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SHORT COMMUNICATIONS

Analysis of the Genetic Structure of Honeybee (*Apis mellifera* L.) Populations

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Abstract—The genetic structure of honeybee populations from the southern part of Bashkortostan was assessed based on an analysis of mtDNA (COI–COII locus) and five nuclear DNA microsatellite loci (Ap243, 4A110, A8, A113, and A28). The data indicate that the examined populations experience a deficit of heterozygotes despite intense interpedigree hybridization. It is suggested that there is a boundary between the population of *Apis mellifera mellifera* L. and the hybrid zone in the examined region.

Keywords: honey bee *Apis mellifera* L., microsatellites, mtDNA locus COI–COII.
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An important condition for preservation of the honeybee gene pool is the demarcation of its distribution. For beekeeping in the forest and forest steppe zones of Eurasia, including the Republic of Bashkortostan (RB), the indigenous subspecies of honeybee is *Apis mellifera mellifera* L. Human intervention in the process of bee dispersal caused the borders between the subspecies to become fuzzy. The hybridization of some bee subspecies with the others has become commonplace on every continent, and only rare purebred populations have been preserved in inaccessible places. For instance, local populations of dark European bee were found in Perm krai and in the north of the Republic of Bashkortostan [1], the British Isles, and Scandinavia [2]. After it was confirmed that a genetically pure population of Central Russian bee remained in the forests of Burzyansky district [3], the study of honeybee populations in Bashkortostan continued. The goal of the research was to clarify the range boundaries of the Burzyansky population and to search for other surviving populations of the Central Russian bee. The objective of the present study was to analyze the gene pool of the bees in the southern part of the Republic of Bashkortostan, to the south of the known range of the Burzyansky population.

Genetic analysis of the honeybee colonies from Zilairsky and Khaibullinsky districts of the Republic of Bashkortostan was carried out based on data for the mtDNA COI–COII locus and microsatellite loci Ap243, 4A110, A8, A113, and A28. The bees were collected during summer 2013 from 91 colonies distributed in Zalairsky district and 130 colonies distributed in Khaibullinsky district. Total DNA was extracted with a guanidinium thiocyanate–phenol–chloroform mixture. PCR analysis of the mtDNA COI–COII locus was performed with a Tsikloterm thermal cycler in a total volume of 15 μL with the primers F-5'-tggcagaataagtgcattgaa-3' and R-5'-cagcataatatgaatttgattctga-3'. Microsatellite loci were amplified with a 96-well T100 thermal cycler. The amplification conditions consisted of initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s; primer annealing at 54°C for 30 s; extension at 72°C for 60 s; and final extension at 72°C for 3 min. Amplificates were separated by means of electrophoresis in 8% polyacrylamide gel in the presence of 1% TBE buffer and were stained with ethidium bromide. The gels were visualized with a Vilber Lourmat UV transilluminator.

The initial gene pool analysis, which was based on the polymorphism of the COI–COII locus (the PQQ variant of which is a marker of the origin of bees from *A. m. mellifera*) and carried out by the previously described method [4], showed that 50% of the studied colonies from Zilairsky maternally originated from the Central Russian breed of bees. The highest frequencies of the PQQ variant were observed in the village of Berdyashevo (0.96, sample size (N) of 27 colonies)) and the village of Dmitrievka (0.9, $N = 20$). In the Khaibullinsky district, the proportion of colonies originating from the Central Russian breed constituted 13%. Relatively high PQQ frequencies were observed in the settlement of Podolsk (0.40), the settlement of Antingan (0.38), and the village of Yantyshevo (0.30).

Based on the geographical location of the sampling sites and the data of primary analysis of the mtDNA

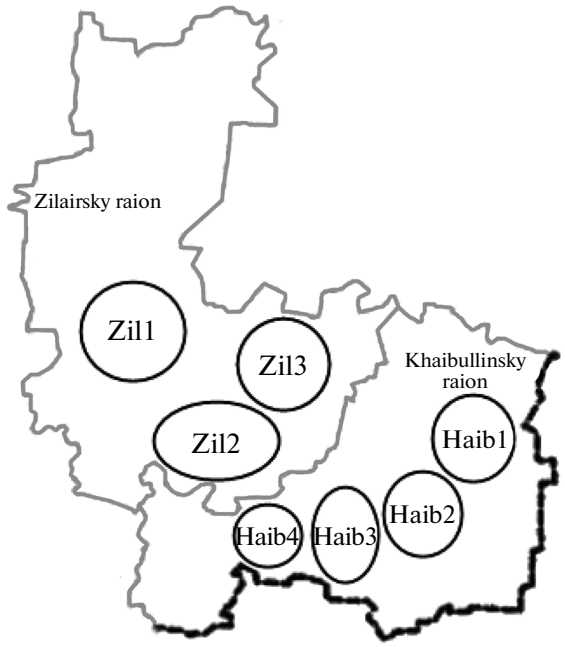

Fig. 1. Grouping of honeybee subpopulations in Zilairsky and Khaibullinsky raions for statistical treatment of the data on nuclear DNA microsatellite loci. See the text for explanations.

polymorphism, samples for a computer analysis of gene diversity at microsatellite loci relatively homogeneous with respect to the mtDNA were formed. The bee colonies from Zilairsky district were grouped into three clusters (Fig. 1). Cluster Zil1 consisted of colonies from the villages of Dmitrievka and Berdyashevo ($N = 48$) with an mtDNA PQQ frequency of 0.94. Zil2 consisted of colonies from the villages of Sidorovka, Sabyrovo, Salyakhovo, Baiguzhino, and Novopreobrazhenskoe ($N = 26$) with a PQQ frequency 0.08; Zil3 included colonies from the settlements of Iskuzhino, Yamansaz, Yumaguzhino ($N = 17$) with a PQQ frequency of 0.41. The bee colonies from Khaibullinsky district were grouped into four clusters. Cluster Haib1 included colonies from the settlements of Vozdvizhenka and Podolsk ($N = 36$) with a PQQ frequency of 0.14. Cluster Haib2 consisted of bee colonies from the settlements of Sadovyi and Makan ($N = 34$), where the PQQ variant was absent. Cluster Haib3 contained bee colonies from the settlements of Yakovlevka and Fedorovka ($N = 36$), where the PQQ variant was also absent, and Haib4 included colonies from the settlements of Yantyshevo and Atingan ($N = 24$) with a PQQ frequency of 0.50.

Based on the analysis of the microsatellite loci polymorphism, the genetic diversity indices (i.e., the observed (H_o) and expected (H_e) heterozygosity and Wright's F statistics (F_{st}, F_{it}, and F_{is})) were calculated (table). The calculations were performed with the FSTAT software package and POPULATION ver. 1.2.32 program.

The data showed that the bee colonies as a whole, despite the intense interpedigree hybridization, experienced some heterozygote deficiency at all loci ($H_o < H_e$). The mean F_{is} value (percentage of inbreeding within subpopulations) for the examined group of honeybee colonies was 0.086, which was considerably lower than the values of this index for the genetically pure Burzyansky population ($F_{is} = 0.421$), as well as for moderately hybrid Iglinsky population ($F_{is} = 0.177$) [5]. These findings indicate a high degree of outbreeding (intensive crossbreeding) in the examined region and are consistent with our data on mtDNA polymorphism.

The F_{st} factor, which characterizes the degree of subpopulation subdivision with the same value (0.086), was actually very high. The value was comparable to that for the genetically pure Burzyansky population and the moderately hybrid Iglinsky population ($F_{st} = 0.080$) [5]. According to the literature data, the genetic differences between pure populations of *Apis mellifera mellifera* L. are even lower. The F_{st} value for the Western European and Scandinavian population

Genetic diversity indices inferred from data on the Ap243, 4A110, A8, A113, and A28 loci for the honeybee populations from Zilairsky and Khaibullinsky districts

Locus	H_o	H_e	F_{is}	F_{st}	F_{it}
Ap243	0.092	0.096	0.026	0.013	0.038
4A110	0.223	0.236	0.038	0.046	0.082
A8	0.531	0.665	0.185	0.090	0.256
A113	0.400	0.438	0.069	0.074	0.137
A28	0.329	0.378	0.113	0.207	0.297
Mean	0.315	0.363	0.086	0.086	0.162

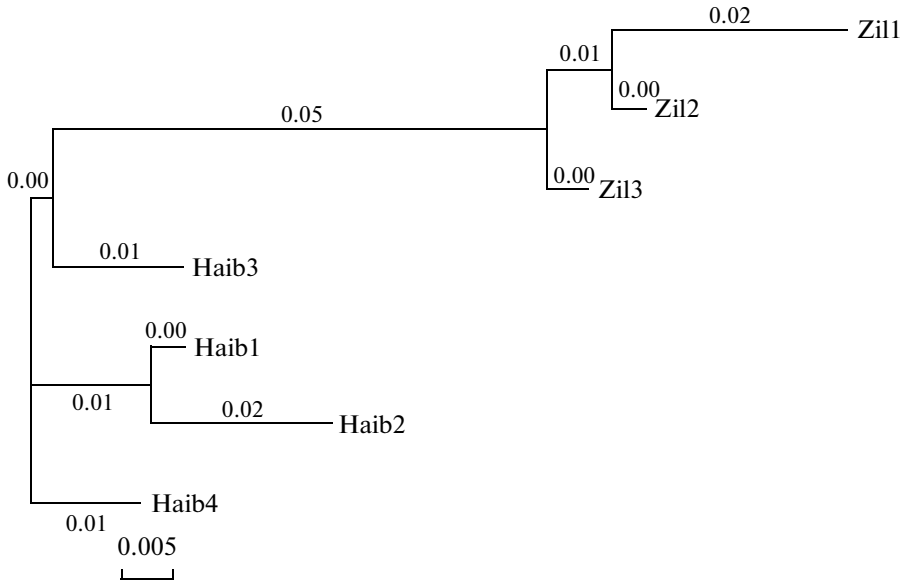

Fig. 2. Dendrogram of genetic relationships of the honeybee subpopulations from Zilairsky and Khaibullinsky districts (according to Nei, 1983).

was 0.050 [6], and the genetic distance between the populations of *A. m. mellifera* within one country (Germany) was 0.029 [7]. Thus, the results of the present study suggest that in the examined region there is a border between the population of *A. m. mellifera* L. and hybrid populations of *A. m. mellifera* L. with *A. m. caucasica* or *A. m. carpatica*.

Based on the genetic distances between the studied honeybee subpopulations, as calculated by Nei's method (1983) in the Population ver. 1.2.32 software program, a dendrogram was constructed by the neighbor joining method as implemented in the MEGA ver. 6 software program (Fig. 2).

The results suggest differentiation of the studied bee colonies into three groups. Clusters Zil1 and Zil2 probably represent a peripheral part of the population of *A. m. mellifera* L. (its relationships with the Burzyansky population remain unresolved). Cluster Haib4 can be also attributed to the peripheral part, but perhaps to other local populations of Central Russian bees. The remaining clusters reflect the location of the interbreeding hybrid zone.

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