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NUCLEOTIDE POLYMORPHISM OF THE GENE *VG* OF HONEY BEES

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Abstract

Conservation of the gene pool of dark European bees *A.m.mellifera* is extremely important for the successful development of beekeeping in the Northern Eurasia. There are many established methods for differentiating honey bee subspecies. This study evaluated single nucleotide polymorphisms (SNP) as a marker for genotyping of honey bees. The gene vitellogenin (*Vg*) is involved in the regulation of social organization of honey bees. We sequenced *Vg* of dark European bees from Ural population and found 26 unique SNP for *A.m.mellifera* in its exons, which can be useful for genetic cataloging of colonies.

Keywords: honey bee, *Apis mellifera mellifera*, Vitellogenin, *Vg*, SNP, single nucleotide polymorphism.

Introduction

The honey bee *Apis mellifera* L. has been genetically subdivided in 26 subspecies in long time evolution (Ilyasov, Poskryakov 2006). This level of genetic divergence does not provide isolation between subspecies. Hybridisation of honey bee subspecies occurred throughout Europe with transportation between countries of different subspecies of honey bees due to spreading of hybrid origin population. Successful breeding of honey bees is possible using clean-breeding lines.

Beekeeping may be successful only when one breed of honey bees is kept. Dark European bees *Apis mellifera mellifera* L. are preferred for beekeeping in North Eurasia countries. This honey bee subspecies is belonging to the evolutionary branch M which adapted to live in a continental climate with long cold winter (Ilyasov et al. 2007). Isolated populations of *A.m.mellifera* remain in small islets in Russia, Switzerland, Denmark, Sweden, Norway, France and Spain (Nikonorov et al. 1998, Jensen et al. 2005). Only genetic control of introduced honey bees in this areas can to provide safety its gene-pool (Nikolenko, Poskryakov 2002).

The most popular genetic markers for monitoring the gene pool of honey bees are the gene ND2 and intergenic locus COI-COII of mitochondrial DNA, and many microsatellite loci and single nucleotide polymorphism loci (SNP) of nuclear DNA (Garnery et al. 1992; Nikolenko, Poskryakov 2002; Whitfield et al. 2006; Whitfield et al. 2006; Pinto et al. 2014).

Our research based on investigation the nucleotide polymorphism of gene vitellogenin (*Vg*) of honey bees. The vitellogenin is 180 kDa phospholipoglycoprotein and a major precursor of egg yolk (Chen et al. 1997; Sappington, Raikhel 1998; Tufail, Takeda 1998). Honey bees have only one copy of gene Vg whereas other insects have more (Kent et al. 2011). Nucleotide sequences of six from the seven exons (from 2 to 7) were deposited in GenBank (http://www.ncbi.nlm.nih.gov) (Fig. 1).

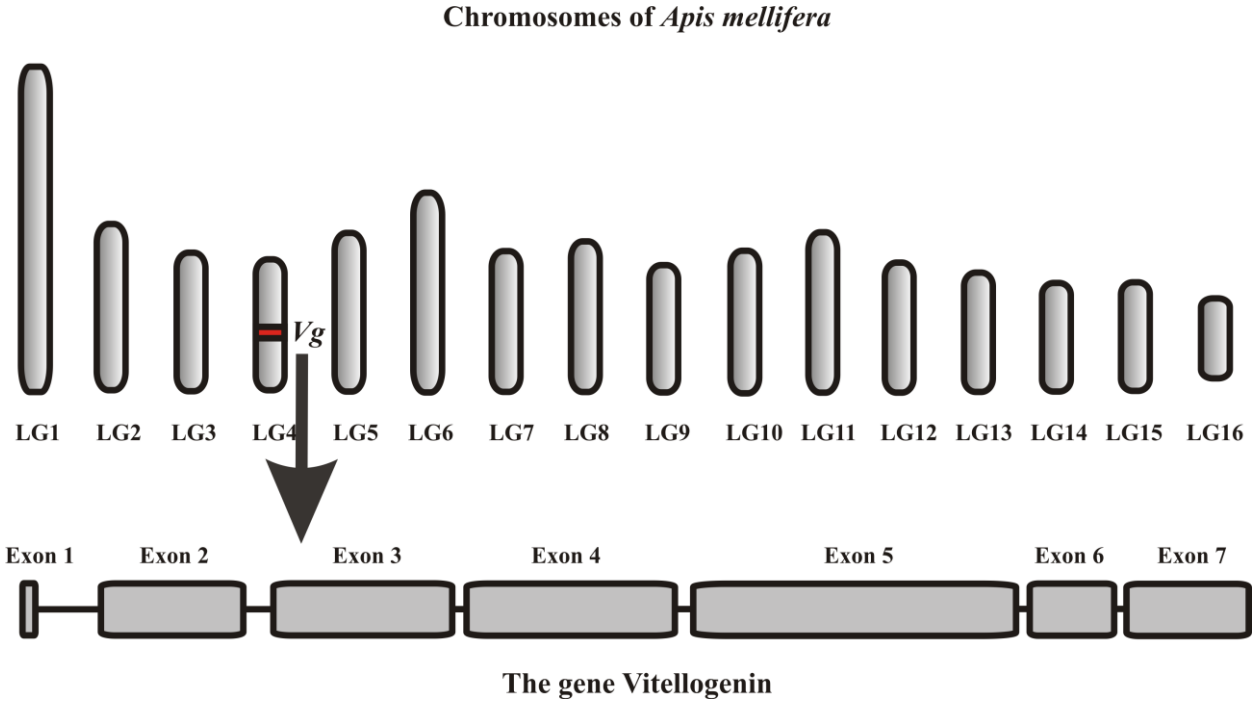

Fig. 1. Localisation of the gene vitellogenin of the honey bee on the fourth chromosome and its exon-intron structure.\

We sequenced six exons of the gene vitellogenin of honey bees of *A.m.mellifera* of evolutionary branch M from twelve isolates from Ural region of Russia. Comparative analysis of sequences of *Vg* of northern bees from Ural (evolutionary branches M) with southern bees from Caucasus (evolutionary branches C) allowed us to discover 26 single nucleotide differences between them. Comparative analysis of sequences of full length DNA of *Vg* of honey bees of three evolutionary branches A, M and C allowed clustering into three major population groups of *A. mellifera* (Whitfield et al. 2006) from Africa (the branch A), from Mediterranean (the branch C) and from Western and Northern Europe (the branch M). Applying single nucleotide differences in exons of gene *Vg* can to help to monitor and solve the problem of intraspecies hybridisation of honey bees.

Materials and methods

We collected the worker bees of *A.m.mellifera* from twelve isolates in Ural region of Russia from Bashkortostan Republic (Kagarmanovo, Kaga, Sermenevo, Galiakberovo, Yaumbaevo, Irgizly, Kustarevka, Sabanchi, Uyadybash) and from Permskii krai (Nytva, Porshakova 1, Porshakova 2) (Fig. 2).

Fig. 2. Geographic distribution isolates of honey bees in Ural region in Russia.

DNA was extracted from thoracic tissue using "DNA extran 2" kit (Syntol) (http://www.syntol.ru). PCR was conducted in 15 μL reactions containing 1 U Taq DNA polymerase (Syntol) 2-4 mM of $MgSO_4$, 200 μM of each dNTP, 0.5 μM of each primer (Kent et al. 2011) and 20-100 ng of DNA (Tab. 1). PCR amplification for the exones 2-7 of the gene *Vg* was performed as follows: 30 cycles at 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C. PCR purification and Sanger sequencing on an automatic sequencer using both forward and reverse primers carried out by Syntol.

All polymorphic sites were manually checked for errors. All sequences were aligned using MEGA 4.1 using the published *Apis mellifera* genome as a reference (LG4, NC_007073.3, AADG06005159.1) (http://hymenopteragenome.org/beebase). The single nucleotide polymorphisms (SNPs) analysed herein were discovered from the alignments as allelic differences between *A. mellifera* subspecies.

Table 1.

Primers for PCR of vitellogenin exons of honey bee *Apis mellifera* (Kent et al. 2011)

Vg exon 2	F	5'-tcttgttcgttccaggttcc-3'
	R	5'-gacagtttcagccgacttcc-3'
Vg exon3	F	5'-cctttcgatccattccttga-3'
	R	5'-gtcaaaacggattggtgctt-3'
Vg exon 4	F	5'-tcgaaggggaagaatttcaa-3'
	R	5'-acgagcaattcctcaacacc-3'
Vg exon 5	F	5'-gtcggacaatttcacgtcct-3'
	R	5'-gttcgagcatcgacacttca-3'
Vg exon 6	F	5'-agagccagggatacgtcaaa-3'
	R	5'-gagtcatctcgaggctcacc-3'
Vg exon 7	F	5'-ttctggctgaggtcaggatt-3'
	R	5'-aatttcgaccacgactcgac-3'

Results

Nucleotide sequences of the six exons (from 2 to 7) of the gene *Vg* of honey bees from the Ural region have been deposited in the GenBank: second exon (KJ572309 - KJ572320), third exon (KJ645883 - KJ645894), fourth exon (KJ572297 - KJ572308), fifth exon (KJ572285 - KJ572296), sixth exon (KJ532136 - KJ532147), seventh exon (KJ532124 - KJ532135). All analysed honey bees were homozygous for the gene *Vg*.

Comparative analysis of sequences of *Vg* of honey bees from Ural with reference sequence from GenBank was allowed us to found 18 transitions (90%) and 2 transversions (10%). In the second exon of *Vg* we are found 2 transitions, in the third exon - 3 transitions, in the fourth exon - 2 transitions, in the fifth exon - 4 transitions and 2 transversions, in the sixth exon - 1 transition and in the seventh exon - 6 transitions. All transversions of gene *Vg* were led to amino acid substitution from Ley to Arg and Ile to Ser. One of the 18 transitions was led to amino acid substitution from Ala to Thr. The deletion of 9 nucleotide long was found in honey bee from isolate Uyadybash. Same deletion was occurred in bees from GenBank JN557265, JN557266, JN557273, JN557274, JN557275, JN557276 (Tab. 2).

Table 2.

Sites of nucleotide substitution of the gene *Vg* of honey bees from Ural region relative to the reference sequence from GenBank

Gene *Vg*	Exon 2		Exon 3			Exon 4		Exon 5							Exon 6	Exon 7					
Polymorphic sites / Samples	526	574	1373	1418	1793	2443	2458	3978	4528	4533	4554	4555	4800	4812	5229	5608	5677	5680	5692	5878	5935
Reference sequence Vg GenBank	A	A	T	A	T	T	T	C	C	G	A	T	G	A	G	T	T	C	C	T	T
RB, Beloretskii, Kagarmanovo	G	G	C	A	T	C	C	C	C	G	A	T	A	A	G	T	T	T	T	T	T
RB, Beloretskii, Kaga	G	G	C	A	T	T	T	C	C	G	A	T	G	A	A***	-	C	T	T	T	C
RB, Beloretskii, Sermenevo	G	G	C	A	T	C	T	C	C	G	A	T	G	G	A***	C	C	T	T	T	C
RB, Burzyanskii, Galiakberovo	G	G	C	G	T	T	T	T	A*	G	A	T	A	A	A***	C	C	T	T	C	T
RB, Burzyanskii, Yaumbaevo	G	G	C	A	C	C	C	C	A*	C**	G	C	G	A	A***	C	C	T	T	C	T
RB, Burzyanskii, Irgizly	G	G	C	A	T	T	T	C	C	G	A	T	A	A	G	C	C	T	T	T	C
RB, Tatyshlinskii, Kustarevka	A	A	C	A	T	T	T	C	C	G	A	T	G	A	A***	C	C	T	T	C	T
RB, Tatyshlinskii, Sabanchi	G	G	C	A	T	T	T	C	C	G	A	T	G	G	A***	C	T	C	C	T	C
RB, Tatyshlinskii, Uyadybash	G	G	C	A	T	T	T	C	C	G	A	T	A	G	A***	C	C	T	T	T	C
PK,Krasnovisherskii, Porshakova 1	A	G	C	A	T	T	T	C	C	G	A	T	G	A	A***	C	C	T	T	T	T
PK,Krasnovisherskii, Nytva	G	G	C	A	T	T	T	C	A*	G	A	T	G	A	A***	C	C	T	T	C	T
PK,Krasnovisherskii, Porshakova 2	G	G	C	A	T	C	C	C	A*	C**	G	C	G	A	A***	C	C	T	T	C	T

RB – Bashkortostan republic; PK – Permskii krai. * - substitution of Ley to Ile; ** - substitution of Arg to Ser; *** - substitution of Ala to Thr. Symbol «-» no data gaps.

Table 3

The single nucleotide differences in the nucleotide sequence of the gene *Vg* of honey bees between subspecies of evolutionary branches M and C

Gene *Vg*	Exon 2			Exon 3					Exon 4					Exon 5							Exon 6					
Position / Branch	964	997	1039	1415	1460	1901	1970	1976	2788	2887	2888	2920	2938	3981	4242	4288	4316	4500	4508	4509	5114	5210	5225	5306	5321	5328
M	T	C	C	T	C	C	G	C	T	A	A	C	T	T	T	C	A	G	G	G	C	C	C	G	T	A
C	C	T	T	C	T	T	A	T	C	T	C	T	C	C	A	T	G	A	A	A	T	T	T	A	A	G

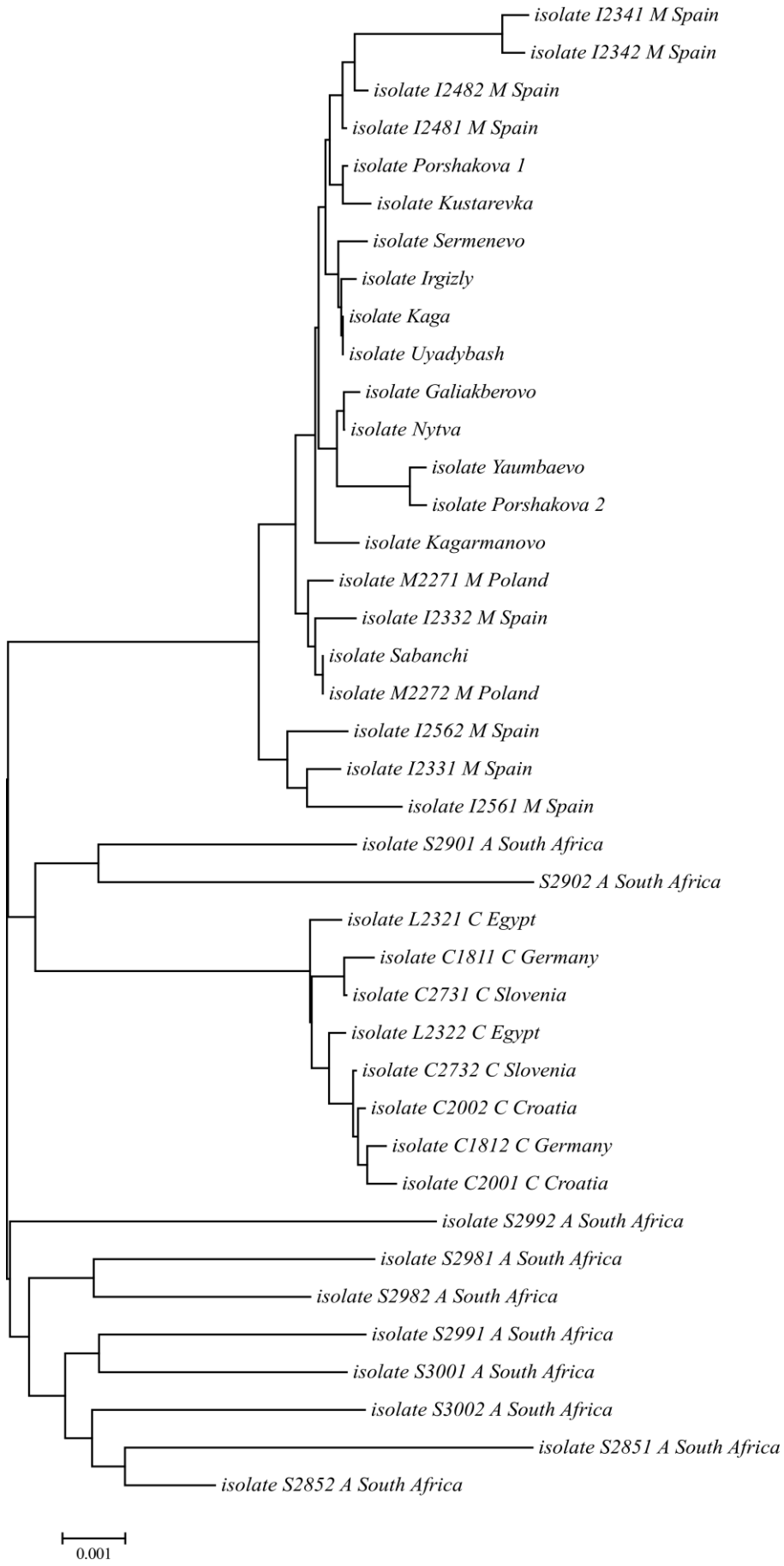

Fig. 3. Dendrogram of genetic relationship of honey bees of tree evolution branches based on comparative analysis of sequences of gene *Vg*.

Discussion

Comparative analysis of sequences of *Vg* of honey bees belonging to two evolutionary branches M and C found 26 single nucleotide differences (SNP) between them (Tab. 3). These 26 SNPs can be used to recognize *A.m.mellifera* from other honey bee subspecies of the evolutionary branch C. In the second exon of *Vg* there were 3 SNPs, in the third exon – 5 SNPs, in the fourth exon – 5 SNPs, in the fifth exon – 7 SNPs, in the sixth exon – 6 SNPs, in the seventh exon – none were found.

Comparative analysis of sequences from full length DNA of *Vg* of honey bees in evolutionary branches A, M and C had allowed us to construct the dendrogram. On the dendrogram honey bee isolates were differentiated into three major population groups of *A. mellifera* (Whitfield et al. 2006) from Africa (the branch A), from Mediterranean (the branch C) and from Western and Northern Europe (the branch M). The honey bee samples from Ural, Spain and Poland were clustered together and had belonged to the evolutionary branch M. The honey bee samples from Egypt, Germany, Croatia and Slovenia were clustered together under evolutionary branch C. Bees from South Africa were clustered separately from all groups under evolutionary branch A (Fig. 3). Only two samples of the honey bees from South Africa were grouped with honey bees of the evolutionary branch C that can be explained by hybrid origin of these samples.

Comparative analysis of the sequence of gene *Vg* of honey bees from the Ural has allowed us to cluster different honey bee subspecies into three groups similar to the earlier works (Ruttner 1988; Whitfield et al. 2006). This has also allowed us to find 26 SNP which can be used to differentiate *A.m.mellifera* and to help solve the problem of the intraspecific hybridization of the honey bees in the Russia and North European countries.

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НУКЛЕОТИДНЫЙ ПОЛИМОРФИЗМ ГЕНА *VG* ПЧЕЛЫ

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Аннотация

Сохранение генофонда темной лесной пчелы *A.m.mellifera* чрезвычайно важно для успешного развития пчеловодства Северной Евразии. Существуют множество методов дифференциации подвидов медоносной пчелы. Наше исследование основано на оценке однонуклеотидного полиморфизма (SNP) и использования его в качестве маркера для генотипирования медоносных пчел. Ген вителлогенина (Vg) участвует в регуляции общественной организации в семье медоносной пчелы. Мы просеквенировали шесть экзонов гена Vg темных лесных пчел уральской популяции и нашли уникальные для *A.m.mellifera* 26 SNP, которые могут быть полезны для генетической паспортизации семей.

Ключевые слова: пчела, *Apis mellifera mellifera*, вителлогенин, *Vg*, SNP, однонуклеотидный полиморфизм