

Local Honeybee (*Apis mellifera mellifera* L.) Populations in the Urals

R. A. Il'yasov^a, A. V. Petukhov^b, A. V. Poskryakov^a, and A. G. Nikolenko^a

^a Institute of Biochemistry and Genetics, Ufa Research Center, Ufa 450054 Bashkortostan, Russia;
fax: (3472)35-61-00; e-mail: apismell@hotmail.com

^b Department of Zoology, Perm State Pedagogical University, Perm, 614600 Russia;
fax: (3422)21-95-86; e-mail: pusha-777@yandex.ru

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Abstract—The COI-COII intergenic region of mitochondrial DNA (mtDNA) was studied in local honeybee (*Apis mellifera mellifera*) L. populations from the Middle and Southern Urals. Analysis of bee colonies in these regions revealed apiaries enriched in families descending from *A. m. mellifera* in the maternal lineage. These results confirm the suggestion of preservation of *A. m. mellifera* refuges in the Urals and provide grounds for work on the preservation of the gene pool of this bee variety, valuable for all Russia.

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About two centuries before, the European dark bee (*Apis mellifera mellifera* L.) inhabited a wide area of Northern Europe from Great Britain to the Ural Mountains. In the 20th century, the *A. m. mellifera* population experienced anthropogenic influence. In particular, honeybee varieties from the Mediterranean and Caucasus were introduced to northern Eurasia, including the Urals. As a result, the native honeybee variety *A. m. mellifera* was displaced by hybrids throughout the area.

It is conceivable that *A. m. mellifera* populations still exist in Russia. The results of morphometric studies suggest the preservation of the Orel [1], Tatar [2], Krasnoyarsk [3], Altai [4], Kama (Vishera and Uya) [5], Sverdlovsk [6], and Bashkir (Burzyan) [7] local populations of *A. m. mellifera*. It is known, though, that hybrid bee colonies cannot be reliably identified by morphometrical methods.

Later, the intergenic COI-COII mtDNA locus as a genetic marker was applied to hybrid bee colonies [8]. By this method, the Burzyan bee population (Bashkortostan) was confirmed to belong to the *A. m. mellifera* variety [9]. We also used this method for testing the current state of existing populations and search for new *A. m. mellifera* populations in the Urals.

Experiments were performed with worker bees *A. mellifera* from 11 apiaries of three districts of Bashkortostan and from 11 apiaries of seven districts of the Perm krai (see table) collected in 2003–2005.

DNA isolation and polymerase chain reaction (PCR) were performed as in [8] with primers CACATTTA-GAAATTCCATTA and ATAAATATAAATCATGTGGA, corresponding to the 3'- and 5'-terminal sequences of the COI and COII genes, respectively. The mtDNA

amplicons are 350 bp long for the simplest combination Q, characteristic of *A. m. caucasica* and other southern varieties, and 600 bp long for the more complex combination PQQ, occurring in *A. m. mellifera*.

We sought for local *A. m. mellifera* populations extant in the Urals. The frequency of combination PQQ varied within 0.57–1.00 (table). The Vishera (Krasnovisherskii District, Perm Krai), Southern Kama (apiaries of the Osinskii, Nytvenskii, Permskii, Chastinskii, and Ordinskii districts of the Perm Krai), Tatyshly (apiaries of the Tatyshlinskii District, Bashkortostan), and Burzyan (apiaries of the Burzyanskii District, Bashkortostan) local populations showed high frequencies of combination PQQ: 0.97–1.00. This fact confirms preservation of *A. m. mellifera* in these populations (see figure).

The Uya (apiaries of the Uinskii District, Perm Krai) and Iglino (apiaries of the Iglinskii District, Bashkortostan) local populations showed low frequencies of occurrence of PQQ (0.71 and 0.57, respectively). This fact points to their hybrid origin.

One of the causes of the preservation of the Vishera and Burzyan *A. m. mellifera* refuges may be geographical isolation of apiaries in the mountainous forest zone of the Southern and Middle Urals, difficult to reach by vehicles. The Southern Kama and Tatyshly populations may have been preserved owing to specific features of local bee-keeping. There, native colonies are grown without delivery of bees from other populations, and therefore anthropogenic isolation takes place.

There are several explanations of the hybrid state of the Uya local population, formerly identified as *A. m. mellifera* by morphometrical methods [5]. The first expla-

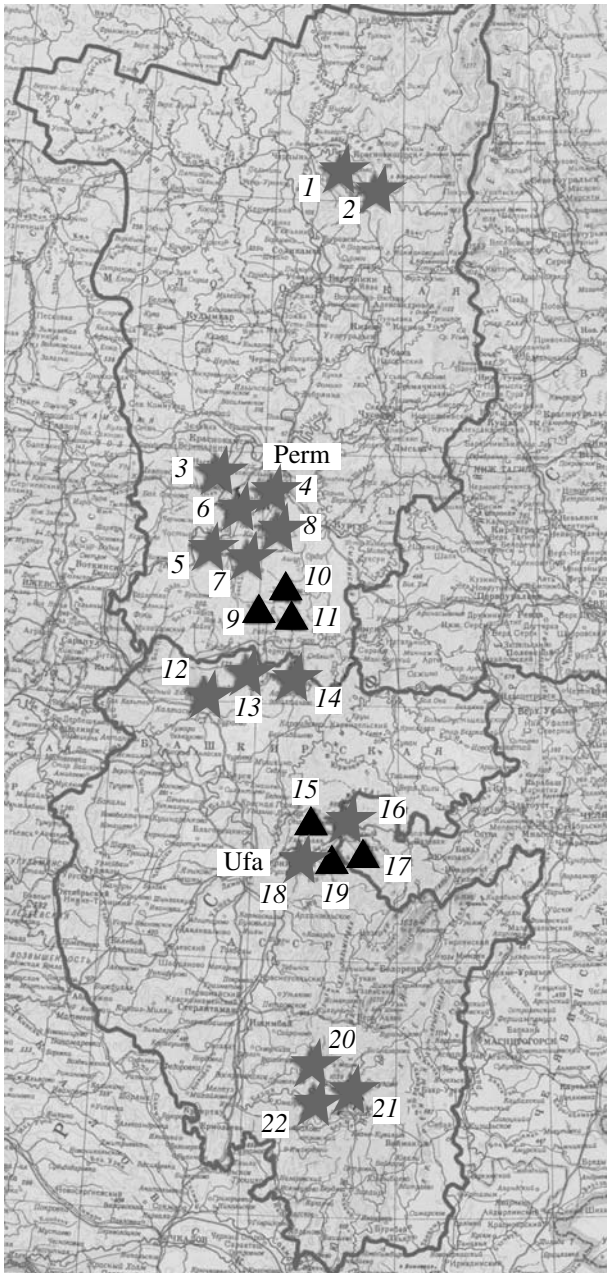
Predominance of the PQQ combination in Uralian honeybee populations

Local population	Apiary name	Number on the map	Number of colonies	PQQ frequency	Mean PQQ frequency
Perm Krai (Middle Urals)					
Vishera	Antipin I.I.	1	20	1.00	1.00
	Antipin N.T.	2	13	1.00	
Southern Kama	Grigor'evckoe village	3	9	1.00	0.99
	Bershet' village	4	23	0.95	
	Osa village	6	9	1.00	
	Chastye village	5	14	1.00	
	Prityk village	7	9	1.00	
	Ashap village	8	11	1.00	
Uin	Gribany village	10	6	0.83	0.71
	Ekaterinovka village	11	15	0.73	
	Upper Tulva village	9	15	0.57	
Bashkortostan (Southern Urals)					
Iglino	Orlovskaya	16	16	0.93	0.57
	Gareeva	15	125	0.27	
	Matkovyvodnaya	17	59	0.33	
	Kugeiko	18	20	0.90	
	Gromova	19	9	0.44	
Tatyshly	Shulganovo village	12	46	1.00	0.98
	Lenin collective farm	13	43	0.93	
	Salavat collective farm	14	22	1.00	
Burzyan	Kapova Peshchera	20	30	0.94	0.97
	Wild Hives	21	10	0.99	
	Koran-Elga village	22	26	0.99	
Total			550	0.87	0.87

nation is that the morphometrical methods cannot precisely discriminate hybrid bees from *A. m. mellifera* in the case of hybridization. Another explanation is that the bee colonies of the Uya local population underwent hybridization after the morphometrical studies. The Iglino local population in general proved to be hybrid, although some apiaries were dominated by the PQQ combination, e.g., Kugeiko and Orlovskaya (0.90 and

0.93, respectively). These apiaries could not be combined into a single local *A. m. mellifera* population, because they are distant from one another and surrounded by apiaries with hybrid colonies, which must have been involved in gene flux mediated by drones.

Thus, use of the COI-COII mtDNA locus as a DNA marker allowed us to reveal at least four local *A. m. mel-*



Geographical location of apiaries examined with the use of the intergenic COI-COII mtDNA locus as a mtDNA marker. Names of the apiaries indicated with numerals are presented in the table. ★, apiaries with families with PQQ > 90%; ▲, apiaries with families with PQQ < 90%.

lifera populations preserved in the Urals. In the future, they can be a source for restoration of the honeybee gene pool within the former area of the variety.

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