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ANIMAL GENETICS

New Approach to the Mitotype Classification in Black Honeybee *Apis mellifera mellifera* and Iberian Honeybee *Apis mellifera iberiensis*

R. A. Ilyasov[a], A. V. Poskryakov[a], A. V. Petukhov[b], and A. G. Nikolenko[a]

[a] *Institute of Biochemistry and Genetics, Ufa Scientific Center, Russian Academy of Sciences, Ufa, 450054 Republic of Bashkortostan, Russia*
e-mail: apismell@hotmail.com

[b] *Department of Zoology, Perm State Humanitarian Pedagogical University, Perm, 614990 Russia*

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Abstract—The black honeybee *Apis mellifera mellifera* L. is today the only subspecies of honeybee which is suitable for commercial breeding in the climatic conditions of Northern Europe with long cold winters. The main problem of the black honeybee in Russia and European countries is the preservation of the indigenous gene pool purity, which is lost as a result of hybridization with subspecies, *A. m. caucasica*, *A. m. carnica*, *A. m. carpatica*, and *A. m. armeniaca*, introduced from southern regions. Genetic identification of the subspecies will reduce the extent of hybridization and provide the gene pool conservation of the black honeybee. Modern classification of the honeybee mitotypes is mainly based on the combined use of the *Dra*I restriction endonuclease recognition site polymorphism and sequence polymorphism of the mtDNA COI–COII region. We performed a comparative analysis of the mtDNA COI–COII region sequence polymorphism in the honeybees of the evolutionary lineage M from Ural and West European populations of black honeybee *A. m. mellifera* and Spanish bee *A. m. iberiensis*. A new approach to the classification of the honeybee M mitotypes was suggested. Using this approach and on the basis of the seven most informative SNPs of the mtDNA COI–COII region, eight honeybee mitotype groups were identified. In addition, it is suggested that this approach will simplify the previously proposed complicated mitotype classification and will make it possible to assess the level of the mitotype diversity and to identify the mitotypes that are the most valuable for the honeybee breeding and rearing.

Keywords: black honeybee, *Apis melifera melifera*, evolutionary lineage M, mtDNA COI–COII intergenic region, mitotypic classification of honeybee, single nucleotide polymorphism (SNP)

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INTRODUCTION

The black honeybee *Apis mellifera melliftra* L. is the only one among 29 subspecies of modern bees perfectly adapted to life in the conditions of the sharply continental climate of Northern Europe. The black honeybee *A. m. mellifera* is a member of the evolutionary branch M, which also includes Iberian honeybee *A. m. iberiensis* [1–7].

The development of agriculture leads to continuous decline of the genetic diversity in Western European populations of black honeybee and Iberian honeybee, despite the ongoing conservation and restoration activities [8]. According to a report of the European Food Safety Authority (EFSA), from 2000 through 2009, the death rate in the surviving populations of black honeybee in Norway, Finland, Sweden, Switzerland, Denmark, the Netherlands, Belgium, Great Britain, and France was 10% higher than the standard level [9]. The increased death rate in the populations of black honeybee populations is caused by the constantly increasing influence of commercial beekeeping, which is characterized by intense import of queens and transport of honeybee colonies over long distances, which leads to hybridization and the loss of the indigenous gene pool purity [10].

The modern distribution range of *A. m. mellifera* is considerably reduced under the influence of hybridization with the honeybee subspecies introduced from southern regions, *A. m. caucasica*, *A. m. carnica*, *A. m. carpatica*, *A. m. armeniaca*, *A. m. ligustica*, representatives of the evolutionary lineage C [10–15]. Hybridization of northern indigenous bees with the introduced southern subspecies leads to the destruction of the valuable indigenous gene pool and the loss of adaptability to extreme conditions of the northern habitat. Beekeeping can be safe, successful, and productive only if one bee subspecies is bred in one region [10–15].

At present, as a result of human activity, most of the populations of black honeybee in Western and Northern Europe have been lost. The remaining populations of this northern subspecies of honeybee *A. m. mellifera* are preserved in the form of small islands in Russia,

Switzerland, Denmark, Sweden, Norway, France, and Spain [1, 16–19].

In Nordic countries, the black honeybee is partially or completely replaced by the Italian bee *A. m. ligustica* [20, 21] and Carniolan bee *A. m. carnica* [21, 22]. The populations of black honeybee in the southwest and northeast of France are also subjected to strong introgression with imported subspecies [16, 17].

The lack of a modern strategy for countering the increased mortality, subspecies introgression, and distant transpositions of the honeybee colonies can lead to the complete disappearance of black honeybee populations in Europe [23].

Successful breeding and reproduction of *A. m. mellifera* requires conservation of the gene pool purity and control of the subspecies affiliation of the exported and imported honeybee colonies [24, 25].

The mitochondrial COI–COII region, located between the genes for the cytochrome C oxidase subunit I (COI) and II (COII), for nearly 30 years has been used as a genetic marker for the assessment of intraspecific mitotype structure of honeybee *A. mellifera* [25]. The mtDNA COI–COII region is composed of repetitive nucleotide sequences, referred to as one P element and from one to five Q elements [1, 12, 13, 18, 26–30]. This region is characterized by high sequence variability and the presence of numerous single nucleotide substitutions (SNPs), deletions, and insertions [1, 27–30].

Up to now, on the basis of the *Dra*I restriction endonuclease digestion of the honeybee mtDNA COI–COII region, in the honeybees of the evolutionary lineage M, 91 mitotypes have been identified (analysis of 6633 honeybee colonies); 30 mitotypes in the bees of the evolutionary lineage A (analysis of 1754 honeybee colonies) [31–36], five mitotypes of the evolutionary lineage C (analysis of 1621 honeybee colonies) [37–41], and seven mitotypes of the evolutionary lineage O (analysis of 83 honeybee colonies) [27, 35, 36, 38, 39, 42] have been identified. Most of the mitotypes were identified only on the basis of visual detection of the restriction fragments of the amplified mtDNA COI–COII region in polyacrylamide gel electrophoresis [16].

In the evolutionary lineage M, mitotypes M4 and M4' were the most frequent, followed by mitotypes M17, M7, M6, M19, M8, M17'. The remaining mitotypes were very rare [16]. Application of the analysis of sequence polymorphism in addition to the *Dra*I restriction endonuclease site polymorphism for mitotypic classification of honeybees of the evolutionary lineage M resulted in the limitless growth of the number of mitotypes [16].

According to the modern classification, mitotypes having similar patterns are designated by the same numbers, but this makes it impossible to discriminate between the samples with different number of Q elements. Therefore, it was decided to use the symbols for three Q ('), four Q ("), and five Q ("') elements. Mitotypes with one and two Q elements are different in patterns and have different numbers. The samples differing in one or two nucleotides are usually denoted by the letters a, b, c, d, e [16].

This classification of mitotypes with the use of alphanumeric symbols with primes greatly complicates their analysis and general perception. It is desirable to perform the classification of the honeybee COI–COII mitotypes on the basis of a single method, in particular, the analysis of sequence polymorphism. Combination of the analysis of the *Dra*I restriction endonuclease site polymorphism and analysis of the mtDNA COI–COII sequence polymorphism can lead to the misclassification of mitotypes. The mitotypic classification with the use of most frequent, informative SNPs and discarding of rare uninformative, single nucleotide substitutions will make it possible to shift to a uniquely interpreted and simplified version of the analysis [16].

The aim of this study was to develop a new mitotypic classification of the honeybee mtDNA COI–COII region based on single nucleotide polymorphism, which will make it possible to simplify the previously proposed complicated classification of the mitotypes, as well as to assess the level of mitotype diversity and to identify the mitotypes valuable for breeding and rearing programs.

MATERIALS AND METHODS

The mtDNA COI–CII intergenic region, located between the genes for the cytochrome C oxidase subunit I (COI) and II (COII), was sequenced in *A. m. mellifera* honeybees representing 20 different colonies from 18 apiaries located in six raions of the Republic of Bashkortostan and Perm krai (Southern and Middle Urals) (Table 1).

Total DNA was isolated from the honeybee thoracic flight muscles using the DNA-EXTRAN-2 extraction kit (Sintol, Moscow).

Polymerase chain reaction (PCR) was performed using the Tertsik MS2 amplifier in 15 μL of the reaction mixture containing one unit of *Taq* DNA polymerase, 1× reaction buffer with 25 mM $MgCl_2$ (Sileks, Moscow), 200 μM of each dNTP, 0.5 μM of each primer; and from 20 to 100 ng DNA. PCR and sequencing were performed using oligonucleotide primers for the mtDNA COI–COII region [25, 36]. Amplificates were purified and sequenced on an Applied Biosystems automated sequencer (United States) at Sintol (Moscow, Russia) and deposited in the GenBank international database.

Comparative sequence analysis of a 510 to 965 bp (relative to the reference sequence of mitotype M4a' (KF274638)) fragment of the mtDNA COI–COII intergenic region was carried out using the MEGA 4.1 software package for 109 honeybee individuals of the evolutionary lineage M. These honeybee individuals

Table 1. The size of the sample from the honeybee colonies of *A. m. mellifera* from the Urals (Republic of Bashkortostan and Perm krai)

GenBank acc. no.	Settlement	Region
KF970918	Osinovka	Republic of Bashkortostan, Birsky raion
KF970919	Pechenkino	
KF970920	Kondakovka	
KF970921	Shestykovo	
KF970922	Uguzevo	
KF970923	Lezhebokovo	
KM242632	Kagarmanovo	Republic of Bashkortostan, Beloretsky raion
KM242633	Kaga	
KM242634	Sermenevo	
KM242635	Galiakberovo	Republic of Bashkortostan, Burzyansky raion
KM242636	Yaumbaevo	
KM242637	Irgizly	
KF970924	Starosubkhangulovo 1	
KF970925	Starosubkhangulovo 2	
KM242638	Kustarevka	Republic of Bashkortostan, Tatyshlinsky raion
KM242639	Sabanchi	
KM242640	Uyadybash	
KM242641	Porshakova 1	Perm krai, Krasnovishersky raion
KM242643	Porshakova 2	
KM242642	Nytva	Perm krai, Nytvensky raion

included 20 individuals from Ural population and 86 individuals from European population of black honeybee *A. m. mellifera* and three individuals from Spanish population of Iberian honeybee *A. m. iberiensis*.

RESULTS

Comparative analysis of the mtDNA COI–COII intergenic region performed among 109 honeybee individuals revealed the presence of 36 single nucleotide substitutions (SNPs), including 13 transversions and 23 transitions (Table 2). On the basis of comparative analysis of the SNP frequencies in different honeybee representatives of the evolutionary lineage M, a median network reflecting the genetic diversity not identified at the levels of phenotypic and restriction polymorphisms was constructed. It seems likely that the presence of the large number of SNPs in the mtDNA COI–COII intergenic region can be explained in terms of the substitution neutrality and the absence of the influence of this region on the adaptiveness and, hence, on the honeybee natural selection.

It should be noted that, in comparative analysis and upon the median network construction, we did not take into the account the insertions and deletions mostly represented by a different amount of the Q element (from one to five copies). These mutations do not contribute significantly to improving the informativeness of the analysis, since we took into the account only one Q element found in the honeybees of all evolutionary lineages. Such an approach, which does not take into account all insertions/deletions of the Q element, will provide the development of a unified mito-

Table 2. Single nucleotide substitutions (SNPs) in the 510 to 965 bp (relative to the KF274638-M4a' reference sequence) fragment of the mtDNA COI–COII intergenic region in the *A. m. mellifera* and *A. m. iberiensis* honeybees of the evolutionary lineage M

Honeybee specimens	SNP relative to the reference sequence KF274638 (M4a')
KF970918 (M) (Birsky raion)	527 T > A, 681 A > G, 727 C > T, *803 C > T, 863 T > C, *912 G > A
KF970919 (M) (Birsky raion)	527 T > A, 681 A > G, 727 C > T, *803 C > T, *912 G > A
HQ260376 (M4e), HQ260377 (M21")	785 T > G, 808 T > C, *817 C > T
HQ337445 (M12)	691 A > T, 795 A > T, *912 G > A
KM242633 (M) (Beloretsky raion), KM242637 (M) (Burzyansky raion)	819 A > G, 900 A > G, *912 G > A
HQ337450 (M17)	585 T > A, 596 T > G, *938 T > A
HQ337438 (M5)	624 A > G, 800 A > G, *938 T > A
HQ260378 (M4c'"), HQ260367 (M26'), HQ260379 (M56'"), HQ337451 (M17')	808 T > C, *817 C > T
HQ260345 (M18)	*817 C > T, *912 G > A
KM242634 (M) (Beloretsky raion)	*857 C > T, *912 G > A
HQ260374 (M59')	514 C > T, 743 G > A
KM242632 (M) (Beloretsky raion)	819 A > G, 900 A > G
HQ260355 (M46)	*555 C > T, 601 T > C
HQ260357 (M48)	774 A > T, 784 A > G
FJ743638 (M5)	*817 C > T
HQ260371 (M55')	694 A > G
HQ260368 (M24')	601 T > C
HQ337437 (M4')	800 A > G
FJ743637 (M4)	*555 C > T
KF274634 (M4k), FJ743634 (M1)	*803 C > T
KF274631 (M4h)	668 C > T
HQ260351 (M40)	832 A > T
HQ260364 (M61)	830 A > G
HQ337448 (M15)	529 T > A
KF274632 (M4i), KF274626 (M4b)	*510 T > C
HQ260352 (M41)	782 T > C
HQ337443 (M10), KF970920 (M) (Birsky raion), KF970921 (M) (Birsky raion), KF970922 (M) (Birsky raion), KM242641 (M) (Krasnovishersky raion), KM242642 (M) (Nytvensky raion), KM242643 (M) (Krasnovishersky raion), KM242638 (M) (Tatyshlinsky raion), KM242639 (M) (Tatyshlinsky raion), KM242640 (M) (Tatyshlinsky raion), KM242635 (M) (Burzyansky raion), KM242636 (M) (Burzyansky raion), KF970923 (M) (Birsky raion), KF970924 (M) (Burzyansky raion), KF970925 (M) (Burzyansky raion)	*912 G > A

Table 2. (Contd.)

Honeybee specimens	SNP relative to the reference sequence KF274638 (M4a')
HQ260358 (M49)	651 A > T
HQ260340 (M30)	513 C > T
HQ260361 (M52)	930 C > A
HQ337457 (M35)	656 A > T
KF274629 (M4f), FJ478008 (M6), FJ478006 (M4)	*857 C > T
HQ337435 (M3), FJ478004 (M3) (*A. m. iberiensis*), HQ337439 (M6)	*938 T > A
KF274638 (M4a') (reference), HQ260370 (M55'), HQ260372 (M57'), HQ260369 (M39'), HQ260365 (M43'), HQ337444 (M10a'), HQ337449 (M16'), HQ337441 (M8'), HQ260366 (M44'), HQ337442 (M9'), HQ260375 (M60'), HQ260373 (M58'), KF274640 (M64), HQ260362 (M53), EF033656 (M4), FJ743635 (M2), FJ743636 (M3), KF274630 (M4g), KF274637 (M4n), KF274636 (M4m), KF274639 (M7a), HQ260353 (M42), KF274633 (M4j), KF274627 (M4d), KF274628 (M4e), HQ260359 (M50), KF274625 (M4a), HQ260349 (M26), HQ260343 (M11), HQ260363 (M54), KF274635 (M41), HQ260342 (M4c), HQ260360 (M51), HQ337456 (M34), FJ478005 (M7) (*A. m. iberiensis*), FJ478007 (M8) (*A. m. iberiensis*), HQ337454 (M32), HQ260350 (M39), HQ260348 (M25), HQ260344 (M17a), HQ260346 (M20), HQ260354 (M45), HQ260356 (M47), HQ337455 (M33), HQ337440 (M8), HQ260347 (M23), HQ337452 (M19), HQ337446 (M13), HQ260341 (M62), HQ337453 (M29), HQ337447 (M14), KF274638 (M4a')	No substitutions

Informative SNPs used for new classification of the honeybee mitotypes are marked by asterisks; transversions are underlined.

type classification for the honeybees of all evolutionary lineages (A, M, C, O).

In the median network, two large central nodes, one of which includes 51 honeybee individuals and the other one includes 15 individuals, can be observed. Smaller groups with rare SNPs are rather randomly distributed around the central nodes. One of the groups includes four individuals, two groups consist of three individuals, four groups have two individuals, and the remaining 25 are the individual honeybee specimens (Fig. 1). Grouping of most of the honeybee individuals from different populations into two large groups point to their considerable homogeneity and common origin. As can be seen from Fig. 1, most of the black honeybee individuals from the Ural and West European populations fell into different groups, differing from one another in the G > A transition at position 912. European black honeybee with mitotype M10 (HQ337443) was grouped together with black honeybees from Ural population.

In molecular phylogenetics, single nucleotide substitutions occurring in more than one individual are recognized as informative. This fact was used to isolate informative SNPs for classification of honeybee M mitotypes. During the isolation of the mitotype groups, only informative single nucleotide substitutions were scored.

DISCUSSION

Hypervariability of the mtDNA COI–COII intergenic region in honeybee leads to the high level of genetic diversity within the evolutionary lineage M. In the framework of the presently existing classification of hypervariable loci, there may be problems associated with the increase in the total number of mitotypes, as even a single SNP has the right to acquire a new mitotype number, which leads to the increase in the number of mitotypes and complexity of their analysis (Fig. 2). This classification, owing to the simultaneous use of several methods of analysis, does not entirely correctly reflect the relationships between the mitotypes, since it can bring together genetically distant mitotypes and remove the genetically close ones. This happens because of the appearance of similar *Dra*I restriction endonuclease recognition sites in genetically distant honeybee representatives along

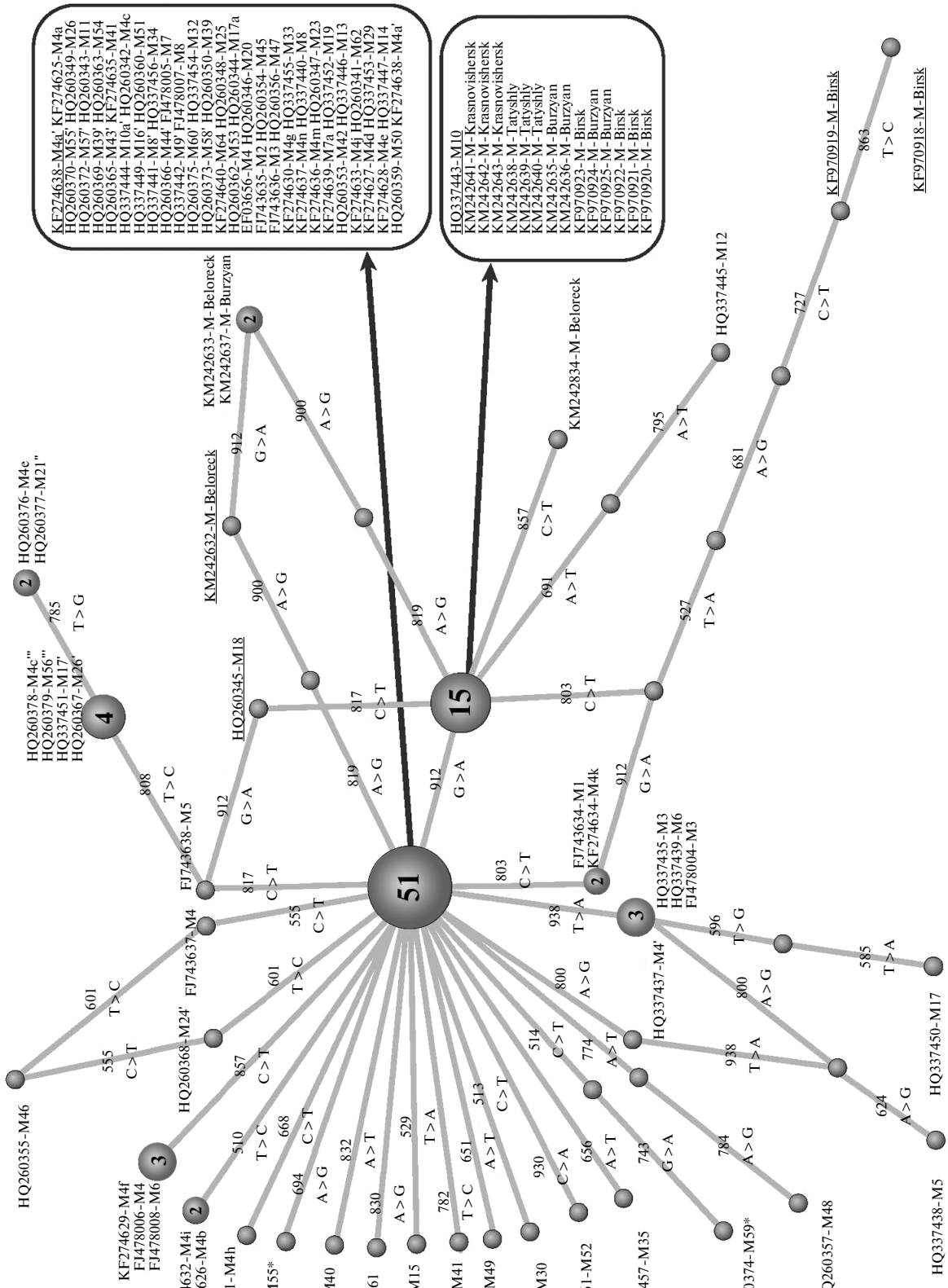


Fig. 1. Median network of the genetic relationships of *A. m. mellifera* and *A. m. iberiensis* honeybees of the evolutionary lineage M constructed on the basis of the discovered 36 SNPs of the mtDNA COI–COII intergenic region relative to the KF274638 reference sequence (M4a'). The specimens mentioned in the text are underlined.

with the disappearance of the *Dra*I recognition sites from the genetically close honeybees as a result of hypervariability of the COI–COII intergenic region.

We suggest a new principle of molecular classification of the honeybee M mitotypes based on the analysis of seven informative SNPs of the mtDNA COI–COII intergenic region. On the basis of the seven most informative SNPs of the mtDNA COI–COII intergenic region, eight honeybee mitotype groups were identified (Fig. 3). The first five groups were distributed in order of decreasing number of individuals and the last three groups were distributed in order of increasing serial number of the position in the nucleotide sequence.

The largest major group I united 67 honeybee individuals of the evolutionary lineage M characterized by the absence of substitutions relative to the reference sequence of mitotype M4a' (KF274638). This group forms the central node of the honeybee evolutionary lineage M (Fig. 3).

Group II united 22 honeybees carrying informative G > A transition at the position 912 relative to the reference sequence of mitotype M4a' (KF274638). This group mostly consisted of black honeybee individuals from the Ural population, as well as of some individuals from the European populations of mitotypes M10 (HQ337443), M12 (HQ337445), and M18 (HQ260345) (Fig. 3). Mitotype M10 (HQ337443) was found to be the most closely related to the honeybees of the Ural population, while mitotypes M12 (HQ337445) and M18 (HQ260345) were more distant from them (Fig. 1). One honeybee individual of the Ural population from Beloretsky raion of the Republic of Bashkortostan (KM242632) was included in group I, which implied close relationships and common ancestry with the representatives of Western European populations of black honeybee (Fig. 3).

Group III united seven honeybee individuals with different M mitotypes carrying informative C > T transition at position 817 relative to the reference sequence of mitotype M4a' (KF274638).

Group IV combined five honeybee individuals with different M mitotypes with informative T > A transversion at position 938 relative to the reference sequence of mitotype M4a' (KF274638).

Group V combined three honeybee individuals of evolutionary lineage M with the C > T transition at position 857 relative to the reference sequence of mitotype M4a' (KF274638).

The last three groups (VI, VII, VIII) contained by two honeybee individuals of evolutionary lineage M with T > C transition at position 510 and C > T transition at positions 555 and 803 relative to the reference sequence of mitotype M4a' (KF274638).

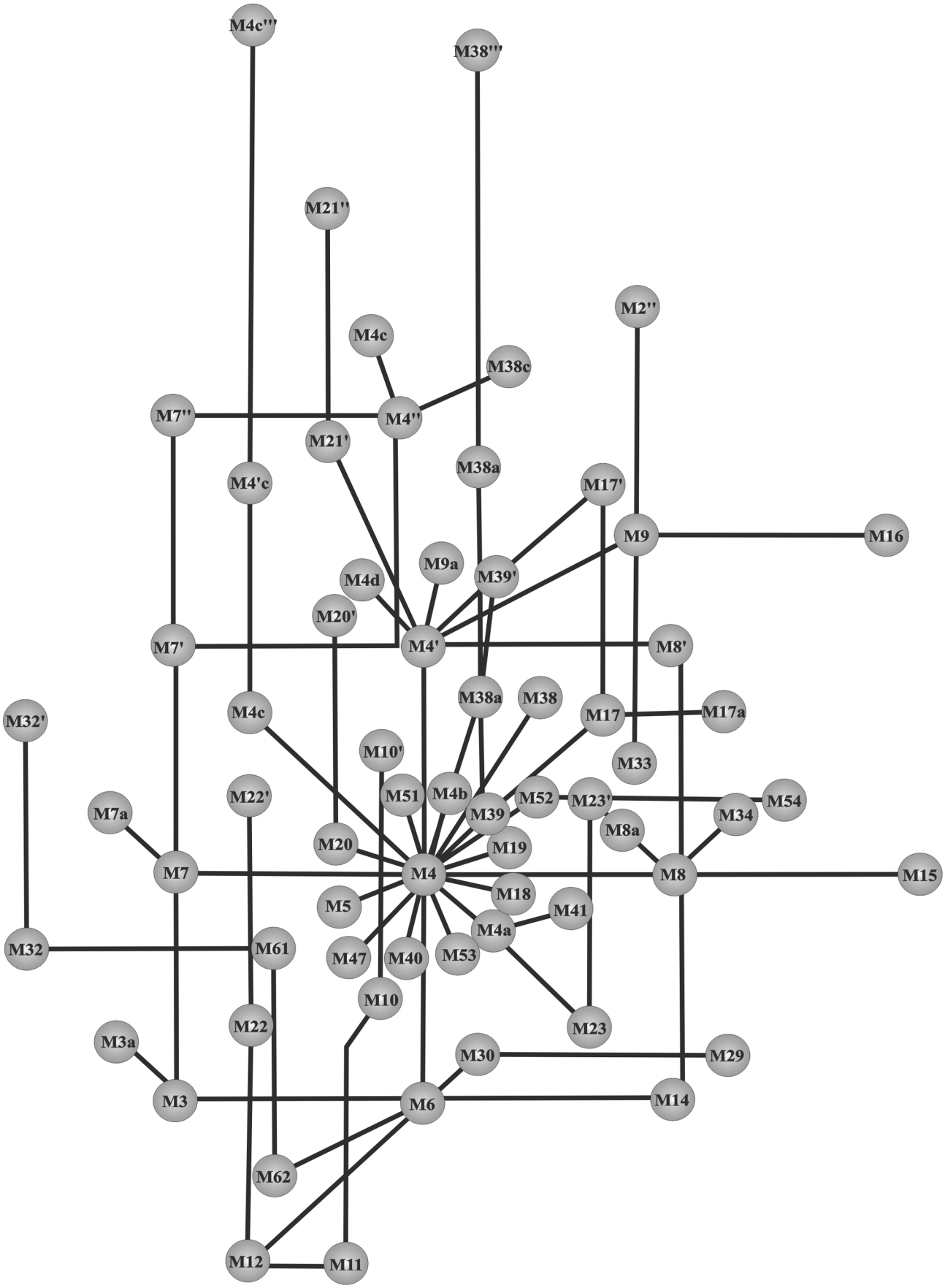
To avoid the appearance of ambiguities and an increase in the number of the mitotype groups during classification, it is important to follow the sequence of the SNP analysis: (1) G > A at position 912; (2) C > T at position 817; (3) T > A at position 938; (4) C > T at position 857; (5) T > C at position 510; (6) C > T positions 555; (7) C > T at position 803; (8) the absence of all substitutions. The first five groups are characterized by considerable robustness, since they contain from 3 to 67 individuals carrying specific single nucleotide substitutions, and therefore, they are grouped according to increasing degree of robustness. The latter three groups contain only two individuals each, and therefore, they are grouped in order of increasing serial number of the SNP position in the nucleotide sequence. In the rare cases of the simultaneous presence of two SNPs (transitional forms), the preference should be given to the substitution which tops the list with the assignment to the corresponding group. We believe that the described approach to create the SNP preference rule in accordance with the degree of robustness and the serial number in the course of grouping will make it possible to include rare transitional forms in the already existing groups and not to create new groups for them.

For instance, the honeybee individual with mitotype M18 (HQ260345) contains the G > A transition at position 912 (Fig. 1), with respect to which it can be included in group II (Fig. 3), and the C > T transition at position 817 (Fig. 1), with respect to which it can be included in group III (Fig. 3). However, according to the suggested rule, the preference will be given to the first G > A transition at position 912, and hence, this honeybee individual will be assigned to group II (Fig. 3).

The other two honeybee specimens of the Ural population from Birsky raion of the Republic of Bashkortostan, belonging to the evolutionary lineage M (KF970918 and KF970919), contained the G > A transition at position 912 (Fig. 1), with respect to which they could be assigned to group II (Fig. 3), and the C > T transition at position 803 (Fig. 1), with respect to which they could be assigned to group VIII (Fig. 3). Since the G > A transition at position 912 is the first, it will be given the preference, and these honeybees will be assigned to group II (Fig. 3).

At present, the black honeybee individuals carrying the combination of two informative SNPs providing their assignment to different groups are very rare. Most of the examined honeybees contained only one out of seven isolated single nucleotide substitutions. In the case where the black honeybee individual contains none of the seven informative SNPs, it will be assigned to group I and will form the basal node of the evolutionary lineage M.

We propose to isolate eight groups of M mitotypes (I–VIII) that can be termed as SNP mitotypes of the mtDNA COI–COII intergenic region and designate them as M-I, M-II, M-III, M-IV, M-V, M-VI, M-VII, M-VIII. The classification itself can be termed SNP mitotyping, similarly to the term genotyping. The transition from the old approach to the mitotype classification based on the combined use of the *Dra*I restriction endonuclease site polymorphism and


M4c'''
M38'''
M21''
M2''
M4c
M38c
M7''
M4''
M21'
M38a
M4'c
M17'
M9
M16
M9a
M4d
M39'
M20'
M7'
M4'
M8'
M38a
M38
M4c
M17
M17a
M32'
M10'
M33
M51
M4b
M52
M23'
M54
M22'
M7a
M39
M34
M8a
M20
M19
M7
M4
M8
M15
M18
M5
M41
M4a
M32
M61
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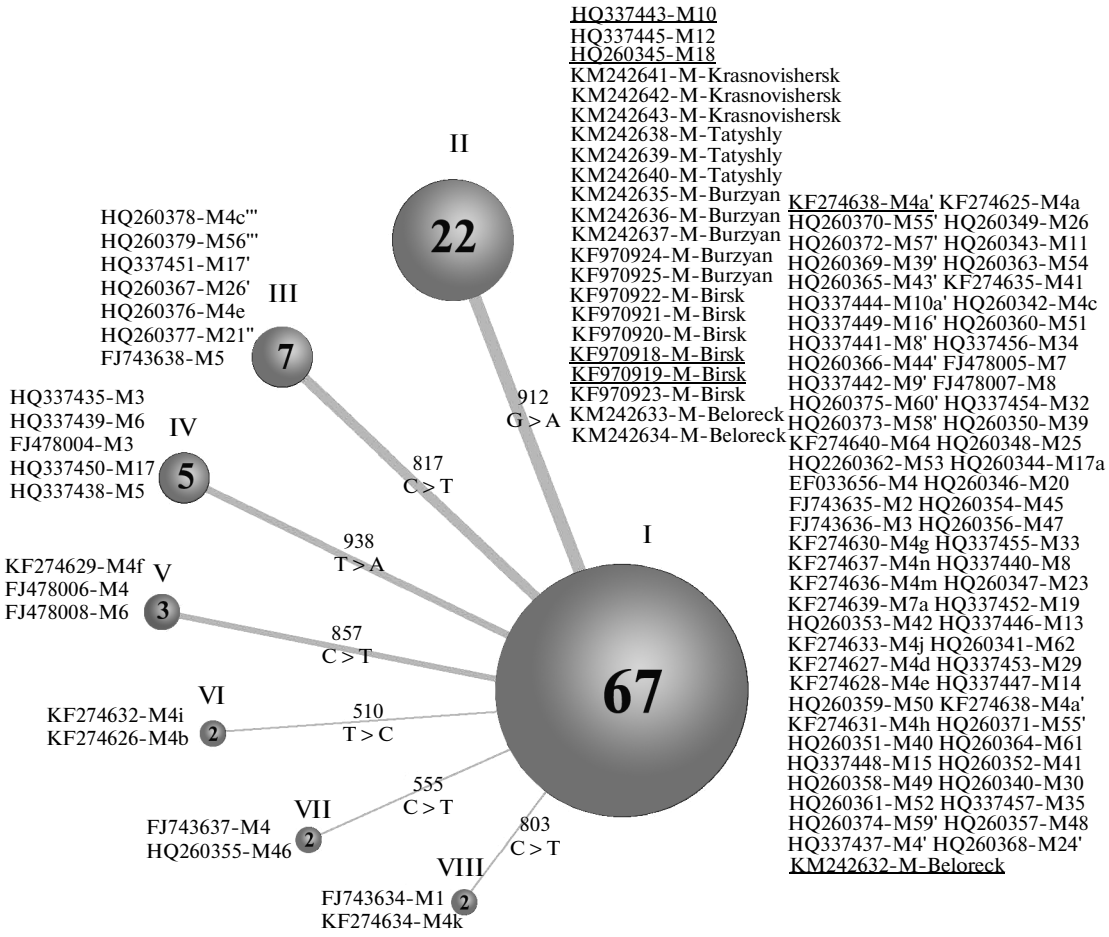

Fig. 3. Newly suggested mitotype classification of *A. m. millefera* and *A. m. iberiensis* honeybees of the evolutionary lineage M constructed on the basis of seven informative SNPs of the mtDNA COI–COII intergenic region relative to the KF274638 reference sequence (M4a'). The specimens mentioned in the text are underlined.

sequence polymorphism of the mtDNA COI–COII intergenic region to the new SNP-mitotype classification based on the analysis of seven informative SNPs will reduce the number from 91 existing mitotypes to eight SNP mitotypes and solve the problem of further increase in the number of mitotypes. The problem of the increasing number of the honeybee mitotypes in terms of the old classification consists in the fact that any new mitotype specimen differing from the already known one even in one nucleotide gets the number with a Latin letter at the end (currently, the 14th letter N is in use, and there are only 26 letters in the English alphabet), supplemented with different number of primes (it is rather difficult to visually perceive more than two primes).

The proposed mitotype classification is a new, at no time in the past used, approach to the mitochondrial genome genetic diversity assessment, based on the SNP data. The SNP-mitotyping approach will provide further development of primers for target SNP detection only with the help of PCR and without determination of the complete nucleotide sequence, which reduces the cost of the analysis and simplifies and accelerates it.

Thus, this approach makes it possible to reclassify the currently existing 91 honeybee M mitotypes of the old classification into eight SNP M mitotypes of the new classification based on the seven most informative SNPs of the mtDNA COI–COII intergenic region. In addition, this approach makes it possible to simplify the complicated prior mitotype classification, assess

Fig. 2. Currently existing mitotype classification of *A. m. millefera* and *A. m. iberiensis* honeybees of the evolutionary lineage M based on the *Dra*I restriction fragment length polymorphism of the mtDNA COI–COII intergenic region and the Q element repeat number. Horizontal link between the two nodes corresponds to the single difference in the restriction fragments or to the presence of either deletion or insertion; vertical link denotes a change in the number of repeats of the Q sequence [16].

the mitotype diversity level, and identify the mitotypes most valuable for breeding and rearing.

We hope that the development of molecular classification techniques for the black honeybee of the evolutionary lineage M will allow a new level of pure breed selection and monitoring in beekeeping, the level of genetic selection. This will make it possible to efficiently use the genetic potential of bee subspecies, optimize the genetic resources of the indigenous gene pool, and preserve its purity. The direction of beekeeping based on breeding according to the SNP genetic markers can be defined as genetically selectable beekeeping.

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