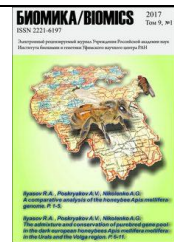




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## THE ADMIXTURE AND CONSERVATION OF PUREBRED GENE POOL IN THE DARK EUROPEAN HONEYBEES *APIS MELLIFERA MELLIFERA* IN THE URALS AND THE VOLGA REGION

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### Resume

The local populations of the dark European honeybees *Apis mellifera mellifera* from the Urals and the Volga region were examined in this work. The genetic analysis of populations and colonies was performed based on the polymorphism of 9 microsatellite loci of the nuclear genome. We found some level of the introgression in the native genetic pool of the *A. m. mellifera* in the Urals and the Volga Region by hybridization with introduced from Caucasus a “southern” subspecies *Apis mellifera carpatica* and *Apis mellifera caucasica*. The greatest proportion of the remaining indigenous gene pool of *A. m. mellifera* is the core of the gene pool of the population of the subspecies *A. m. mellifera*, which distributed over the entire territory of Perm Krai and the north of the Republic of Bashkortostan. Finally, we found biggest reserves of native gene pool of *A. m. mellifera* in the Urals and Volga region, which contain about thousand colonies of purebred dark European honeybees.

**Keywords:** dark European honeybees *Apis mellifera mellifera*, native gene pool, microsatellite loci, extant reserves, introgression, genetic standard, heterozygosity

### Introduction

The dark European honeybees *A. m. mellifera*, a representatives of the evolutionary lineage M [Jensen et al., 2005; Soland-Reckeweg et al., 2009], is currently recognized as an endangered subspecies as a result of mass introgression of the gene pool of “southern” subspecies of honeybees of the evolutionary lineage C [Muñoz et al., 2009; Nedić et al., 2014]. Interregional movements of different bee subspecies lead to the loss of the indigenous gene pool purity as a result of hybridization [Meixner et al., 2011]. The gene pool of the native dark European honeybees *A. m. mellifera* is considered lost in many European countries [Jensen and Pedersen, 2005; Ilyasov et al., 2014]. Unfortunately, the preference of beekeepers from Western and Northern Europe in the breeding of honeybees of the evolutionary lineage C (*A. m. carnica*, *A. m. ligustica*, and hybrid Buckfast bee) because of their cheapness, availability, and early maturation of queens compared to dark European honeybees

contributed to the loss of *A. m. mellifera* range integrity and introgression with the gene pool of “southern” subspecies [Jensen et al., 2005; Aronstein et al., 2016; Fried, Fried, 2016; Madras-Majewska et al., 2016; Pritchard, 2016; Skonieczna, 2016; Thunman, 2016]. The objective of this study was to analyse a local populations of dark European honeybees from the Urals and the Volga region, to assess their genetic characteristics, levels of introgression of “southern” genes based on of analysis of the polymorphism of 9 microsatellite loci of nuclear DNA.

### Materials and methods

Worker bees of *A. m. mellifera* were collected from 2729 colonies from 447 apiaries in the Urals (the Republic of Bashkortostan (RBa), the Permskii Krai (PKr), the Sverdlovskaya Oblast (SOB)) and from 330 colonies kept in 35 apiaries in the Volga region (the Republic of Udmurtiya (RUd), the Republic of

Chuvashiya (RCh), the Republic of Tatarstan (RTa), the Kirovskaya Oblast (KOb)) (tab. 1, fig. 1). Amplification of nine microsatellite loci (AP243, 4A110, A24, A8, A43, A113, A88, AP049, A28) of nuclear DNA [Garnery et al., 1993; Estoup et al., 1995; Haberl and Tautz, 1999; Solignac et al., 2003] was performed using a BIO–RAD T100 thermal cycler (United States) according to the Sileks (Moscow) protocol (www.sileks.com.ru).

The level of introgression of “southern” genes in nuclear genomes of local populations of *A. m. mellifera* honeybees from the Urals and the Volga region was evaluated based on the polymorphism of the microsatellite loci. The analysis was carried out in the STRUCTURE 2.3.4 software program on the basis of Bayesian analysis with Monte Carlo Markov chain (MCMC) clustering [Pritchard et al., 2000] with the given number of clusters  $K =$  from 2 to 5 using the admixture model and 50 000 MCMC iterations. We performed 10 replicates for each of  $K = 1$  to 5. We used STRUCTURE HARVESTER v0.6.94 [Earl, Vonholdt 2012] to estimate the most appropriate fit of  $K$  and to implement Evanno’s method for estimating DK [Evanno et al. 2005].

### Results and discussion

To characterize local populations from the Urals, the Volga region the heterozygosity values and the coefficients of inbreeding were calculated based on the polymorphism of the 9 microsatellite loci.

The genetic standards for the populations ( $H_o = 0.24$ ,  $H_s = 0.23$ ,  $H_t = 0.28$ ,  $F_{is} = 0.06$ ,  $F_{it} = 0.14$ ,  $F_{st} = 0.17$ ) and for colonies ( $H_o = 0.20$ ,  $H_s = 0.16$ ,  $H_t = 0.15$ ,  $F_{is} = 0.23$ ,  $F_{it} = 0.35$ ,  $F_{st} = 0.11$ ) of the dark European honeybees *A. m. mellifera* obtained based on the polymorphism of the 9 microsatellite loci of nuclear genome. A best way for the pure breeding the dark European honeybees *A. m. mellifera* is selection of colonies under following genetic characteristics obtained using polymorphisms of microsatellite loci ( $H_o \leq 0.30$ ,  $H_s \leq 0.30$ ,  $H_t \leq 0.30$ ,  $F_{is} \leq |0.45|$ ,  $F_{it} \leq |0.55|$ ,  $F_{st} \geq |0.11|$ ). The quality of the selecting genetic material can be determined by accordance to these genetic characteristics. Such selection of colonies allows maintaining the genetic and genotypic diversity within the genetic standard of subspecies.

We calculated the proportions of genes of “southern” subspecies for local dark European honeybees populations from the Urals and the Volga

region on the basis of 9 microsatellite loci polymorphism data (tab. 1). Honeybees from “southern” region - “southern” subspecies (*A. m. carpatica* and *A. m. caucasica*) have “southern” genes and belongs to the C–lineage, whereas native bees in the Urals and the Volga region - “northern” subspecies (*A. m. mellifera*) belongs to the M–lineage.

The minimum degree of introgression of “southern” genes into the nuclear genome was characteristic of local dark European honeybees populations from the Republic of Bashkortostan (Burzyanskaya, Tatyshlinskaya, Yanaul’skaya, Baltachevskaya, Karaidel’skaya, Mishkinskaya, Kushnarenkovskaya), the Permskii Krai (Ordinskaya, Osinskaya, Chastinskaya, Dobryanskaya, Krasnovisherskaya, Yusvenskaya, Nytvenskaya, Usol’skaya, Uinskaya, Permskaya), the Republic of Udmurtiya (Kambarskaya, Mozhginskaya, Yakshur–Bodyinskaya, Malopurginskaya), the Republic of Tatarstan (Mamadyshskaya), the Republic of Chuvashiya (Cheboksarskaya), and the Kirovskaya Oblast (Kilmezskaya). All other local populations have damaged the native genetic structure and gene pool of *A. m. mellifera*.

To perform gene geographic analysis, pie charts reflecting the proportions of introgression of genes of “southern” subspecies of evolutionary lineage C in local populations of dark European honeybees of evolutionary lineage M from the Urals and Volga region were placed at the sites of their location on a geographical map (fig. 1).

In the Urals and the Volga region, the genes of dark European honeybees *A. m. mellifera* were preserved nonrandomly. There is a trend toward increasing proportion of genes of evolutionary lineage M regarding the microsatellite loci from south to north. In the “southern” part of the Urals and the Volga region, there is only one local population of dark European honeybees surviving in purity, Burzyanskaya, which is under protection of the Altyn Solok Reserve, Shulgan–Tash Reserve, and the Bashkiria National Park.

The data on spatial distribution of local populations characterized by minimum introgression of genes of “southern” subspecies regarding the nuclear genome enabled identification in the Urals and the Volga region remaining populations of the dark European honeybees *A. m. mellifera* predominantly on the territory of the Republic of Bashkortostan, the Republic of Udmurtiya and the Permskii Krai.

Table 1.

The proportions of the genes of M and C–lineages in the local populations of honeybees from the Urals and the Volga region based on 9 microsatellite loci polymorphisms

No.	Local populations	Number of colonies, N	M–lineage	C–lineage
1	Abzelilovskaya (RBa)*	90	0.23	0.77
2	Alsheevskaya (RBa)	35	0.21	0.79
3	Baimakskaya (RBa)	70	0.34	0.66
4	Baltachevskaya (RBa)	36	0.98	0.02
5	Belebeevskaya (RBa)	16	0.36	0.64
6	Birskaya (RBa)	91	0.75	0.25
7	Burzyanskaya (RBa)	326	0.96	0.04
8	Beloretskaya (RBa)	114	0.62	0.38
9	Gafuriiskaya (RBa)	62	0.35	0.66
10	Zilairskaya (RBa)	141	0.30	0.70
11	Iglinskaya (RBa)	197	0.62	0.38
12	Ishimbaiskaya (RBa)	226	0.35	0.65
13	Karaidelskaya (RBa)	132	0.80	0.20
14	Kyshnarenkovskaya (RBa)	37	0.86	0.14
15	Kuyurgazinskaya (RBa)	61	0.08	0.92
16	Meleuzovskaya (RBa)	73	0.12	0.88
17	Mishkinskaya (RBa)	55	0.93	0.07
18	Tatyshlinskaya (RBa)	200	0.97	0.03
19	Yanaulskaya (RBa)	100	0.98	0.02
20	Ufinskaya (RBa)	30	0.36	0.64
21	Uchalinskaya (RBa)	10	0.19	0.81
22	Chishminskaya (RBa)	15	0.17	0.83
23	Chekmagushevskaya (RBa)	62	0.61	0.39
24	Haibullinskaya (RBa)	130	0.06	0.94
25	Uinskaya (PKr)	59	0.85	0.15
26	Ordinskaya (PKr)	25	0.95	0.05
27	Chastinskaya (PKr)	28	0.99	0.01
28	Nyvenskaya (PKr)	18	0.99	0.01
29	Osinskaya (PKr)	38	0.99	0.01
30	Permskaya (PKr)	76	0.93	0.07
31	Yusvenskaya (PKr)	37	0.99	0.01
32	Krasnovisherskaya (PKr)	41	0.99	0.01
33	Usolskaya (PKr)	20	0.97	0.03
34	Dobryanskaya (PKr)	20	0.96	0.04
35	Krasnoufimskaya (SOB)	58	0.31	0.70
36	Kukmorskaya (RTa)	24	0.60	0.41
37	Nizhnekamskaya (RTa)	12	0.16	0.85
38	Mamadyskaya (RTa)	16	0.97	0.03
39	Malopurginskaya (RUd)	26	0.92	0.08
40	Sharkanskaya (RUd)	34	0.79	0.21
41	Kambarskaya (RUd)	46	0.98	0.02
42	Mozhginskaya (RUd)	22	0.99	0.01
43	Zavyalovskaya (RUd)	39	0.64	0.36
44	Yakshur–Bodyinskaya (RUd)	33	0.90	0.10
45	Cheboksarskaya (RCh)	14	0.98	0.02
46	Kirovo–Chepetskaya (KOb)	20	0.23	0.77
47	Darovskaya (KOb)	20	0.37	0.63
48	Orlovskaya (KOb)	14	0.53	0.48
49	Kilmezskaya (KOb)	10	0.94	0.06

\*The following abbreviations mean: RBa - the Republic of Bashkortostan, PKr - the Permskii Krai, SOB - the Sverdlovskaya Oblast, RUd - the Republic of Udmurtiya, RCh - the Republic of Chuvashiya, RTa - the Republic of Tatarstan, KOb - the Kirovskaya Oblast.

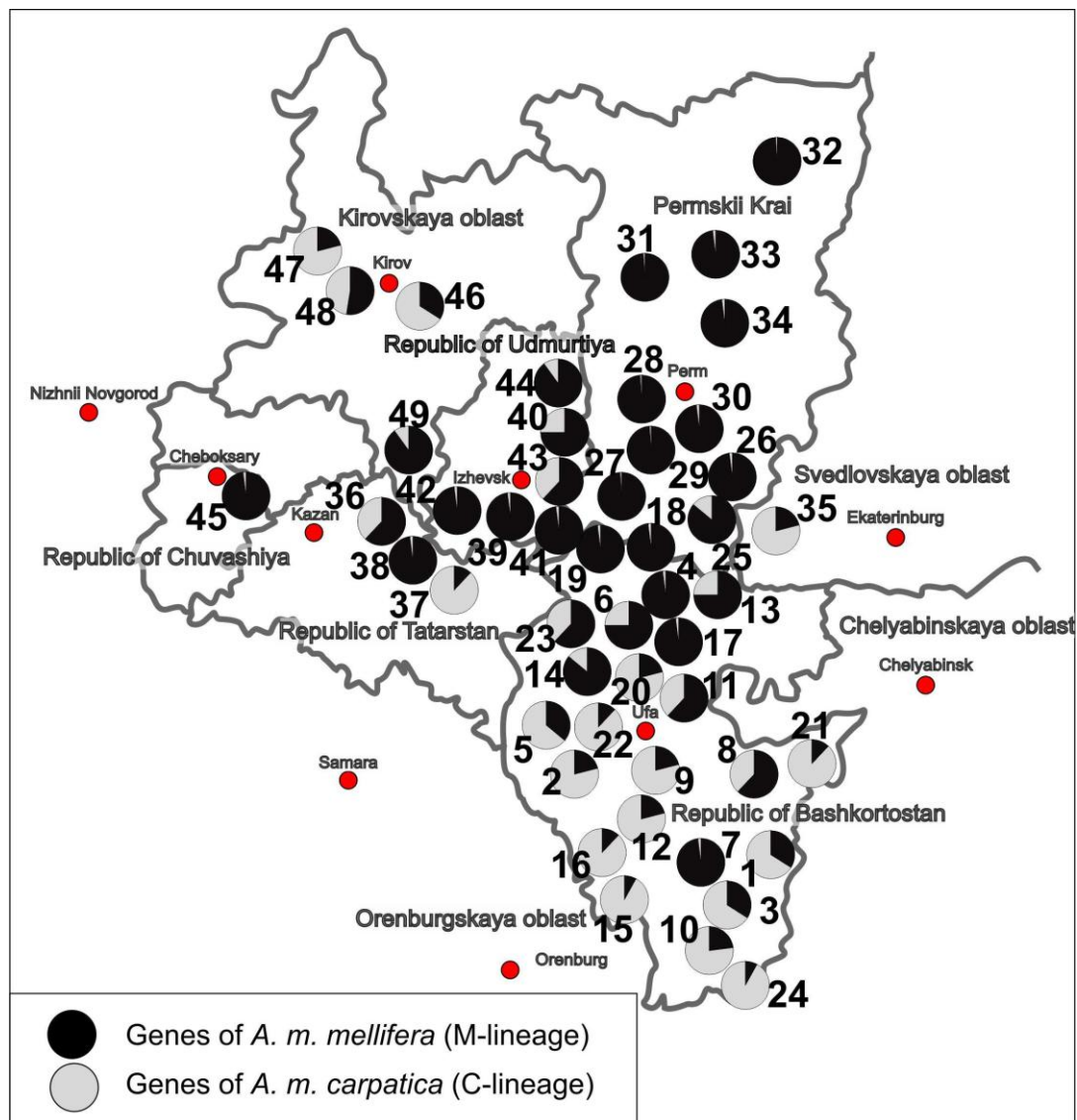


Figure 1. Spatial distribution of the genes of honeybees from M-lineage and C-lineage in the Urals and the Volga Region based on 9 microsatellite loci polymorphisms

These populations are currently characterized by sufficient number, stable and balanced genetic and genotype structure, and a small deviation of the genotype frequency distributions from the Hardy–Weinberg proportions. We found some level of the introgression in the native genetic pool of the *A. m. mellifera* in the Urals and the Volga region by hybridization with introduced from Caucasus a “southern” subspecies *A. m. carpatica* and *A. m. caucasica*. The greatest proportion of the remaining indigenous gene pool of *A. m. mellifera* is the core of the gene pool of the population of the subspecies *A. m. mellifera*, which distributed over the entire territory of Permskii Krai and the north of the Republic of

Bashkortostan. Finally, we found biggest reserves of native gene pool of *A. m. mellifera* in the Urals and Volga region, which contain about thousand colonies of purebred dark European honeybees.

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## СМЕШЕНИЕ И СОХРАНЕНИЕ ЧИСТОПОРОДНОГО ГЕНОФОНДА ТЕМНОЙ ЛЕСНОЙ ПЧЕЛЫ *APIS MELLIFERA MELLIFERA* НА УРАЛЕ И В ПОВОЛЖЬЕ

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### Резюме

В работе представлены локальные популяции темной лесной пчелы *Apis mellifera mellifera* Урала и Поволжья. Генетический анализ был выполнен на основе полиморфизма 9 микросателлитных локусов ядерного генома. Мы обнаружили определенный уровень интрогрессии в генофонде

природной популяции *A. m.mellifera* Урала и Поволжья в результате гибридизации с интродуцированными с Кавказа «южными» подвидами *Apis mellifera carpatica* и *Apis mellifera caucasica*. Большая доля сохранившегося аборигенного генофонда *A. m. mellifera* является ядром генофонда подвида *A. m. mellifera*, который имеет распространенность по всей территории Пермского края и северной части Республики Башкортостан. В результате исследований мы обнаружили наибольшие резерваты естественного генофонда *A. m. mellifera* на Урале и в Поволжье, которые содержат около тысячи семей чистопородной темной лесной пчелы.

**Ключевые слова:** темная лесная пчела *Apis mellifera mellifera*, естественный генофонд, микросателлитные локусы, сохранившиеся резерваты, интрогрессия, генетический стандарт, гетерозиготность.