of gene pool populations. Particularly important is the solution of this issue in connection with the solution of the main problem - the identification of the most typical representatives of populations and the creation of genetically valid programs for their conservation and rational use [1, 2]. It is

obvious that the creation of polyolecular systems of genetic markers will provide valuable information on the population-genetic structure of aboriginal honey bee breeds and the range of their local populations, and the effectiveness of the genetic approach can be greatly enhanced by comparing and correcting the results obtained by the traditional method- we morphometry.

The purpose of the research was to create information systems for DNA genotype genotyping for the determination of genetic polymorphism at the interspecific level and the establishment of criteria for purebredness, the nature of differentiation and the elimination of the negative effects of the methytization of the gene pool of bees of Ukrainian breed. In order to achieve this goal, methodological optimization of the DNA extraction procedure from the ima working bees has been carried out, the ISSR and RAPD primer nucleotide structure, which is suitable for the study of the genetic polymorphism at the interspecific level, is experimentally established an optimal mode of amplification of fragments of genetic loci of bees located between inverted-oriented sequences of microsatellite primers was determined, genetic monitoring of the most typical pure-breeding populations of bees of Ukrainian, Carpathian and gray mountain Caucasian breeds was made, genetic passports were developed.

Research methodology. In this work, samples of the imago of working bees (24 individuals) were used: Ukrainian breed from the Plebuche Medobory plant in Letychiv district, Khmelnytsky region, private beeps of Poltava, Mykolaiv and Sumy regions; Carpathian breed of the type "Vuchkivsky" from the Mukachevo Plemchjolozavod of the Transcarpathian region. and private enterprise (PP) "Honeyfields" of the Kyiv region; Gray mountain Caucasian breed from the Bee Field "Chervona Polyana" (Georgia) and the private enterprise of the Kyiv region.

The research was carried out on the basis of the Genetics Laboratory of the Institute of Pig Production and ATV NAAS and the Institute of Animal Breeding and Genetics named after MV Zubets of NAAS during 2011-2014.

Genomic DNA was isolated from tissues of imago working bees using commercial DNA "Sorb B "manufactured by Amplilisens (Russian Federation) in accordance with the recommendations of the manufacturer and using their own modifications [7]. For polymerase chain reaction (PCR) with ISSR, RAPD primers, a 25 μl reaction mixture was used: reaction buffer (16.6 mmol / ml (NH4) 2SO4; 67 mmol / ml Tris-HCl; 0.01% Tween-20; 2 mmol / ml MgCl2; 2 mmol / ml of each dNTP) - 2.5 μl; 100 μM primer (0.5-1 μl); from 2 to 4 units. Taq polymerase activity (0.1 - 0.2 μl); 1 - 2 ng DNA sample (1-3 μl); deionized water.

The temperature regime and the number of cycles of PCR amplification for each primer were determined separately. The structure of primers and the mode of their burning have been established (Table 1).

1. Concentration and temperature regime of ISSR, RAPD firing primers suitable for DNA-certification

Electrophoresis of DNA amplification products obtained with RAPD primers, ISSR was carried out in 2% agarose gel followed by staining in a solution of bromide etidium [6]. Visualization of the patterns was carried out on a transilluminator for a length of UV radiation of 340 nm followed by photocodification by a digital camera. The control of the size of the obtained PCR products was carried out using molecular weight markers of 1 kb-Ledder plus ("Fermentas", Lithuania).

For the analysis of the genetic structure of the groups of animals, polyolexic DNA markers used specialized mathematical algorithms for the use of standard computer programs GELSTATS [15], GENALEX-6 [13], TREE [13], Statistica, BIOSYS-1 [17], MEGA-4 [12]. The statistical probability of the obtained results was calculated according to the criteria $\chi 2$, Student, Fisher [5, 8, 9].

Research results and their discussion. The procedure for extracting DNA from the imago of working bees has been optimized and the nucleotide structure of ISSR primers suitable for the study of the genetic polymorphism at the interspecific level has been established. The methodological approach for determining the suitability of primers in RAPD, ISSR technologies for identifying inter-breeding differences is based on the use of equivalent DNA mixtures of typical breed representatives and the mean-temperature optimum of the amplification regimen + 58 ° C. The calculation of the parameters of the informativity of primers based on the analysis of three breeds of bees (Ukrainian, Carpathian and gray Mountain Caucasian) shows that the primers for reproduction of 17 to 33 amplitudes and characterized by 3.14 - 7.23 genomic locus were found to be the most suitable for conducting genetic-population studies of bees at interspecies level: ISSR-S1 genetic systems, S2, S4, S7, S9 and RAPD markers based on OPA-1, OPA-4, and B15 seedlings.

According to the calculated variables of marker indices, the most informative systems for inter-breeding equations of bees are ISSR-S4 with a marker index (MI) of 3,05 and RAPD-OPA-4 (MI = 4,59).

Three types of bees were subjected to DNA testing, previously characterized by morphometric indices corresponding to the phenotypic purity criteria for 5 ISSR systems and 3 RAPD markers. The genetic testing method used by us after population-genetic research with the use of fingerprinting RAPD-, ISSR-technologies was based on the establishment of absolute and relative markers with a high frequency of distribution and reliable criteria for differences within the analyzed populations. This approach was used to develop genetic passports. The genealogical passport formula contains alphanumeric primers with striker indices - the size of the identification DNA fragments of the breed (Table 2).

2. Genetic formulas of three breeds of bees under DNA analysis with 3 primers RAPD and 5 ISSR

The highest number of highly informative rock-specific markers for Ukrainian breed of bees was detected by the ISSR-S4 primer, Carpathian RAPD-OPA-1 (with 3 identifying DNA fragments), the Caucasian-ISSR-S1 and S2, which generally detected 14 identification alleles. An amplification with a microsatellite with an anchor primer S1 in the ISSR technique leads to the formation of a finerprint with a total number of bands of 32 with dimensions in the range of molecular weights of 1430-220. This genetic system is suitable for the establishment of specific characteristics of bees in the Caucasian breed - in general, 7 DNA markers have been identified. Particular attention deserves to be a DNA band of 1000 bp which occurs in all investigative representatives of the Caucasian breed and not found at all in Ukrainian bees, therefore it can be considered an absolute diagnostic marker for these two breeds. The small concentration of this DNA fragment found in the sample of Carpathian breed bees (25%) does not allow unambiguously to separate the representatives of the Carpathian and Caucasian breeds at the level of one individual or a limited sample. The largest number of unique breed markers was discovered for gray mountain Caucasian bees - a total of 24, one of which was characterized as absolute and monomorphic (with a frequency in the population of 1,000), since it was characteristic only to individuals of the Caucasian breed and did not occur in populations of others breeds of bees used in this experiment.

This marker (labeled in bold in the formula - G_{800}), established by means of an inter-microsatellite analysis with the S7 primer, is a reliable tool for identifying pure-breed bees of the Caucasian family and families of Ukrainian and Carpathian breeds of hybrid origin. In general, the maximum of specific species markers established for bees of the Caucasian breed is explained by their geographical isolation and minimization of the influence on the structure of their gene pool of individuals of Ukrainian breeding. The beekeepers of Ukrainian and Caucasian breeds are the most contrasting in most genetic-population indicators.

The average number of amplification products detected in total due to the typing on 8 polyolefic systems in bees of the Ukrainian breed was 80.84 versus 69.50 in the gray Caucasian ($P < 0.001$), the value of this parameter in the Carpathian breed bees was 73, 71

The calculated heterozygosity in the population of bees of the Caucasian breed was 0.441 and was significantly lower ($P < 0.001$) than the value of this parameter in individuals of the Carpathian (0.461) and Ukrainian (0.465) breeds. This may indicate moderate inbreeding and understated effective numbers of populations used in the study.

To determine the nature of the phylogenetic relationships between bees in the Ukrainian, Carpathian and Caucasian breeds, the available sample of animals was divided into separate subpopulations and based on the conducted genetic-population analysis, 8 genetic distances were calculated according to the M. Ney algorithm [15]. According to the received data, bees of Ukrainian and Carpathian breeds were characterized by a high degree of genetic similarity (0.578): according to genetic monitoring results involving more than 50 polymorphic genomic loci in the analysis, the value of the index was 0.457 between bees of Ukrainian and Caucasian breeds for Carpathian and Caucasian bees - 0,458. Calculated values of genetic distances between populations of bees of different breeds were used to construct a dendrogram consisting of two separate clusters (figure).

The structure of one of them consisted of a subcluster consisting of populations of the Ukrainian genus Khmelnytsky type and bees of the Carpathian breed from the apiaries of the Kyiv region. Individuals of the Carpathian breed and the Ukrainian "Novoukrainsky" type formed separate branches as part of a single cluster.

In another (separate) cluster of dendrograms included populations of one breed - gray mountain Caucasian. The formation of a common subcluster for populations of Ukrainian breeds (such as Khmelnytsky and bees from the Kyiv region) may partly indicate that it was Ukrainian bees that were repeatedly imported into the Kyiv region. This explains the nature of the grouping of subcluster branches and proves the unclean-like origin of Carpathian bees from the apiary chosen for the experiment, despite their morphometric compliance with the standards of the Carpathian breed.

Conclusions

The obtained data of the cluster analysis of three-breed bee colonies suggest that the bees of the Carpathian and Ukrainian breeds belong to the same species (Apis mellifera carnica) and can be considered as separate ecotypes with the existence of a certain level of genetic differentiation and the presence of unique determinants at the phenotypic and genetic levels. In order to enhance the uniqueness of the Ukrainian and Carpathian breeds, it is necessary to create natural isolated reserves under conditions of strict morphometric control, molecular-genetic methods of identification of pure-bred individuals, geographic isolation and the use of breeding methods in semi-closed populations. The results of the research, the cluster analysis and the genetic passports of three breeds of bees were created, can be used to develop effective measures for the rational use and restoration of the number of autochthonous Ukrainian bees.

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