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POLYMORPHIC STR MARKERS AS A TOOL FOR POPULATION-GENETIC STUDIES OF *Apis mellifera* L. HONEYBEES
(review)

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Abstract

The relevance of honeybee biology comprehensive study is increasing every year. Primarily, this is caused by the decline of honeybee populations which occurs all over the world including the Russian Federation. Historically, the Europe and Africa continents were the habitat of the only representative of genus *Apis*, the honeybee *Apis mellifera* L. from which a significant number of freely interbreeding races (subspecies) derived during evolution. Nowadays, due to human introduction of honeybees to other continents, *Apis mellifera* are found all around the world. The loss of unique gene pools and purebred status of native honeybee subspecies due to uncontrolled hybridization is a matter of great concern worldwide (P. de la Rúa et al., 2009). Therefore, evolutionary relationships and population genetics of *A. mellifera*, genetic control of domestic and imported breeding stock purity, breed authentication, genome-wide association mapping for traits of apicultural interest (e.g., queen performance, flight activity, honey and wax productivity, resistance to parasites, winter hardiness, royal jelly components, bee venom, etc.), and breeding value estimation are the key points of approach to biodiversity conservation in honeybees. The set of parameters characteristic of the population/line as a whole is the necessary base to preserve and maintain polymorphism as a component of population stability (N.I. Krivtsov et al., 2011). Genetic structure of breeding populations and relations between geographically isolated populations are relevant to characterize breed gene pool and optimize selection programs. The paper discusses general aspects of microsatellite structure, the main models of evolution (H. Fan et al., 2007) and putative mechanisms of origin in eukaryotic genomes (A.V. Omelchenko, 2013). Microsatellites are tandem repeats of short (2-6 bp) noncoding sequences that are dispersed throughout the nuclear genome (W.S. Sheppard et al., 2000). Microsatellites are located in both protein-coding and non-coding regions, including regulatory sequences (I. López-Flores et al., 2012). It is believed that microsatellites emerge and spread via formation of various non-canonical DNA structures that favor the slipping of replication forks (R.D. Wells, 1996). Microsatellite loci are a very convenient tool to analyze the genetic structure of populations, estimate genomic inbreeding and the level of heterozygosity, calculate genetic similarity coefficients, and determine the level of introgression. This paper overviews the use of STR markers for reconstruction of the honeybee evolutionary history. The principal research papers on population genetics of various *A. mellifera* subspecies from Europe, Asia, America, and Africa are considered. Special attention is paid to the Russian honeybee breeds and populations. To summarize, the STR markers due to the large number of alleles, the high frequency of mutational events and codominant type of inheritance continue to be extremely powerful tool for genomic mapping, verification of the genomic authenticity, and in genetic and evolutionary studies of populations.

Keywords: honey bee, microsatellite markers, STR markers, evolution, population genetics, gene pool, introgression

Honeybees (*Apis mellifera* L., *Hymenoptera*: *Apidae*) are the main pollinating insects on the planet, vital for the existence of many crops (rapeseed, sunflower, legumes), as well as for the preservation of natural plant biodiversity. From 1961 to 2007, agricultural dependence on pollinators in developed and developing

countries increased by 50% and 62%, respectively [1].

The relevance of a comprehensive study of the biology of the honeybee increases every year, which is caused by the emerging negative processes occurring in the populations of these insects, both in the Russian Federation and around the world. First, the pressure of biotic and abiotic environmental factors on bee colonies is increasing, resulting in a decrease in their number. For example, in Europe, the number of bee colonies has decreased by 16% in 20 years [2]. In the US, the annual loss of bee colonies is close to 50% [3]. The mass death of bee colonies, according to many researchers, in the short term can lead to irreparable consequences, up to the complete disappearance of beekeeping. Second, the import and reproduction of *Apis mellifera* subspecies, which are not scientifically justified, unsystematic and uncontrolled, lead to mass hybridization of bees and loss of purebredness [4]. With a high degree of confidence, these processes are associated with the mass collapse of bee colonies.

Bees of hybrid lines have less resistance to adverse environmental factors, such as abiotic stress factors [5, 6] and exposure to pesticides [7, 8], and are characterized by reduced immunity [9-11], which increases their susceptibility to parasites [12, 13] and pathogens [14, 15]. The result of interbreeding hybridization is a decrease in the adaptation of hybrid bee colonies to changing environmental conditions, which inevitably leads to an increasing death of bees. Ectoparasitic mites *Varroa destructor*, which causes varroosis [16], and microsporidia *Nosema ceranae*, which causes nosematosis type C [17], are recognized among the key factors causing the death of bee colonies in winter in Europe.

During hybridization and loss of purebredness, the gene pools of native bee subspecies are lost [18-20]. Currently, the dark forest bee *Apis mellifera mellifera* L., one of the unique subspecies of the honeybee, is recognized as endangered in Europe [21]. Thus, the issues of preserving the gene pool and native populations of the honeybee *A. mellifera* acquire worldwide significance.

Due to the frightening scale of interbreed hybridization, an important task of Russian beekeeping is to preserve the gene pools of populations of domestic breeds and subspecies of bees. Russia has some unique opportunities to preserve native honeybee populations [18, 22, 23]. The Central Russian honeybee breed *A. mellifera mellifera*, which is the most adapted to a long winter with low temperatures and resistant to some diseases, is of considerable interest and recommended for breeding in most of the country [24]. To restore the gene pool of the Central Russian breed, two large populations of the Russian honeybee that have been preserved in the Krasnoyarsk Territory and Bashkortostan can be used – the Yenisei and Burzyansk bees.

The purpose of this review is to consider some aspects of the molecular nature of microsatellite loci and the mechanisms of their evolution, as well as a retrospective analysis of the use of microsatellite markers in the population genetics of bees.

The evolution of the honeybee *A. mellifera* in its natural geographic range occurred in different climatic zones, which in the Old World led to the division of the species into 30 subspecies (according to some data, 31), of which only *A. m. mellifera* is adapted to exist in the climatic conditions of Northern Europe [25]. At the same time, Europe was the evolutionary cradle of the honeybee, which was reflected in the formation of 10 subspecies, which represent a significant part of the total genetic diversity of *A. mellifera* [26, 27].

Subspecies of the honeybee are divided into at least five evolutionary lines, the A (Africa), M (Western Europe), C (Eastern Europe), O (Middle East), and Y (North-East Africa) [28]. European subspecies are grouped into two evolutionary lines — M and C. The latter currently includes a large number of subspecies,

including two subspecies that are widely used in world commercial beekeeping – the Italian honeybee *A. mellifera ligustica* and the carnica *A. mellifera carnica*.

In their natural area, European honeybees are exposed to factors that are not related to beekeeping activities (the use of agrochemicals, destruction, and fragmentation of habitats), and those that are directly related to it (the import of parasites and pathogens, the targeted introduction of foreign queens) [29]. Of all the European subspecies of the honeybee, the subspecies *A. m. mellifera* is the most susceptible to the pressure of these factors, among which introgression plays the most important role [21, 30, 31]. The growing awareness of the importance of native subspecies as a valuable source of genetic material for the sustainable development of beekeeping has led to the creation of protected areas in Northern Europe to preserve the genetic integrity of the dark European honeybee [30-33].

It was previously thought that the dark forest bee was not in danger of passing away, as its demographics are supported by the activities of beekeepers. However, it has recently been shown that human activity is not able to compensate for the loss of honeybee biodiversity, and the conservation status of *A. m. mellifera* in Europe requires revision [34]. Over the past 200 years, the range of this subspecies in Eurasia has significantly narrowed due to the intensive reduction of forest areas, the introduction of various southern subspecies to their usual habitats, and the accompanying widespread of new invasive and infectious diseases (varroatosis, nosematosis type C, ascospherosis, etc.). In some European countries, the gene pool of the dark forest bee *A. m. mellifera* is recognized as gone. The disturbance of the native subspecies' areal continuity was caused by the introgression of southern subspecies in Western and Northern Europe, which is associated with the preferred breeding of bees of the evolutionary C branch. For example, in Europe, *A. m. mellifera* is partially, and in some cases completely, replaced by non-indigenous bees, for example, *A. m. ligustica* in Northern Europe [21] and *A. m. carnica* in Germany [35, 36]. In most of the Russian areal, *A. m. mellifera* has been replaced by subspecies *A. m. carpatica* and *A. m. caucasica* [37]. Soon, the gene pool of *A. m. mellifera* may be irretrievably lost due to the active introgression of genes of other evolutionary lines and a general decrease in the effective population of the subspecies. If a conservation strategy for this subspecies is not implemented, the collapse of bee colonies, family introgression, and population shifts will lead to the extinction of the dark forest bee, which has repeatedly happened to other species [38].

The study of the genetic structure and evolutionary relationships of *A. mellifera* populations in the territory of the Russian Federation, the determination of the purebredness of breeding stock of bees available and imported into the country, genetic certification, the identification of genomic associations with economically useful traits (in particular, with the egg production of queens, the flight activity of bees, honey and wax productivity, resistance to parasites, winter hardiness, components of royal jelly, bee venom), as well as the development of methods for assessing the breeding value of honeybees and the practical use of a genomic selection of *A. mellifera*, are of paramount importance in preserving the natural genetic diversity of domestic honeybee breeds.

When assessing the state of the breeding gene pool of bees and optimizing the selection of source material for breeding, it becomes mandatory to study the genetic structure of breeding farm populations and identify the evolutionary relationships between geographically isolated populations. To preserve the gene pool, the genetic certification of bees is necessary. It includes identification of their individual and/or group genetically determined parameters using morphological and/or molecular markers. To preserve and maintain polymorphism as a component of population stability, it is necessary to determine the parameters and group

characteristics of populations and lines [39].

It is generally assumed that populations of both eusocial and solitary representatives of the order *Hymenoptera* are characterized by an extremely low degree of polymorphism of allozyme loci [40-43]. In populations with relatively low allozyme variability, such as the majority of honeybee populations, population genetics and sociobiological analyses are difficult to perform [44]. In the search for adequate polymorphisms, much attention was paid to DNA markers, especially those associated with length variability, namely, minisatellites (DNA fingerprint) [45] and microsatellites (STR, short tandem repeats) [46]. Minisatellites are tandem repeats with a length of 15 bp or more, usually located in intergenic regions. Microsatellites consist of very short tandem repeats with a monomeric repetitive unit of 2-6 bp dispersed throughout the nuclear genome [47]. Microsatellites can be localized in both non-coding regions (including regulatory regions) and coding regions [48]. Microsatellites located inside the protein-coding regions are expected to be trinucleotide repeats, since otherwise the DNA reading frame is disrupted. The length of microsatellite clusters is on average from 20 to 60 bp (an exception is some hereditary human diseases, in which there is an expansion of triplet repeats).

Molecular mechanisms and characteristics of genome instability processes remain one of the most relevant issues in the biochemistry and molecular biology of nucleic acids. The factor of this kind of instability is, in particular, microsatellite DNA sequences. Due to the high rate of mutation processes in microsatellite sites (from 10^{-2} to 10^{-5} events per locus per generation), depending on the type of microsatellite [49], population-specific mutations accumulate quite quickly in them, which makes it possible to use information about the variability of microsatellite loci in the analysis of the population structure [50]. Studies of microsatellite regions of DNA have shown that the changes occurring in them are very diverse and depend on the types of repeats, alleles, species, and sex of living organisms, as well as the age of individuals [51].

Several models explain the variability of microsatellite loci [52-55]. Most of them fit into the so-called "stepwise mutational model", in which changes in the length of microsatellite loci occur sequentially by increasing or decreasing the repeat length by a single nucleotide. One of the main mechanisms that lead to the emergence and promote the expansion of microsatellites is the replicative fork slippage during the formation of thyroids [56]. The presence of complementary interactions between DNA/DNA duplexes of nucleotides in the regions of the DNA molecule flanking the microsatellite locus ensures the stability of these structures [57, 58]. Thus, the formation of a hairpin and a loop, and subsequent replication slippage of DNA chains during replication, are the key provisions of the “step-by-step” mutation model [59].

Computer modeling of the secondary structure of DNA molecules made it possible to establish a relationship between the number of monomeric units in microsatellite clusters and the ability of DNA molecules to form non-canonical secondary structures. It is shown that the appearance of non-canonical structures is also associated with the types of single nucleotide substitutions in microsatellite units and the types of microsatellite clusters [60-62]. There is evidence of the polarity of mutations within microsatellite DNA [63] and an increased frequency of single-nucleotide substitutions in the microsatellite-flanking regions of DNA [64]. Using the example of invertebrate dinucleotide microsatellites, it was shown that the frequency of mutations in the flanking regions may exceed the frequency of mutations in the microsatellite cluster itself [65].

Tandem repeats in general and microsatellite sequences, in particular, are considered to play an important role in the functioning of the genome at the

subcellular, biochemical, and molecular levels [66]. Currently, the most studied microsatellites are those of humans, some animals, and plants [67, 68]. At the same time, the dinucleotide microsatellite regions widely represented in the eukaryotic genome are the most interesting [69], which at the same time serve as the most evolutionarily conservative genetic markers of DNA.

Until about the mid-1990s, most information about population structure and relatedness in social insects was based on data on allozymes [70]. The first precedent for the use of DNA markers was to study the polymorphism of ribosomal RNA gene restriction sites in *Polistes* wasps [71], and since then the use of such markers has expanded rapidly. It is since microsatellite loci have some advantages: they are numerous, hypervariable, extremely informative, and widely represented throughout the genome. Microsatellite loci are a very convenient tool for analyzing the genetic structure of populations, assessing heterozygosity, the degree of inbreeding, determining the coefficients of genetic relation, calculating the genetic distances between populations and subspecies, and evaluating the inclusion of foreign genes of some species in the gene complexes of others. The first microsatellite loci in *Apis mellifera* were described in 1993 [72]. Microsatellite markers, due to inheritance from both parents, provide a more complete picture of population events, so they are actively used to assess introgressive hybridization as a result of mating drones and queens [32].

Thus, the analysis of polymorphism of microsatellite loci has become an important and popular method of population genetics studies of *A. mellifera* worldwide; to date, about 552 polymorphic genetic markers have been described [73]. Microsatellites are abundantly represented in the honeybee genome, which made it possible to create the first linkage map based on them for *A. mellifera* L. It was obtained mainly using the offspring of two hybrid queens (*A. m. ligustica* × *A. m. mellifera*). During the project implementation, 541 loci were mapped, including 474 microsatellite markers, and 24 linkage groups were identified. The average density of markers reached 7.5 cM, and the resolution was one marker for every 300 kbp of the genome [74]. In the honeybee genome, 60% of all microsatellites are located in the coding region, with 50% of the trinucleotide and 25% of the dinucleotide repeats located in the exons [75]. All these loci are polymorphic. Moreover, many of them are successfully amplified in three other species of the genus *Apis* — *A. cerana* (58%), *A. dorsata* (59%), and *A. florea* (38%). To obtain a statistically significant estimate of the structure of the honeybee population, as well as to assign individuals of unknown origin to particular populations based on the genetic distance between individuals and populations, it is sufficient to study the polymorphism of 10 microsatellite markers in 30-50 workers [76]. When using morphometric methods, processing of 200 to 750 workers is required to achieve the same degree of resolution [77].

To date, based on the data on the STR loci polymorphism level, introgression areas have been identified between bees of the subspecies *A. m. mellifera* and *A. m. ligustica* in the Alps, in Norway, and Switzerland [30], in Poland [31], and populations of Africanized honey bees in Central America [78]. The provinces of hybridization between the subspecies *A. m. ligustica* and *A. m. mellifera* in the territory of Northwestern Europe were determined [21]. The structure of honeybee populations in Spain was studied [79-81]. The phylogenetic analysis confirmed the data on the existence of evolutionary branches in *A. mellifera* corresponding to the geographical origin of its subspecies, previously obtained based on morphometric data and mtDNA analysis [82, 83]. The origin of honeybee populations in Europe [84, 85], the Middle East [86], and Africa [87] has been established. Methods of differentiation of bee populations and subspecies are proposed [88].

Studies involving the analysis of polymorphism of microsatellite markers

have been widely carried out not only to solve the problems of population genetics of the genus *Apis* but also to study other biological aspects, such as mating frequency [89], anarchy syndrome [90], and control of reproductive dominance [91].

Currently, there is a growing interest in studying the genetic structure of *A. mellifera* populations in developing countries as well. Thus, populations of the subspecies of the honeybee *A. m. jemenitica* native to Saudi Arabia [92] were studied using microsatellite markers A7, A24, A28, A88, A113, B124, Ap43, and Ap81 to determine the levels of introgression and hybridization with bees of subspecies actively imported into the country [93]. As a result, a slight deficit of heterozygotes in the subpopulations and a higher deficit of them in the general population of *A. m. jemenitica* (F_{IS} = 0.123, F_{ST} = 0.009, and F_{IT} = 0.13) were revealed. Introgression was bi-directional and more frequent in some regions than in others. At the same time, the structural analysis did not reveal different subpopulations among the samples of native bees. The high genetic diversity of local honeybees requires the urgent adoption of a program to preserve the integrity of the population.

Using the analysis of eight microsatellite markers, the polymorphism of three populations of the Iranian honeybee *A. m. meda* in the northwest of Iran was studied. Seven, five, and four polymorphic microsatellite markers were found in populations from Ardabil, Ardabil sharqi, and Ardabil gharbi provinces, respectively [94]. The total number of observed alleles is 42. Bees from the Ardabil sharqi province had the highest level of heterozygosity (0.563), and the lowest was determined for the population from the Ardabil gharbi province (0.438). In general, based on the F_{ST} assessment, the authors identified a low degree of genetic divergence between honeybee populations in Northwestern Iran.

Interesting results were obtained when studying the genetic characteristics of the population of the honeybee of the island of Rodriguez, located in the southwestern part of the Indian Ocean. In a study of 524 bee colonies from 20 different areas of the island using 18 microsatellite markers, all individuals were successfully genotyped at least 10 loci [95]. The number of observed alleles per locus ranged from three (for AP273) to 15 (for A029). Genetic diversity expressed as the representation of alleles varied between different sample collection sites from 4.75±1.58 to 5.09±1.38. Thus, the analysis of nuclear DNA showed that the honeybees on the island of Rodriguez represent a single genetically homogeneous population. It may be since the distances between settled families are extremely small to create genetic isolation (from 0.6 to 13.8 km). However, the level of genetic diversity in the studied population is comparable to that in the populations of *A. m. ligustica* and *A. m. carnica* in continental Europe. At the same time, the population of the Rodrigues Island bees, unlike the rest of the world, did not experience strong biological pressure caused by parasites and pathogens [96], which may explain the fact of its much higher heterozygosity compared to the populations of other island systems where *A. m. ligustica* was introduced [79, 97].

The genetic diversity of island populations was also studied by the example of the Balearic Islands, where 98 bee colonies from 22 areas of the archipelago were analyzed using eight polymorphic microsatellite loci – B124, A113, A7, A35, A24, A28, A88, and A8. At the same time, low variability was found, determined both based on the observed number of alleles and heterozygosity, which is expected for island populations [81]. Despite the low degree of genetic differentiation within the islands, there is a significant shortage of heterozygotes, indicating the existence of a subpopulation genetic structure. The honeybee populations of the Balearic Islands are divided into two clusters, the Gimnesias (the islands of Mallorca and Menorca) and Pitiusas (the islands of Ibiza and Formentera), which is consistent with the biogeographic hypothesis postulated for this archipelago. Phylogenetic

analysis confirmed the Iberian origin of the honeybees of the Balearic Islands, thus supporting the evolutionary scenario for *Apis mellifera* in the Mediterranean basin, according to which *A. m. Iberica* is a hybrid between the African subspecies *A. m. intermissa* and the dark European bee *A. m. mellifera* [83, 87].

When studying the genetic structure and diversity of 414 worker bees from eight Algerian populations using 14 polymorphic microsatellite loci, significant genetic diversity was found both in the number of alleles and in the degree of heterozygosity. The number of alleles in the studied loci varied from two (B24) to 22 (Ap43). Most of the populations were in the Hardy-Weinberg equilibrium. It was found that Algerian bees were represented by two subspecies – *A. m. intermissa* and *A. m. sahariensis* [98]. The conducted phylogenetic analysis placed them in a group separated from the evolutionary lines M, C, and O [99]. Data on the polymorphism of microsatellite loci in Algerian honeybee populations, as well as in reference populations studied earlier [82, 85, 98, 100], allowed clustering these populations, resulting in five groups depending on their origin: the lines M (France, Belgium), O (Armenia, Georgia), C (Greece, Italy), and A (Morocco, Guinea), as well as the Algerian group belonging to the African evolutionary branch A. At the same time, African honeybee populations are characterized by a high degree of polymorphism of microsatellite DNA loci, which was the result of pronounced migratory behavior and a tendency to swarming [83]. For some Algerian populations, a slight introgression of the M and C evolutionary lines was found.

Polymorphic STR loci are actively used in the study of the genetic structure of autochthonous honeybee populations in various regions of the Russian Federation: populations of hybrid bees of the Tomsk Region [101] and populations of *A. m. mellifera* of the Perm Region [102], the Republic of Bashkortostan [103], the Arkhangelsk and Vladimir Regions, the Krasnoyarsk Territory and the Republic of Tatarstan [104]; populations of *A. m. carpatica* of the Republic of Adygea [105]; populations of *A. m. caucasica* of the Orel Region and Krasnodar Territory [105], hybrid bees of the Novosibirsk Region [107].

To assess the variability of microsatellite loci A008, Ap049, AC117, AC216 in honeybees living in the Tomsk Region, four sample sets (Central Russian and Carpathian bees, hybrids of various origins) were formed based on previously conducted mtDNA study and morphometric analysis. In the studied loci, the samples of the Central Russian and Carpathian breeds differed in the observed allelic variants and the frequencies of their occurrence. At the same time, the spectrum of alleles identified for bees of the Central Russian breed was fully observed in hybrids based on the Central Russian and Carpathian breeds [101]. Based on the analysis of polymorphism of nine microsatellite loci of nuclear DNA among more than 300 DNA samples of bee families collected in the north of the Republic of Tatarstan, the Republic of Bashkortostan, and the Perm Territory, the population and genetic structure of the honeybee subspecies *A. m. mellifera* was studied. The results of molecular genetic analyses suggest the existence in the Urals of a fairly stable preserved population system of the dark forest bee, possibly the last in the world [102].

The results of the analysis of the genetic structure of the honeybee population in the southern part of Bashkortostan based on the polymorphism of five microsatellite loci of nuclear DNA (Ap243, 4A110, A8, A113, and A28) indicate that intensive interbreeding hybridization, which is indicated by the average F_{IS} value, has not yet led to the disappearance of the heterozygote deficit. The value of the degree of subdivision of subpopulations obtained by the authors suggested the presence of a border between the hybrid zone and the population of *A. m. mellifera* localized in the studied region [103]. The studied bee families were

differentiated into three groups. The Zil1 and Zil2 clusters likely correspond to the peripheral part of the *A. m. mellifera* population, but the question of its relationship with the Burzian population remains open. The Haib4 cluster can be attributed to the peripheral part of another local population of the Central Russian bee. The location of the hybrid interbreed zone reflects the other clusters.

To assess the variability of the allelic fund of STR markers during the formation of specialized honeybee lines of the Prioksky type of the Central Russian breed, microsatellite profiles were studied in six bee families of each of the two lines — Klever (selected due to pollination efficiency of meadow clover) and IV-ZT (selected due to winter hardiness) [104]. In a sample set of 88 individuals, the observed number of alleles per locus averaged 6.29±1.51 and 8.71±1.61, respectively. The high probability of using inbreeding in the breeding of Prioksky type bees is strongly evidenced by the lack of heterozygotes, which reached 24.5 and 10.8%, respectively. It was found that 85.7% of individuals of the Klever line and 86.8% of individuals of the IV-ZT line could be genetically assigned to their populations based on the analysis of microsatellites. As follows from the calculation of the R_{ST} fixation index (AMOVA), 23% of all variability is due to inter-population differences, 77% − to intra-population variability. It is convincingly shown that microsatellite analysis is fully applicable to the creation of specialized honeybee lines since the selection of such lines is always accompanied by a change in the allele-fund of microsatellites.

The information content of the test system developed for the analysis of seven microsatellite loci (A024, A88, A113, AP043, HB-C16-05, HB-THE-03, and HB-C16-01) was also studied. It was used to study the main parameters of the allelic fund of populations of honeybees of the gray mountain Caucasian (n = 70) and Central Russian (n = 65) breeds, as well as the Prioksky type of the Central Russian breed (n = 88) [105]. It was found that the average number of alleles per locus is 7.48±1.02, the number of effective alleles is 3.38±0.56, and the number of informative alleles is 3.62±0.71. Compared with the populations of the Central Russian and gray mountain Caucasian breeds that participated in the breeding of the Prioksky type, the latter revealed an increased genetic diversity of the allelic fund (9.57±1.88 versus 6.86±1.55 and 6.00±1.84). The introduction of alleles of the original breeds into the allelic fund of bees of the Prioksky type has been confirmed, the process of genetic consolidation of which, however, has not yet been completed. It was found that the share of inter-population differences accounts for 8% of the total allelic diversity.

The purebredness and differentiation of the main breeds of honeybees bred in the territory of the Russian Federation were evaluated based on the polymorphism of microsatellite markers of nuclear DNA, using multiplex analysis of eight loci − AO24, A88, A113, APO43, APxO1, HB-C16-05, HB-THE-03, and HB-C16-01 [106]. The high degree of isolation of the Carpathian bee breed was indicated by the presence of the largest number of private alleles. At the same time, there were no significant differences in the number of private alleles between the Central Russian and gray mountain Caucasian breeds. The analysis of STR markers demonstrated on average a high identity of individuals in the studied breeds (99%). The lowest degree of consolidation was characterized by the Carpathian breed (97.0%), and the most consolidated was the Central Russian breed (100%). The calculation of genetic distances showed that the gray mountain Caucasian and Carpathian honeybee breeds, which form a single cluster on the phylogenetic tree, are the closest to each other.

The comparison of the allelic fund in the Far Eastern honeybee population introduced to the Novosibirsk Region (n = 90) and in the populations of the Central Russian (n = 191, *A. m. mellifera*), gray mountain Caucasian (n = 113, *A. m. cau-*

casica), Carnica (n = 61, *A. m. carnica*), and Carpathian (n = 184, *A. m. carpatica*) breeds was performed using seven microsatellite loci [107]. The degree of genetic differentiation of the Novosibirsk population was estimated using the F_{ST}, R_{ST} (AMOVA) indices, and Nei genetic distances. As a result, it is shown that the Novosibirsk population of Far Eastern bees is characterized by a high degree of genetic diversity and, being a half-breed, is the closest in origin to the Carnica. Taking into account the origin of the Far Eastern bees from the Ukrainian steppe breed, the data obtained can be considered as an indirect confirmation of the close relationship of the Ukrainian steppe and Carnica breeds [107].

The analysis of microsatellite profiles for molecular genetic differentiation of the lines and families of the honeybee *A. m. caucasica* bred in the Sochi area revealed similar trends in the assessment of intra-and inter-family variability [106]. As an indication of the high heterogeneity of the first line, the observed excess of heterozygotes (F_{IS} = –0.048) can be considered. Representatives of this line were characterized by maximum inter-family (F_{ST} = 0.124) and minimum individual (F_{IT} = 0.052) variability. The 2nd-5th lines were characterized by relatively high individual variability (F_{IT} from 0.143 to 0.189) with the observed heterozygote deficiency (F_{IS} from 0.062 to 0.128), as well as significantly lower values of inter-family variability concerning the first line (F_{ST} from 0.095 to 0.104). The smallest inter-family differences (F_{ST} = 0.096 and F_{ST} = 0.095) were observed in the third and fourth lines among all the studied groups. The differentiation of the studied lines by morphometric features and STR markers revealed some differences in the structure of the family tree. The geographical distance of the lines from each other was reflected in a dendrogram based on the analysis using microsatellite markers.

The most important condition for the development and increase in the productivity of the beekeeping industry is the maintenance of the biodiversity of the honeybees. Regional populations can represent a significant reserve for its replenishment. Using seven microsatellites (A024, A88, A113, AP043, HB-C16-05, HB-THE-03, and HB-C16-01), the key characteristics of the allelic fund of the Primorsky population of the Far Eastern bee were determined and the level of its genetic differentiation was estimated [109]. The material was the worker bees of the Far Eastern population (DALN) (n = 143). In the pairwise comparison, the values D and F_{ST} were used. Forming comparison groups, purebred bees were selected based on the similarity coefficient Q. Its values averaged 98.0±0.1; 97.9±0.2; 98.1±0.1 and 95.8±0.4%, respectively, for the gray mountain Caucasian (SGK, n = 70), Central Russian (SR, n = 61), Carpathian (KARP, n = 55), and Carnica (CAR, n = 30) breeds. The relatively high genetic diversity characteristic of the comparison groups (12.43±2.71 alleles per locus for KARP, 11.29±2.49 alleles per locus for SR, and 10.00±2.07 alleles per locus for CAR) was comparable to that in the studied sample of DALN (11.14±1.30 alleles per locus). The effective number of alleles calculated for the DALN group exceeded the value typical for the other groups (4.94 alleles vs. 3.19-4.51 alleles). The deficiency of heterozygotes was the greatest in the population of Far Eastern bees (F_{IS} = 0.32); almost the same indicator was observed in the Central Russian breed (F_{IS} = 0.31). The DNA analysis data became the basis for assigning 96.5% of the DALN sample individuals to their population. The high degree of genetic consolidation of the Far Eastern breed can be an indicator of the almost complete absence of gene flow between this and the other studied breeds. Far Eastern bees form an independent branch on the family tree, which confirms their different origin compared to the other breeds in the sample. Based on the results obtained, the Far Eastern bee was included in the Russian State Register of Breeding Achievements in 2018 as an independent breed of honey bees (application No. 8356497, patent holder Chaika Far Eastern Federal Research Center for Agrobiotechnologies).

Microsatellite loci are also considered as a tool for studying the reproduction features of honeybees, in particular, polyandry. Polyandry is a specific phenomenon that provides an increase in genetic diversity. In an experiment to determine the degree of polyandry and the contribution of drones to genetic diversity, microsatellite profiles were compared at three loci (A008, Ap049, AC117) in hybrid and purebred bee families of *A. mellifera* (Central Russian and Carpathian breeds, Tomsk Region) [110]. It turned out that the share of alleles introduced into the bee family by the paternal line was 6.67-28.0%. At the same time, hybrid bee colonies were characterized by the greatest genetic diversity (a higher proportion of introduced alleles in the male line is shown – 25-28%).

So, the honeybee is a species that has a worldwide distribution (except for Antarctica) and is of the most important economic, agricultural, and environmental importance. However, in the previous few years, there has been a global decline in the total number of honeybee hives (from 21 to 15.5 million), which poses a threat not only to beekeeping but also to some crop production sectors, as well as to many natural ecosystems, the stability of which is supported by the participation of bees in the pollination of wild plants. The reasons for this decline are not fully understood but may be related to the loss of genetic diversity, the synergistic effects of parasite infestations (varroatosis and nosematosis), viral and bacterial infections, as well as the widespread use of pesticides in agriculture. Under these conditions, the determination of genetic diversity in honeybee populations using molecular methods is of primary importance. Microsatellites are represented by short tandem repeats (the size of the monomeric repetitive unit is from two to six base pairs), scattered throughout the nuclear DNA. They can be localized both in non-coding (including regulatory) regions and in the regions of the genome that encode proteins. Microsatellite loci are a very convenient tool for analyzing the genetic structure of honeybee populations, the degree of inbreeding and heterozygosity, calculating genetic relation coefficients, and determining the level of introgression. Using microsatellite markers, the evolutionary history of honeybee subspecies was revealed, the structure of a large number of their populations in the Old and New Worlds was studied, the inclusion of foreign genes of some subspecies in the gene complexes of others was evaluated, and methods for differentiating subspecies and populations were developed. A large number of alleles typical for microsatellite loci due to the high frequency of mutational events occurring in them, and the codominant type of inheritance make STR markers extremely powerful tools for genomic mapping, determining the reliability of origin, and conducting population genetic and evolutionary studies.

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