

Genetic diversity of honeybees in different geographical regions of Siberia

Nadezhda V. Ostroverkhova, Aksana N. Kucher, Olga L. Konusova, Tatjana N. Kireeva & Igor V. Sharakhov

To cite this article: Nadezhda V. Ostroverkhova, Aksana N. Kucher, Olga L. Konusova, Tatjana N. Kireeva & Igor V. Sharakhov (2017) Genetic diversity of honeybees in different geographical regions of Siberia, *International Journal of Environmental Studies*, 74:5, 771-781, DOI: [10.1080/00207233.2017.1283945](https://doi.org/10.1080/00207233.2017.1283945)

To link to this article: <http://dx.doi.org/10.1080/00207233.2017.1283945>



Published online: 31 Aug 2017.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)



Genetic diversity of honeybees in different geographical regions of Siberia

Nadezhda V. Ostroverkhova^a, Aksana N. Kucher^a, Olga L. Konusova^a,
Tatjana N. Kireeva^a and Igor V. Sharakhov^{a,b}

^aInstitute of Biology, Tomsk State University, Tomsk, Russia; ^bDepartment of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

ABSTRACT

An assessment of the genetic diversity of nine microsatellite loci in honeybees of different origin (evolutionary lineages M, C, and hybrids) from different geographical regions of Siberia was conducted in order to find the genetic markers of the Siberian ecotype of dark-coloured forest bee (*Apis mellifera mellifera*). The greatest genetic diversity (both in the number of alleles and the calculated expected heterozygosity) was shown for loci A008, A113, Ap049, and A043 in honeybees from the Tomsk region, as bees from the southern districts had higher genetic diversity than bees from the northern districts. Honeybees from the Eastern region of Siberia (Yenisei population) had the lowest level of genetic diversity on these loci compared to Tomsk bee populations. Allele 162 pb of the locus A008, allele 126 bp of the locus A043, and allele 218 bp of the locus A113 can be considered as genetic markers of the *A. m. mellifera* Siberian ecotype.

KEYWORDS

Honeybee; *Apis mellifera*;
COI-COII mtDNA locus;
microsatellites; Siberia

Introduction

The honeybee, as a natural pollinator and one of the important elements of the ecosystem, is of great interest for ecological, biocenological, and population studies [1,2]. It is necessary to monitor the population genetic structure of the honeybee and analyse its gene pool, stability, and dynamics for a number of generations in order to understand how changes to habitat may have affected the functioning and evolution of ecosystems (agricultural systems).

The honeybee *Apis mellifera* L. has a large natural habitat covering the whole of Africa, Europe, and the Middle East. In Europe, there are several subspecies of honey bee, but only the subspecies *Apis mellifera mellifera* (or the dark-coloured forest bee) mastered the forest steppe and forest zones and has a natural range along the northern border of Eurasia, up to about 60° N. In Siberia, the honeybee was introduced about 230 years ago. It was the dark-coloured forest bee *A. m. mellifera*, or the Middle Russian race (a term adopted in Russia), which is well adapted to the local climate and plant communities and is an artificial population whose wintering is controlled by people [3].

In recent decades, as a result of human activity, a dramatic decrease in the number of populations of the unique bee *Apis m. mellifera* and massive bee hybridization (mainly, the dark-coloured forest bee and southern subspecies) have been observed in Europe and Russia, including Siberia. The observed bee hybridization reduces the range of native subspecies and modifies the genetic pool of local bee populations leading to the loss of their genetic identity [4]. Hybrid populations are less adapted to environmental conditions and are characterized by a higher morbidity and low immunity [5–8].

In Siberia, in connection with the active importation (since the end of the last century) of the southern subspecies of bees, such as the Carpathian race or *A. m. carpatica* (a derivative of *A. m. carnica*) and Gray Mountain Caucasian bee (*A. m. caucasica* Gorb.), hybrid populations began to be registered in the different districts of the region [9]. In a comprehensive study of some populations of honeybee (332 colonies) in Siberia (Tomsk region, Krasnoyarsk Krai, and Altai) by morphometric and molecular genetic methods (analysis of COI-COII mtDNA locus and microsatellite loci) the following was established: (1) 64% of bee colonies from the Tomsk region and all colonies studied from the Krasnoyarsk and the Altai territories originate from *Apis m. mellifera* on the maternal line; (2) according to the morphometric study, the majority of bee colonies of the Tomsk region are hybrids; in some colonies, the mismatch of morphometric and mtDNA data was observed when testing bees for compliance with the breed standard; (3) Yenisei population may be considered as a unique *Apis m. mellifera* population; and (4) microsatellite analysis (loci A008, Ap049, AC117, AC216, Ap243, H110, A024, A113) showed the specific distribution of genotypes and alleles for some loci in the bees, which differ by geographical location (Siberia, the Urals, Europe) (see details in reference [9]).

The goal of this work is to study the genetic diversity of honeybees living in the different climatic conditions of Siberia and to assess the influence of ecological factors on the formation of the gene pool structure of the bee population.

Materials and methods

Bees and bee colonies were investigated in 3 different geographical regions of Siberia: the northern and southern districts of the Tomsk region (Western Siberia) and the Yenisei population (Krasnoyarsk Krai, Eastern Siberia) (Figure 1). The northern and southern districts of the Tomsk region differ in ecological features and climatic characteristics (hydrothermal conditions, duration of the winter season, the species composition of plants, and degree of anthropogenic load). The climatic characteristic of the northern region is a more severe and prolonged winter season; the southern districts are characterized by a high anthropogenic pressure; in contrast to the northern district with a low apicultural activity, the southern district is characterized by a high activity in beekeeping and active importation of southern bee races. The Yenisei population of Krasnoyarsk Krai is the unique isolated Old Believers' population of *A. m. mellifera*, which existed for more than 60 years in forest without the importation of new honeybees.

In all, 43 bee colonies (15 apiaries) in 8 districts of the Tomsk region were investigated by molecular genetic methods. The total included 25 bee colonies (9 apiaries) from five northern districts (Parabelsky, Kolpashevsky, Chainsky, Molchanovsky, and Bakcharsky) and 18 bee colonies (6 apiaries) from three southern districts (Asinovsky, Zyryansky, and Tomsky), and 10 bee colonies (2 apiaries) of the Yenisei population of Krasnoyarsk Krai.

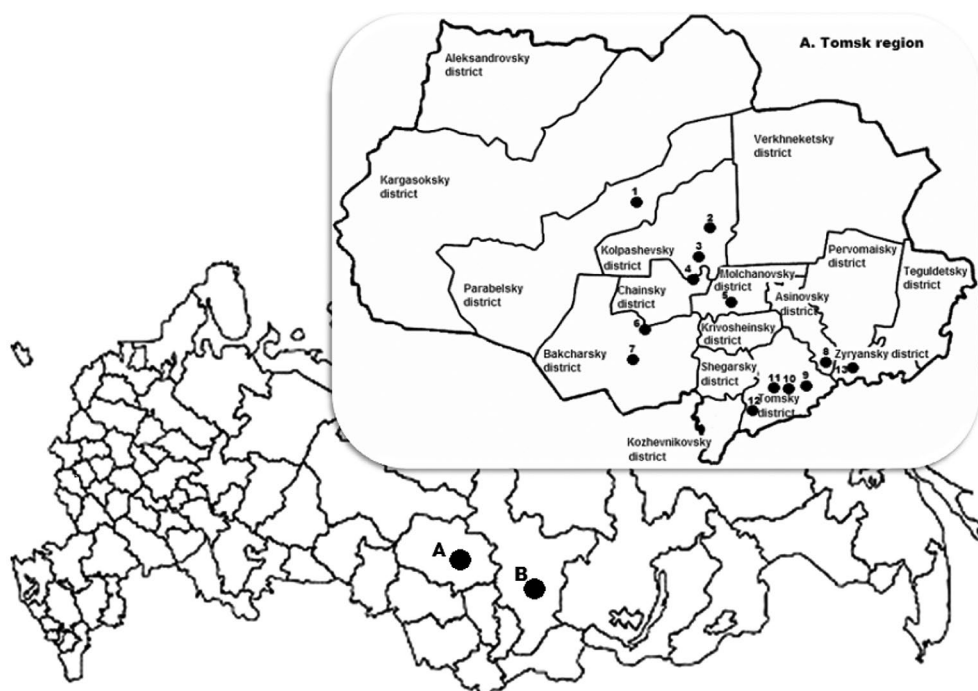


Figure 1. Map of localization of studied areas of Siberia (dots A and B) and apiaries of the Tomsk region (dots 1–13): A, the Tomsk region; B, the Krasnoyarsk Krai. 1, s. Parabel; 2, vicinity of g. Kolpashevo; 3, d. Novoabramkino; 4, s. Leboter; 5, s. Mogochino; 6, s. Vysoky Yar, d. Krylovka; 7, s. Bakchar, s. Parbig; 8, d. Tsvetkovka; 9, p. Zarechnyi (Malinovskoe rural settlement); 10, vicinity of Tomsk; 11, p. Sinii Utes, 12, s. Kurlek; 13, d. Dubrovka.

Note: Apiaries located at a distance less than 15 km from each other are marked as a single point.

Each bee colony has been studied using the mtDNA analysis (locus COI-COII) to determine the origin of the colony on the maternal line (5–6 individuals from colony) and microsatellite analysis (from 10 to 30 bees from colony for different loci). All bee colonies of the Yenisei population, and some bee colonies from the Tomsk region, were studied by morphometric analysis (morphometric parameters of wing, including the cubital index, the hantel index, and the discoidal shift were analysed) (see details in references [9–11]). In total, 721 bees (53 bee colonies) were studied.

DNA isolation and polymerase chain reaction (PCR) was carried out according to standard techniques with some modifications [12,13]. To amplify the COI-COII mtDNA locus, the following sequences of primers were used: 3'-CACATTTAGAAATTCCATTA, 5'-ATAAATATGAATCATGTGGA [12]. Amplification products were fractionated in 1.5% agarose gel, and the results were documented with the use of Gel-Doc XR+.

Variability of nine microsatellite loci was studied: A008 (=A8), Ap049, AC117, Ap243, H110, A024, A043, A113, and SV185. PCR was performed using specific primers and reaction conditions according to Solignac et al. [14]. Amplification products were analysed with ABI Prism 3730 Genetic Analyser (Applied Biosystems, Inc., Foster City, CA), and GeneMapper Software (Applied Biosystems, Inc.). Two microlitres of PCR products were mixed with GeneScan500-ROX size standards (Applied Biosystems, Inc.) and deionized formamide. Samples were run according to the manufacturer's recommendations.

The genetic parameters calculated were allelic frequencies, expected heterozygosity, and its standard error.

Results and discussion

Geographical differentiation of bee colonies for microsatellite loci

Variability of nine polymorphic microsatellite loci, including seven earlier studied microsatellite loci (A008 (=A8), Ap049, AC117, Ap243, H110, A024, and A113 – see details in references [9,15,16]) and two new (previously uncharacterized) microsatellite loci (A043 and SV185), in honeybees from different Siberian populations were analysed. For each locus, the range and frequency of alleles and expected heterozygosity were determined (Tables 1 and 2).

Among the 9 microsatellite loci examined, the greatest genetic diversity (both in the number of alleles and the calculated expected heterozygosity) was shown for loci A008, A113, Ap049, and A043 (Tables 1 and 2; shown in bold) in honeybees from the Tomsk region, as bees from the southern districts had higher genetic diversity than bees from the northern districts. Honeybees from the Eastern region of Siberia (Yenisei population) had the lowest level of genetic diversity on these loci in comparison with Tomsk bee populations,

Table 1. Allele diversity at 9 microsatellite loci in honeybee from different Siberian populations (Tomsk region and Krasnoyarsk Krai).

Locus	Northern districts of the Tomsk region			Southern districts of the Tomsk region			Eastern region of Siberia (Krasnoyarsk Krai, Yenisei population)		
	Q+PQQ+PQQQ ¹			Q+PQQ+PQQQ ¹			PQQ, <i>Apis mellifera mellifera</i> ²		
	Number of alleles, total	Prevalent alleles	Allele frequency	Number of alleles, total	Prevalent alleles	Allele frequency	Number of alleles, total	Prevalent alleles	Allele frequency
A008	8	162	0.74	10	162	0.58	1	162	1.00
		170	0.09		174	0.17			
AC117	3	176	0.15	3	180	0.10	3	176	0.14
		184	0.76		184	0.83		184	0.83
A113	7	212	0.24	8	212	0.55	4	218	0.83
		218	0.60		218	0.25		220	0.13
		220	0.09		220	0.08			
A024	6	94	0.61	4	94	0.33	7	94	0.15
		102	0.26		102	0.36		96	0.50
		104	0.10		104	0.28		104	0.19
Ap049	4	127	0.73	7	127	0.48	6	127	0.74
		130	0.14		130	0.22		130	0.23
		139	0.12		139	0.24			
H110	3	162	0.74	3	162	0.86	2	162	0.56
		170	0.18		166	0.10		170	0.44
SV185	5	261	0.38	5	261	0.27	4	261	0.09
		264	0.21		267	0.27		264	0.30
		267	0.31		270	0.42		267	0.57
Ap243	5	255	0.59	5	255	0.73	4	255	0.29
		262	0.34		262	0.13		262	0.59
					271	0.10		268	0.10
A043	4	126	0.82	6	126	0.49	1	126	1.00
		140	0.14		140	0.45			

Notes: Here and in Table 2: ¹ – bees from colonies of different origin (three variants of the COI-COII mtDNA locus were registered: PQQ, PQQQ (typical for Middle Russian race), and Q (typical for southern races)); ² – subspecies determined according to the morphometric and mtDNA analysis (see details in reference [9]).

Table 2. Gene diversity* of the honeybees in the Siberian population (Tomsk region and Krasnoyarsk Krai) of different geographical locations.

Locus	Northern districts of the Tomsk region	Southern districts of the Tomsk region	Eastern region of Siberia (Krasnoyarsk Krai, Yenisei population)
	Q+PQQ+PQQQ ¹	Q+PQQ+PQQQ ¹	PQQ, <i>A. m. mellifera</i> ²
A008	0.442 ± 0.030	0.619 ± 0.024	0
AC117	0.394 ± 0.027	0.293 ± 0.028	0.286 ± 0.024
A113	0.568 ± 0.021	0.620 ± 0.027	0.298 ± 0.024
A024	0.556 ± 0.022	0.681 ± 0.008	0.684 ± 0.021
Ap049	0.429 ± 0.027	0.663 ± 0.014	0.404 ± 0.027
H110	0.409 ± 0.024	0.253 ± 0.035	0.492 ± 0.006
SV185	0.702 ± 0.011	0.677 ± 0.019	0.577 ± 0.016
Ap243	0.539 ± 0.025	0.435 ± 0.046	0.558 ± 0.020
A043	0.312 ± 0.030	0.559 ± 0.014	0
Mean value	0.483 ± 0.013	0.533 ± 0.015	0.367 ± 0.010

*Expected heterozygosity (H_e) with the standard error (s.e.).

and microsatellite loci such as A008 and A043 were monomorphic (only one homozygous genotype was registered in all the studied bees). One possible reason for the low genetic diversity is the isolation of the Yenisei population (genetic drift), although some microsatellite loci show a high level of genetic diversity. Thus, for the locus A024, the maximum number of alleles (7) was registered in bees of Yenisei population; expected heterozygosity (H_e) was 0.684. Consequently, not only the genetic drift, but also the adaptive advantage (specific adaptation to the environment) of these alleles, can cause the loss of genetic diversity in the isolated population.

For the loci AC117, H110, SV185, and Ap243, the common allele spectrum with different allele frequency was registered in bees of different locations, and the expected heterozygosity was higher in bees from the northern and/or eastern districts of Siberia than in bees from the southern districts.

It would be expected that a higher genetic diversity will be shown for bees from southern districts than for bees from northern districts, and especially for an isolated population, because the southern districts of the Tomsk region show more developed beekeeping and imported bee colonies from different origins to this territory, so that there are different races of bees in the same area, and thus a heterogeneous population. But only half of the studied loci show high genetic diversity in bees of southern districts; other loci show a high diversity in bees from northern and eastern districts. These differences may reflect the role of geographical and ecological factors (specific adaptation to local conditions).

Geographical differentiation of bee colonies of different origin by microsatellite loci

The different subspecies (race) of honeybee and their hybrids inhabit Siberia (see details in Table 1). On the one hand, the races have specific genetic features; on the other hand, bee races are differently represented in the northern and southern regions of the Tomsk region. This may be the reason for identified genetic differences between bees of northern and southern populations according to the microsatellite analysis. In order to identify the effects of subspecies composition and the influence of environmental factors (north – south) on the genetic diversity of bees from different geographical regions, the comparative analysis of the variability of the studied loci was carried out for three bee groups (from the Tomsk

region) of different origin on the maternal line according to mtDNA analysis: bees originating from *A. m. mellifera* (PQQ or PQQQ variants of the COI-COII locus, branch M), southern race *A. m. carpatica* (Q variant, branch C), and hybrids (different variants were registered in the same colony – PQQ/Q or PQQQ/Q) (Table 3).

Microsatellite loci are different in variability, allele spectrum, the frequency of alleles, the dominant alleles, and geographical differentiation. For example, when comparing bee groups of different species and/or different geographical localization for the loci AC117 and H110, the clear differentiation of bees has not been shown. For all studied bee groups, allele 184 bp of locus AC117 and allele 162 bp of locus H110 (except Parabelsky district) were predominant. For locus SV185 having a wide allele spectrum (from 2 to 5 alleles), the similar pattern (no specific character, the absence of a dominant allele) was also found, and probably indicates the random nature of the variability of genetic diversity on this marker (no adaptive significance).

For loci A008, Ap049, and A043, the differentiation between bees originating from *A. m. mellifera* (PQQ or PQQQ, evolutionary branch M) and bees of southern races (Q, branch C, mainly *A. m. carpatica*) was registered. However, hybrids, for which we would expect the combination of genetic diversity of the two subspecies, alleles specific to *A. m. mellifera* (branch M) are registered with greater frequency. Thus, for the locus A008, the allele 162 bp specific for *A. m. mellifera*, was registered in hybrids with a frequency of more than 0.72. For the locus Ap049, allele 127 bp specific for *A. m. mellifera*, was also detected in hybrids with high frequency (≥ 0.71), but allele 139 bp typical for the southern subspecies was rare (frequency was from 0.12 to 0.28). Finally, for loci A043 and A113, various dominant alleles were detected in the bees of different evolutionary branches: alleles 126 bp (A043) and 218 bp (A113) are specific for bees of branch M; alleles 140 bp (A043) and 212 bp (A113) are characteristic for bees of branch C.

These results are consistent with those obtained for bees of different origins from the European population [17], i.e. the loci A043 and A113 may be candidate DNA markers for differentiation of bee subspecies of different evolutionary branches. But for the hybrids of Siberian populations, the allele 126 bp of the locus A043 and the allele 218 bp of the locus A113 are dominant (the frequency is ≥ 0.69 and ≥ 0.59 , respectively). Interestingly, only allele 126 bp of locus A043 was registered in bees of the Yenisei population. Our results may indicate the adaptive significance of these alleles to environmental conditions. This assumption was confirmed by a study of European bee populations with a variable level of introgression, mainly by alleles from the C lineage. A good correlation between the frequency of C haplotypes and C nuclear genes was established [17,18]. In our study, according to the values of genetic distances (on a set of microsatellite loci) between different races ($r = 0.7208$) and hybrids of different origins (both from *A. m. mellifera* ($r = 0.8200$), and from the Carpathian race ($r = 0.5712$)), in the process of hybridization, the honeybees of southern origin (the Carpathian race, C haplotype) quickly lose their genetic specificity and are becoming more similar to the *A. m. mellifera* (M haplotype) ($r = 0.0584$) with respect to genetic diversity.

Previously, we compared the genetic variability of some microsatellite loci in honeybees *A. m. mellifera* from different geographic regions (Siberia, the Urals, and Europe) using our own data (Tomsk region and Krasnoyarsk Krai) and literature data (see details in reference [9]). Of considerable interest in terms of the adaptive significance of some alleles to the environmental conditions is locus A008 for which the differences in the spectrum of alleles and the frequency of allele registration were revealed in honeybees of Siberian, the Ural,

Table 3. Allele diversity at 9 microsatellite loci in honeybee populations from the Tomsk region, taking into account their origin of the maternal line (variants of the COI-COII mtDNA locus).

Geographical location, district		Microsatellite locus (the total number of alleles, prevalent alleles (allele frequency))								
		A008	AC117	A113	A024	Ap049	H110	SV185	Ap243	A043
PQQ or PQQQ, branch M (the origin from <i>A. m. mellifera</i>)										
Northern districts										
Bakcharsky		3 162(0.73) 172(0.23)	3 176(0.31) 180(0.22) 184(0.47)	4 218(0.56) 220(0.17) 226(0.23)	4 94(0.69) 102(0.11) 104(0.17)	3 127(0.53) 130(0.10) 139(0.37)	2 162(0.93)	5 261(0.30) 264(0.10) 267(0.53)	2 255(0.83) 262(0.17)	No data
Parabelsky		2 162(0.41) 170(0.59)	3 176(0.16) 180(0.30) 184(0.55)	4 212(0.74) 218(0.17)	5 94(0.61) 96(0.15) 102(0.13)	2 127(0.52) 130(0.48)	3 162(0.25) 166(0.27) 170(0.48)	4 261(0.17) 264(0.28) 267(0.22) 270(0.33)	No data	2 124(0.16) 126(0.84)
Molchanovsky		3 162(0.94)	3 176(0.09) 180(0.10) 184(0.81)	5 212(0.14) 218(0.74) 220(0.09)	3 94(0.83) 102(0.16)	3 127(0.81) 130(0.10) 139(0.09)	3 162(0.78) 170(0.14)	2 261(0.63) 267(0.37)	5 255(0.43) 262(0.31) 271(0.19)	2 126(0.83) 140(0.18)
Asinovsky		4 152(0.05) 162(0.83) 170(0.05) 172(0.08)	3 180(0.20) 184(0.78)	5 218(0.35) 220(0.31) 226(0.23)	4 94(0.60) 100(0.13) 102(0.23)	2 127(0.24) 130(0.76)	2 162(0.94)	No data	2 255(0.91) 262(0.09)	2 126(0.73) 140(0.27)
Tomsky		6 162(0.81) 174(0.09)	3 176(0.25) 184(0.70)	7 212(0.58) 218(0.25)	2 94(0.59) 102(0.41)	5 127(0.58) 130(0.30)	3 162(0.94)	5 261(0.52) 267(0.24) 270(0.18)	5 255(0.11) 262(0.39) 268(0.18) 271(0.29)	3 126(0.88) 138(0.08)
Zyryansky		4 162(0.85) 170(0.05) 172(0.07)	3 176(0.07) 184(0.88)	No data	No data	2 127(0.80) 130(0.20)	No data	No data	No data	No data

(Continued)


Table 3. (Continued).

Geographical location, district		Microsatellite locus (the total number of alleles, prevalent alleles (allele frequency))									
		A008	AC117	A113	A024	Ap049	H110	SV185	Ap243	A043	
Q, branch C (the origin from southern races, mainly <i>A. m. carpatica</i>)											
Northern districts											
	Bakcharsky	6 162(0.29) 172(0.12) 174(0.10) 176(0.29) 178(0.15)	3 176(0.09) 184(0.89)	3 212(0.81) 218(0.15)	3 94(0.17) 102(0.45) 104(0.38)	4 121(0.14) 127(0.29) 130(0.11) 139(0.46)	3 162(0.90)	4 261(0.63) 264(0.27)	2 255(0.76) 262(0.24)	No data	
Southern districts											
	Asinovsky	3 174(0.55) 178(0.43)	1 184(1.00)	1 212(1.00)	3 102(0.48) 104(0.47)	3 130(0.30) 139(0.63)	3 162(0.86) 166(0.13)	No data	1 255(1.00)	3 140(0.91)	
	Tomsky	6 162(0.07) 170(0.21) 174(0.60)	3 180(0.10) 184(0.85)	2 212(0.93)	3 102(0.35) 104(0.63)	6 130(0.09) 139(0.66) 152(0.19)	2 162(0.75) 166(0.25)	3 267(0.32) 270(0.66)	1 255(1.00)	4 136(0.17) 140(0.74)	
PQQ/Q, PQQQ/Q (branches M+C, hybrids)											
Northern districts											
	Kolpashhevsky	5 162(0.85) 172(0.05) 174(0.05)	3 176(0.15) 184(0.80)	5 218(0.78) 220(0.13)	3 94(0.48) 102(0.43)	2 127(0.88) 130(0.12)	3 162(0.80) 170(0.17)	4 261(0.37) 264(0.26) 267(0.36)	2 255(0.43) 262(0.57)	4 126(0.86) 140(0.10)	
	Chainsky	5 152(0.08) 162(0.87)	3 176(0.18) 184(0.80)	4 212(0.11) 218(0.70) 220(0.13)	No data	3 127(0.83) 139(0.12)	2 162(0.72) 170(0.28)	4 261(0.34) 264(0.16) 267(0.41) 270(0.09)	2 255(0.55) 262(0.45)	2 126(0.69) 140(0.31)	
Southern districts											
	Tomsky	4 152(0.15) 162(0.72) 172(0.08)	3 180(0.14) 184(0.85)	3 212(0.39) 218(0.59)	3 94(0.65) 102(0.25) 104(0.10)	3 127(0.71) 139(0.28)	3 162(0.72) 170(0.22)	No data	3 255(0.39) 262(0.22) 271(0.39)	4 126(0.78) 140(0.17)	

and European populations. For honeybees of the Ural and Europe populations, shorter alleles of locus A008 were predominant (154 and 148 bp, respectively), whereas for bees from Siberia, allele 162 bp was the most specific (in bees of the Yenisei population, only this allele has been registered). Probably this locus should be considered as a marker related to geographic and environmental conditions (specific adaptation to local conditions) [5–8,19].

Conclusion

Expanding the habitat area of any organism, including honeybees, is accompanied by their adaptation to the new conditions of the habitat and the formation of ecotypes which differ from each other in characteristics, important for adaptation to specific environmental conditions – the behavioural, morphological, and physiological, which are based on genetic factors [6].

The evolution of the dark-coloured forest bee occurred in various climatic conditions, including the harsh conditions of Siberia. In contrast to other subspecies of honeybee, *A. m. mellifera* is characterized by a high level of adaptation to adverse environmental factors and greater resistance to diseases. It is expected that such a vast territory cannot be inhabited by only one subspecies of *A. m. mellifera* honeybee, with a similarly structured gene pool in all local populations. Most likely, there are ecological groups (ecotypes).

The present study was aimed at finding significant genetic markers for the adaptation of *A. m. mellifera* to the environmental and geographical conditions of Siberia. Accordingly, the research examined a variety of 9 microsatellite loci in bees that live in three regions of Siberia (northern and southern districts of the Tomsk region, Krasnoyarsk Krai), as well as bees which differ in origin (*A. m. mellifera*, southern races, hybrids), living in the southern and northern districts of the Tomsk region. The study has allowed us to allocate microsatellite loci, which are potentially important for the definition of ecotypes of the dark-coloured forest bees. First of all, it is locus A008, for which a specific spectrum of alleles was detected for *A. m. mellifera*, living in Siberia, as compared to bees of this subspecies from other regions (Europe, the Urals), as well as hybrids of *A. m. mellifera* and the southern race of the Tomsk region. In addition, allele 126 bp of the locus A043 and allele 218 bp of the locus A113 may be considered as markers of adaptation to the conditions of Siberia; the frequency of these alleles was high in individuals of *A. m. mellifera* living in Siberia and in hybrids of *A. m. mellifera* and southern subspecies; and these alleles are not specific for southern subspecies. Microsatellites AC117, H110, and SV185 were not informative loci for revealing markers of the Siberian ecotype of *A. m. mellifera*.

For assessing the adaptive and selective potential of honeybees, it is necessary to continue the gene-geographical research of honeybees, taking into account not only the bee race, but also climatic conditions (ecological factors) that influence the formation of specificity in the gene pool structure of honeybees in different regions.

Acknowledgements

We thank Melissa Wade for preliminary editing of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the FASIE (Program START, research) [grant number 696/22897].

References

- [1] Klein, A.-M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. and Tscharntke, T., 2007, Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B. Biological Sciences*, **274**(1608), 303–313. doi:10.1098/rspb.2006.3721.
- [2] Brown, M.J.F. and Paxton, R.J., 2009, The conservation of bees: a global perspective. *Apidologie*, **40**, 410–416. doi:10.1051/apido/2009019.
- [3] Konusova, O.L., Pogorelov, Y.L., Ostroverkhova, N.V., Nechipurenko, A.O., Vorotov, A.A., Klimova, E.A. and Prokopiev, A.S., 2009, Honey bee and bee-farming in the Tomsk region: past, present and future [in Russian]. *Tomsk State University Journal of Biology*, **4**(8), 15–28.
- [4] Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M.D., Conte, Y., Uzunov, A., Berg, S., Bienkowska, M., Bouga, M., Drazic, M., Dyrba, W., Kryger, P., Panasiuk, B., Pechhacker, H., Petrov, P., Kezić, N., Korpela, S. and Wilde, J., 2014, The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. *Journal of Apicultural Research*, **53**(2), 205–214. doi:10.3896/IBRA.1.53.2.03.
- [5] De la Rúa, P., Jaffé, R. and Dall’Olio, R. Muñoz, I. and Serrano, J., 2009, Biodiversity, conservation and current threats to European honey bees. *Apidologie*, **40**(3), 263–284. doi:10.1051/apido/2009027.
- [6] Meixner, M.D., Costa, C., Kryger, P., Hatjina, F., Bouga, M., Ivanova, E. and Büchler, R., 2010, Conserving diversity and vitality for honey bee breeding. *Journal of Apicultural Research*, **49**(1), 85–92. doi:10.3896/IBRA.1.49.1.12.
- [7] Meixner, M.D., Pinto, M.A., Bouga, M., Kryger, P., Ivanova, E. and Fuchs, S., 2013, Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. *Journal of Apicultural Research*, **52**(4), 1–28. doi:10.3896/IBRA.1.52.4.05.
- [8] Meixner, M.D., Büchler, R., Costa, C., Francis, R.M., Hatjina, F., Kryger, P., Uzunov, A. and Carreck, N.L., 2014, Honey bee genotypes and the environment. *Journal of Apicultural Research*, **53**(2), 183–187. doi:10.3896/IBRA.1.53.2.01.
- [9] Ostroverkhova, N.V., Konusova, O.L., Kucher, A.N. and Sharakhov, I.V., 2016, A Comprehensive Characterization of the Honeybees in Siberia (Russia). In: E. Dechechi Chambo (Ed.) *Beekeeping and Bee Conservation – Advances in Research*, pp. 1–37. ISBN 978-953-51-2412-2. doi:10.5772/62395.
- [10] Ostroverkhova, N.V., Konusova, O.L., Kucher, A.N., Kireeva, T.N., Vorotov, A.A. and Belikh, E.A., 2015, Genetic diversity of the locus COI-COII of mitochondrial DNA in honeybee populations (*Apis mellifera* L.) from the Tomsk region. *Russian Journal of Genetics*, **51**(1), 80–90. doi:10.1134/S102279541501010X.
- [11] Konusova, O.L., Ostroverkhova, N.V., Kucher, A.N., Kurbatskij, D.V. and Kireeva, T.N., 2016, Morphometric variability of honeybees *Apis mellifera* L., differing in variants of the COI-COII mtDNA locus [in Russian]. *Tomsk State University Journal of Biology*, **1**(33), 62–81. doi:10.17223/19988591/33/5.
- [12] Nikonorov, Y.M., Ben’kovskaya, G.V., Poskryakov, A.V., Nikolenko, A.G. and Vakhitov, V.A., 1998, The use of the PCR technique for control of the pure-breeding of honeybee (*Apis mellifera mellifera* L.) colonies from the Southern Urals. *Russian Journal of Genetics*, **34**(11), 1344–1347.
- [13] Ostroverkhova, N.V., Konusova, O.L., Kucher, A.N., Pogorelov, Y.L., Belykh, E.A. and Vorotov, A.A., 2013, Population genetic structure of honey bee (*Apis mellifera* L.) in the village of Leboter in Chainsky district of the Tomsk region [in Russian]. *Tomsk State University Journal of Biology*, **1**(21), 161–172.
- [14] Solignac, M., Vautrin, D., Loiseau, A., Mougél, F. and Baudry, E., 2003, Five hundred and fifty microsatellite markers for the study of the honey bee (*Apis mellifera* L.) genome. *Molecular Ecology Notes*, **3**, 307–311. doi:10.1046/j.1471-8286.2003.00436.x.

- [15] Ostroverkhova, N.V., Konusova, O.L., Kucher, A.N. and Kireeva, T.N., 2016, Investigation of polyandry in honey bees (*Apis mellifera*) using microsatellites. *Entomological Review*, **96**(4), 389–394. doi:10.1134/S0013873816040011.
- [16] Ostroverkhova, N.V., Konusova, O.L., Kucher, A.N., Kireeva, T.N. and Baghirov, R.T-o., 2015, Characterization of the genetic diversity of honey bees (*Apis mellifera* L.) in Tomsk population using mtDNA and microsatellite markers [in Russian]. *A.I. Kurentsov's Annual Memorial Meetings*, **26**, 227–240.
- [17] Garnery, L., Franck, P., Baudry, E., Vautrin, D., Cornuet, J.M. and Solignac, M., 1998, Genetic diversity of the West European honey bee (*Apis mellifera mellifera* and *A. m. iberica*). II. Microsatellite loci. *Genetics Selection and Evolution*, **30**(1), S49–S74.
- [18] Franck, P., Garnery, L., Solignac, M. and Cornuet, J.M., 1998, The origin of West European subspecies of honeybees (*Apis mellifera*): New insights from microsatellite and mitochondrial data. *Evolution*, **52**(4), 1119–1134. doi:10.2307/2411242.
- [19] Hatjina, F., Costa, C., Büchler, R., Uzunov, A., Drazic, M., Filipi, J., Charistos, L., Ruottinen, L., Andonov, S., Meixner, M.D., Bienkowska, M., Dariusz, G., Panasiuk, B., Conte, Y., Wilde, J., Berg, S., Bouga, M., Dyrba, W., Kiprijanovska, H., Korpela, S., Kryger, P., Lodesani, M., Pechhacker, H., Petrov, P. and Kezic, N., 2014, Population dynamics of European honey bee genotypes under different environmental conditions. *Journal of Apicultural Research*, **53**(2), 233–247. doi:10.3896/IBRA.1.53.2.05.