

MICROSATELLITES AS A TOOL FOR EVALUATION OF ALLELE POOL DYNAMICS WHEN CREATION OF PRIOKSKY TYPE OF MIDDLE RUSSIAN HONEY BEE

N.A. Zinovieva¹, N.I. Krivtsov², M.S. Fornara^{1,3}, E.A. Gladyr^{1,3},
A.V. Borodachev², S.A. Berezin², V.I. Lebedev²

¹ All-Russia Research and Development Institute for Livestock Husbandry, RAAS,
Moscow province, Podolsk region, Dubrovitsy 142132, Russia
e-mail: n_zinovieva@mail.ru

² All-Russia Research and Development Institute of Beekeeping, RAAS, Ryazan province, Rybnoe 391110, Russia
e-mail: bee@email.ryazan.ru

³ LLC "Biostrim", Moscow province, Podolsk region, Dubrovitsy 142132, Russia

Received August 23, 2011

Summary

The informative capacity of the test system for the analysis of seven microsatellites (A024, A88, A113, AP043, HB-C16-05, HB-THE-03, HB-C16-01) for the allele pool characteristics of Middle Russian ($n = 65$), Gray Mountain Caucasian ($n = 70$) and Prioksky type of Middle Russian ($n = 88$) honey bee breeds was studied. The average number of alleles, the number of informative alleles and the number of effective alleles per loci were 7.48 ± 1.02 , 3.62 ± 0.71 and 3.38 ± 0.56 , respectively. The increase of allele pool genetic diversity in Prioksky type comparing to Middle Russian and Grey Mountain Caucasian breeds used in type breeding was observed: 9.57 ± 1.88 allele per loci as against 6.86 ± 1.55 and 6.00 ± 1.84 , respectively. The allele introduction of initial breeds was evaluated. It was shown that 7.9 per cent of common microsatellite diversity was due to intra population differences.

Keywords: genetic markers, microsatellites, heterogeneity, honey bee breeds.

Creation of new breed types of honeybees is one of promising techniques aimed at improving the productivity and breeding qualities of bee colonies. There are new breed types of honeybees recognized as selection achievements – Orlov type of Central Russian breed (1), Maikop type of Carpathian breed (2), Krasnopolyansky type of Gray Mountain Caucasian breed (3). The new highly-productive Prioksky type of Central Russian breed was created in the All-Russia Research and Development Institute of Beekeeping through the crossing Central Russian bee queens with Gray Mountain Caucasian drones and further inbreeding (4).

The development of DNA-labeling technique opens up the possibility for monitoring and control of diversity in populations of honeybees. It has been shown the use of markers based on RAPD and ISSR as a tool for assessing differentiation of breeds and populations of honeybees (5, 6) and mitochondrial markers used to determine gene exchange between populations (7) and breed purity of honeybee families on maternal line (8, 9). Microsatellites are one of the most informative genetic markers whose applied importance has been confirmed at characterization of new types of animals (7, 10, 11). Informativity of genome analysis based on microsatellites is largely determined by the number of studied loci and their polymorphism degree. The authors suggest a multi-locus test system for analysis of the honeybee genome using seven polymorphic loci (A024, A88, A113, AP043, HB-C16-05, HB-THE-03 and HB-C16-01).

The purpose of this research was to evaluate informativity of the proposed microsatellite test system as a tool for assessing the allelic pool of honeybees and determine the degree of introgression of source breeds in the Prioksky breed type.

Technique. The material for genetic analysis were samples obtained from 88 worker bees of Prioksky type of Central Russian breed. Comparison groups were honeybees of Central Russian ($n = 65$) and Gray Mountain Caucasian ($n = 70$) breeds whose genetic identity to the given breeds had been established earlier (12) as well as Q values averaged to 98.6 ± 0.14 and $99.0 \pm 0.05\%$, respectively.

Genotyping the bees was performed using test system for DNA analysis of honeybees including a set of reagents for DNA isolation DNA-ELYUT (Russia) and a set of reagents for genotyping seven microsatellite loci of honeybees APIS-7 (Russia). Separation and detection of amplification products was performed on genetic analyzer AVI3130xl ("Applied Biosystems", USA). Calculation of population-genetic parameters was performed using GenAIEx software (v. 6.4). Individual similarity to the given populations was found according to J.K. Pritchard et al. (12) in the software Structure (v. 2.3.1). Genetic "contributions" of source breeds in Prioksky type were determined implying the number of source breeds ($k=2$), while the consolidation of its allelic pool was assessed considering the number of studied populations ($k=3$).

The data were calculated as described (13) with admixture model and correlation model without introducing the prior data about individuals' similarity to given populations and at the threshold value of this parameter accepted as the level of exception $Q = \geq 75\%$. Genetic distances were established as described by M. Nei et al. (14).

Results. The analysis of allelic profiles showed the number of alleles per one microsatellite locus ranging from 5 (A024) to 17 (HB-C16-01). The average number of alleles per locus was 7.48 ± 1.02 , the number of informative alleles - 3.62 ± 0.71 , the number of effective alleles - 3.38 ± 0.56 . Prioksky type of honeybees was found to be genetically more diverse than the source breeds: the average number of alleles per locus in this type was equal to 9.57 ± 1.88 vs. 6.86 ± 1.55 (Central Russian) and 6.00 ± 1.84 (Caucasian Gray Mountain). Detailed analysis of allelic profiles revealed the introduction of source breeds' alleles in Prioksky type and their combination resulting in higher allelic diversity of this new breed type. Thus, in Gray Mountain Caucasian breed, locus A024 doesn't contain allele 103, locus HB-C16-05 – allele 75, while in the corresponding loci of Central Russian bees there were not found alleles 107 and 77 (Fig. 1). Participation of these breeds in creation of Prioksky type was indicated by intermediate frequency of most of the analyzed microsatellite alleles. For example, the frequency of alleles 97, 105 (A024), and 69 (HB-C16-05) in Prioksky type amounted to 0,256; 0,517 and 0,599, respectively, while in source breeds, the corresponding values were 0,531; 0,385 and 0,458 (Central Russian); 0,114; 0,614 and 0,786 (Gray Mountain Caucasian) (Fig. 1). Similar patterns were observed in allelic profiles of other microsatellite loci. Thus, the analysis of microsatellite allelic profiles reveals the introduction of alleles of source breeds during

the creation of new types of honeybees.

Evaluation of variability in allelic pool of the microsatellite loci by analysis of molecular variance (AMOVA) showed a significant proportion of intra-population differences (92,1% of the total variability, including 87,4% inter-individual and 3,7% intra-individual diversity), while inter-population differences amounted to 7,9% of total variability ($R_{st} = 0,079$ at $p = 0,01$).

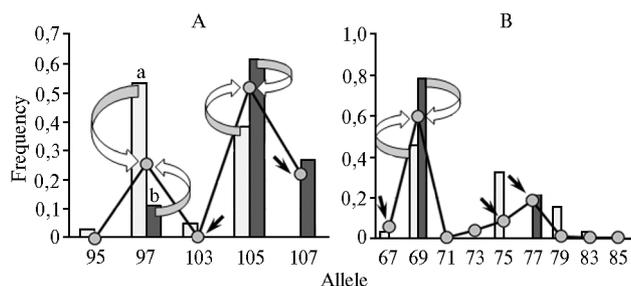


Fig. 1. Allelic profiles of microsatellite loci A024 (A) and HB-C16-05 (B) in honeybees of Prioksky type of Central Russian breed and in source breeds-participants of its creation: a – Gray Mountain Caucasian breed, b – Central Russian breed. Rounded arrows show intermediate values of allele frequency in Prioksky type, straight arrows - introduced alleles missing in one of the source breeds.

The analysis of observed heterozygosity showed a similarity of Prioksky type and Gray Mountain Caucasian breed (0,583 vs. 0,581). However, high genetic diversity of Prioksky type was reflected by the higher value of expected heterozygosity (0,711 vs. 0,529) resulting in a deficit of heterozygotes ($F_{is} = 0,177$) against the excess of heterozygotes in the studied group of Gray Mountain Caucasian breed ($F_{is} = -0,084$). Deficit of heterozygotes while the low value of observed heterozygosity (0,385) was also found in Central Russian breed ($F_{is} = 0,116$). Apparently, the deficit of heterozygotes along with high level of genetic diversity detected in Prioksky type is the result of inbreeding used at its creation in order to consolidate a desired exterior and commercially valuable properties.

Creation of a new breed type is more than just a union of allelic pools of source breeds at interbreed crosses. The next step is breeding “within” a resulting form including the selection in each generation of best individuals satisfying the desired exterior, behavior, honey production, which eventually forms a unique allelic pool of the new synthetic type. The contributions of genes of the source breeds were assessed as recommended by J.K. Pritchard et al. (12) implying the number of populations equal to the number of source breeds ($k = 2$). Prioksky type of honeybees was found to carry the contributions of Central Russian and Gray Mountain Caucasian breeds amounted to, respectively, $29,6 \pm 3,5$ and $71,4 \pm 3,5\%$ with variations between individual 0,6-98,7 and 1,3-99,4% (Fig. 2A). When $k = 3$, the average value of Q exception level in Prioksky type was $72,9 \pm 3,3\%$ at inter-individual diversity 0,9-98,8%; at the same time, in 58 of 88 individuals (65 9%) Q criterion value exceeded 75%, which fact, according to D'O. Raf-faele et al. (13), indicates genetic isolation of this group. 90% Individuals of Prioksky type were considered as identical to this population (Fig. 2, B). The obtained results suggest the formation of own unique allelic pool in Prioksky breed type, though its genetic consolidation is not yet complete.

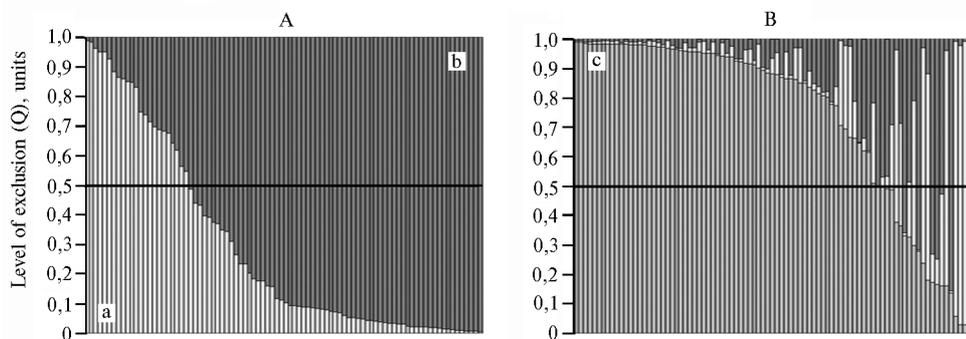


Fig. 2. Individual identity of honeybees to populations of source breeds (A) — Central Russian (a), Gray Mountain Caucasian (b) and the new breed type created upon them (B) — Prioksky type of Central Russian breed (c). Analysis performed according to J.K. Pritchard et al. (12) considering the number of populations = number of source breeds-participants in creation of the new breed type ($k = 2$) (A), and the total number of studied populations ($k = 3$) (B). Individuals ($n = 88$) are distributed along abscissa in descending order of Q values.

Genetic distances found according to M. Nei et al. (14) indicated greater genetic similarity of Prioksky type to the group of Gray Mountain Caucasian breed (0,256) than to Central Russian breed (0,350). Genetic distance between Gray Mountain Caucasian and Central Russian breeds was equal to 1,055. Paired comparisons of populations of Gray Mountain Caucasian and Central Russian breeds with Prioksky type using R_{st} revealed inter-population differences in microsatellite profiles: respectively, 10,8 and 2,2% ($p = 0,01$). Differences between Gray Mountain Caucasian and Central Russian breeds were characterized as 17,2% ($p = 0,01$). Knowing the breeding strategy of creation of Prioksky type allows considering R_{st} as more adequate criterion for assessing the differences between populations upon their microsatellite allelic profiles

Thus, microsatellite analysis is an informative tool that allows to assess allelic diversity during the creation of new types of honeybees. The new Prioksky breed type of Central Russian breed has the unique allele pool distinct from allelic pools of source breeds – Central Russian and Gray Mountain Caucasian honeybees. The studied population of Prioksky type of honeybees has high genetic diversity and relative isolation from the source breeds (genetic consolidation of this new type is yet incomplete).

REFERENCES

1. Grankin N.N., Orlovsky Type of Central Russian Breed of Honeybees, *Pchelovodstvo*, 2008, no. 4, pp. 8-9.
2. Mal'kova S.A. and Vasilenko N.P., Maikop Type of Carpathian Breed of Honeybees, *Pchelovodstvo*, 2008, no. 3, pp. 18-19.
3. Krivtsov N.I., Savushkina L.N., Sokol'sky S.S. and Lubimov E.M., Selection of Krasnopolyansky Type of Gray Mountain Caucasian Breed of Honeybees, *Vest. RASKhN*, 2008, no. 5, pp. 69-71.

4. Borodachev A.V. and Krivtsov N.I., A New Prioksky Type of Central Russian Breed of Honeybees, *Vest. RASKhN*, 2000, no. 4, pp. 70-72.
5. Suazo A., McTiernan R. and Hall H.G., Differences between African and European Honey Bees (*Apis mellifera* L.) in Random Amplified Polymorphic DNA (RAPD), *J. Hered.*, 1998, vol. 89, pp. 32-36.
6. Sylvester H.A., Inter-Simple Sequence Repeat Restriction Fragment Length Polymorphisms for DNA Fingerprinting, *Biotechniques*, 2003, vol. 34, no. 5, pp. 942-944.
7. Kraus F.B., Franck P. and Vandame R., Asymmetric Introgression of African Genes in Honeybee Populations (*Apis mellifera* L.) in Central Mexico, *Heredity*, 2007, vol. 99, pp. 233-240.
8. Nikonorov Yu.M., Ben'kovskaya G.V. and Poskryakov A.V., The Use of PCR for Testing Breed Purity of *Apis mellifera mellifera* L. Families in Southern Urals, *Genetika*, 1998, vol. 34, no. 11, pp. 1574-1577.
9. Krivtsov N.I., Goryacheva I.I., Udina I.G., Borodachev A.V. and Monakhova M.A., Identification of Race and Populations of Honey-Bee with the Use of PCR-Method, *S.-kh. biol.*, 2010, no. 6, pp. 26-29.
10. Tautz D., Hypervariability of Simple Sequences as a General Source for Polymorphic DNA Markers, *Nucl. Acids Res.*, 1989, vol. 17, pp. 6463-6471.
11. Strekozov N.I., Zinovieva N.A., Gorelov P.V., Listratenkova V.I., Konovalova E.N., Chernushenko V.K. and Ernst L.K., Genetic Characterization of New Cattle Types of Brown Swiss and Sychevskaja Breeds Using Polymorphism of Microsatellites, *S.-kh. biol.*, 2009, no. 2, pp. 10-15.
12. Pritchard J.K., Stephens M. and Donnelly P., Inference of Population Structure Using Multilocus Genotype Data, *Genetics*, 2000, vol. 155, pp. 945-959.
13. Raffaele D'O., Alberto M., Marco L. and Robin F.A.M., Genetic Characterization of Italian Honeybees, *Apis mellifera ligustica*, Based on Microsatellite DNA Polymorphisms, *Apidologie*, 2007, vol. 38, pp. 207-217.
14. Nei M., Tajima F. and Tatenko Y., Accuracy of Estimated Phylogenetic Trees from Molecular Data, *J. Mol. Evol.*, 1983, vol. 19, pp. 153-170.