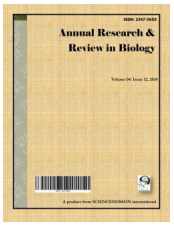

Annual Research & Review in Biology

28(5): 1-9, 2018; Article no.ARRB.42772
ISSN: 2347-565X, NLM ID: 101632869

Genetic Characterisation of Honey Bees (*Apis mellifera*) Populations from Kurdistan Region of Iraq via ISSR Markers

Kamal Mohammad-Said Ahmad[1*]

[1]Department of Animal Science, College of Agriculture - Kifri, Garmian University, Kalar, As Sulaymaniyah, KRG, Iraq.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/ARRB/2018/42772
Editor(s):
(1) Dr. Rajeev Kumar, Department of Veterinary Public Health & Epidemiology, Vanbandhu College of Veterinary Science & A. H, Navsari Agricultural University, Navsari, India.
(2) Dr. George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) S. Sheik Asraf, Kalasalingam Academy of Research and Education, Kalasalingam University, India.
(2) Marcos Siqueira, Universidade do Sagrado Coração, Brazil.
(3) António A. N. De Alcochete, Agostinho Neto University, Angola.
Complete Peer review History: http://www.sciencedomain.org/review-history/26339

Original Research Article

Received 02 June 2018
Accepted 21 August 2018
Published 22 September 2018

ABSTRACT

In the present study, to investigate genetic diversity and phylogenetic relationships among honey bee populations of Iraq ISSR markers were used. Sampling was carried out during summer 2017 from 5 cities of Iraq (Dahuk, Arbil, Sulaymaniyah, Kirkuk, and Kafri). Total DNA was extracted from the head and thorax sections of each worker honey bee, using salting out method with minor modifications. PCR amplification of genomic DNA was performed using 10 ISSR marker primers (A1, A2, A3, A4, A5, A6, A7, A8, A9 and A10). The primers yielded 50 polymorphic bands and number of bands were variable from 8-12 (average 9.62), and percentage of polymorphic loci was 73.6. The estimated genetic diversity for the populations ranged from 0.39 in Kafri population to 0.47 in Arbil population, and total genetic diversity among loci was calculated as 0.47 while average within population genetic diversity was 0.44. GST value was 0.085. The Phylogenetic tree showed two main clusters; the first one comprised of three populations (Dahuk, Arbil, and Sulaymaniyah), and the second one included two communities (Kirkuk and Kafri). Heterozygosity values, Shannon index and the number of alleles of honey bee populations were minimal that could be caused by low

**Corresponding author: E-mail: Kamalzangana52@gmail.com;*

definite geographic structure of honey bee populations. This research provided new information regarding genetic diversity in selected local honeybee in Kurdistan region of Iraq and will be useful for selection, future local biodiversity conservation and controlled breeding programs.

Keywords: ISSR markers; Iraq; genetic diversity; heterozygosity; honeybee.

1. INTRODUCTION

Honey bees (*Apis mellifera* L.) are the most economically valuable pollinators of crops worldwide [1-3]. One-third of the total human diet is dependent on plants which are pollinated by insects; predominately by honey bees [4]. The honey bees evolved and adapted to a large variety of climatic and ecological conditions and are an essential part of both our agricultural economy and the overall ecosystem including homeowners, wildlife, and anyone which interest in nature. In biology and biomedicine, honeybees are popular research model organisms in diverse areas including allergic disease, development, gerontology, neuroscience, social behaviour, and venom toxicology.

The western honey bee, *Apis mellifera* L., is native to Africa, the Near and Middle East, and Europe. Based on morphometric analysis, Ruttner [5] grouped honey bee subspecies into four geographic branches: a south and central African branch (A), a north African and west European branch (M), an East European and North Mediterranean branch (C), and a Near and Middle Eastern branch (O). Subsequent studies using morphometry, along with mitochondrial DNA and microsatellite marker analysis, have confirmed or modified the species distributions within these groups and have added a new Middle Eastern branch from Yemen (Y) [6-12]. Based on morphometrics, the Near Eastern subspecies, Anatolian (*A. m. anatoliaca*), Caucasian (*A. m. caucasica*) and Iranian (*A. m. meda*), had been grouped within the O branch [5,8,13]; however, mtDNA analysis showed that they, in fact, belonged to the C lineage [9-12,14-16]. After morphometric analyses, Ruttner [5] concluded that *Apis mellifera meda* exist north and northeast in Iraq. In morphological methods, a variety of proteins and DNA fingerprinting are used for determining the status and resolution of honey bee populations in this region [17].

The north and northeast cities, Dahuk, Arbil, Sulaymaniyah, Kirkuk, and Kafri of Iraq comprised an essential centre of honey production and provide a considerable amount of the country's honey needs. This region is a well known modernised bee keeping area in Iraq. These cities have well defined ecological characteristics, and because of favourable environmental conditions, they are major destinations for seasonal colony migrations from other regions of the country. Due to desirable and almost similar climates, there are fewer reciprocal colony migrations among these provinces, and it appears that these areas have their exclusive groups of honey bees. Because of the fundamental role of this area in the country's honey production, we focused our study mainly on these cities. These authors quoted that this species is native of Iraq and has similarities with *Apis mellifera ligustica*, *Apis mellifera anatolica* and honey bees in Iraq. There is no published report concerning the genetic diversity of honeybee populations of Iraq via ISSR marker. Presently, microsatellites play an important role in determining genetic diversity and kinship ties between animals, and especially insects [2]. Usually used ISSR markers for genetic diversity of plants and in the insects world now use these markers to assess the genetic diversity of Lepidopteran orders, especially from two families Noctuidae and Bombycidae, Order dipteral and Hymenoptera have been the focus of the scientific community [18-20]. Inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance between two identical microsatellite repeat regions oriented in the opposite direction. The technique uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter-SSR sequences of different sizes. The microsatellite repeats used as primers can be di-nucleotide, tri-nucleotide, tetranucleotide or penta-nucleotide. The primers used can be either unanchored [21-23] or more usually anchored at 3’ or 5’ end with 1 to 4 degenerate bases extended into the flanking sequences [24]. This study explores the usefulness of Inter Simple Sequence Repeat (ISSR) molecular marker to identify and discriminate several populations of honey bee *Apis mellifera* L. from Iraq. The goal of the present study was to investigate the genetic diversity and phylogenetic relationship between honey bee populations of the five cities

of Iraq via the inter-simple sequence repeats (ISSR) marker.

2. MATERIALS AND METHODS

2.1 Honey Bee Samples

A total of 100 adult worker honey bees from 100 colonies of *Apis mellifera meda* were sampled from 10 different localities distributed in Dahuk, Arbil, Sulaymaniyah, Kalar, and Kafri cities of Kurdistan region of Iraq during summer 2017 (Fig. 1.) Samplings were carried out from honey bee colonies of most active apiaries, two regions were chosen in each city, and one apiary was selected in each region (10 worker honey bees per region). Honey bees were collected directly from the comb and were stored in 96% ethanol and kept at –20°C until DNA extraction.

2.2 DNA Extraction

Total DNA was extracted from the thorax and head sections of each worker honey bee, using salting out method described by Aljanabi and Martinez [25] with minor modifications.

2.3 PCR Amplification

PCR amplifications were performed in 20 µl volume containing 40 - 80 ng extracted DNA, 2.5 µl reaction buffer 1X, 400 µM of mix dNTP, 1 µM of each primer and 1 Unit of *Taq* DNA polymerase. Ten suitable primers were used for PCR reaction (Table 2). The PCR schedule adopted was 1 cycle of 94°C for 3 min, followed by 45 cycles of 94°C for 45 s, 51°C for 1 min, 72°C for 3 min and a final extension of 15 min at 72°C. The PCR products were loaded on 1.5%

Fig. 1. Geographical locations of 10 region of 5 cities in Kurdistan region of Iraq. N is the number of sampled bees per population

Table 1. DNA sequence of ISSR primers

No	Primer	Sequence 5'_3'
A_1	$(AGAC)_4GC$	5'-AGACAGACAGACAGACGC-3'
A_2	$AC(GACA)_4$	5'-ACGACAGACAGACAGACA-3'
A_3	$(GACA)_4GT$	5'-GACAGACAGACAGACAGT-3'
A_4	$(GACA)_4CT$	5'-GACAGACAGACAGACACT-3'
A_5	$(ATG)_5GA$	5'-ATGATGATGATGATGGA-3'
A_6	$(TCC)_5GT$	5'-TCCTCCTCCTCCTCCGT-3'
A^7	$(CTC)_5GT$	5'-CTCCTCCTCCTCCTCGT-3'
A_8	$(AC)_8G$	5'-ACACACACACACACACG-3'
A_9	$(CT)_8A$	5'-CTCTCTCTCTCTCTCTA-3'
A_{10}	$(AC)_8AT$	5'-ACACACACACACACACAT-3'

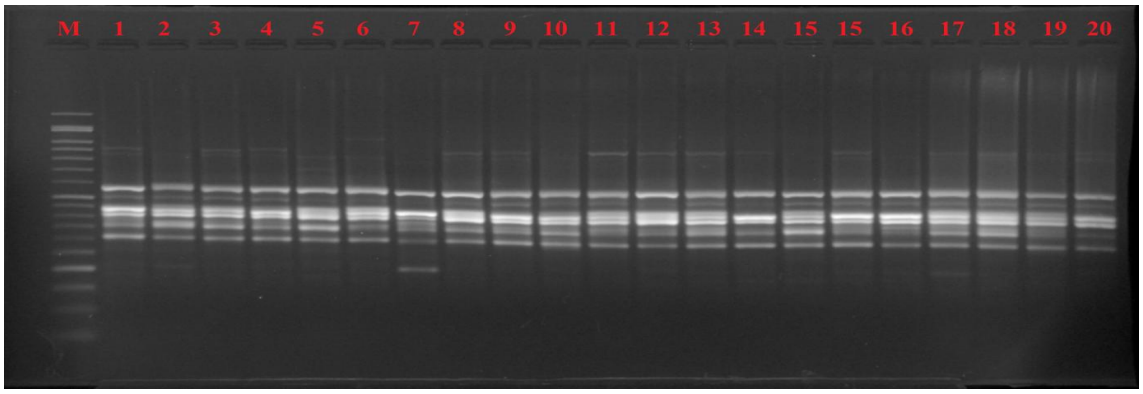

Fig. 2. DNA fragments profile of the honey bee population genotypes amplified with ISSR primer (Primer A6). Namely; (M): size molecular marker 100 base-pair ladder, (1-20): samples of honey bee (1-10 (samples of Kafri city) and 11-20 (samples of Kalar city))

agarose gel in Tris-boric acid/EDTA buffer (1X TBE) and electrophoresis was carried out with a constant voltage of 80 V for 3 h. The gel was stained with ethidium bromide (0.6 g/ml) and photographed with the gel documentation system (Fig. 2) for scoring as present (1) and absent (0) same other dominant molecular markers. The POPGEN software version 1.31 [26] was used for estimating population parameters. Cluster analyses were conducted using UPGMA (Unweighted pair group method with arithmetic mean) and the resulting cluster was expressed as dendrogram using TREEVIEW [27] software. An analysis of molecular variance (AMOVA) was performed using GENALEX 6.3 [28] to partition the total molecular variance between and within populations. The significance level was obtained via permutation test (n = 1000). Population groups were also defined according to the principal coordinate analysis (PCoA).

3. RESULTS

One hundred honey bee genotypes were used for DNA genotyping. These samples were selected according to different geoclimatic location. All used primers showed polymorphism in amplified loci (Table 2). The total number of detected fragments was 90 with an average of 9 fragments per primer, and the range was between 6 to 12 fragments per primer (Table 2). 50 loci were found to be polymorphic and showed an average of 55.52% polymorphism. Primer No. A_6 exhibited the highest number of polymorphic fragments (7 fragments). Primers No. A_1 showed the lowest number of polymorphic fragments. Sizes of the polymorphic bands approximately ranged from 100 bp to 850 bp. Gene diversity (H_E) ranged from 0.287 to 0.35, and total gene diversity among loci was calculated as 0.376 while within a population average genetic diversity was 0.315. *Gst* value was 0.085 among the studied honey bee populations. Shannon indices (*I*) were also calculated to find genetic variation within the population. It ranged from 0.36 for Kafri population to 0.52 for Arbil population, and the average of gene flow (*Nm*) was 4.98 (Table 3). The Genetic distance of honey bee populations according to Nei's (1978) unbiased genetic

distances between populations indicated that Kafri and Kalar populations had the closest distance, while the Dahuk population exhibited a distinct location from the other four populations (Table 4). Cluster analysis using UPGMA method classified honey bee populations into two main groups. The first group included the honey bees collected from Dahuk, Arbil and Sulaymaniyah cities. The honey bees from Kalar and Kafri cities represented the second group (Fig. 3). The AMOVA analysis revealed that 95% of the molecular variance among individuals was found within a single population (Table 5), whereas 5% was due to the differences between populations. Results from PCoA showed that populations are basically separated into two groups by PCo 1 (Fig. 4).

Table 2. Primers used for the analysis of the genetic diversity of honey bee populations of Iraq using ISSR Markers, indicating their sequences, number of polymorphic bands and percentage of polymorphism

No	Primer	Total number of fragments	Number of polymorphic fragments	Percent of polymorphic fragments	Range of fragment sizes (bp)
A_1	(AGAC)4GC	6	3	50	110-700
A_2	AC(GACA)4	9	5	55.5	100-300
A_3	(GACA)4GT	8	3	37.5	140-850
A_4	(GACA)4CT	9	5	55.5	100-340
A_5	(ATG)5GA	10	6	60	250-400
A_6	(TCC)5GT	12	7	58.33	200-450
A^7	(CTC)5GT	9	5	55.5	180-500
A_8	(AC)8G	8	6	75	200-430
A_9	(CT)8A	8	5	62.5	250-500
A_{10}	(AC)8AT	11	5	45.45	140-450
Total	-	90	35		-
Mean		9	3.5	55.52	170-490

Table 3. Population parameters of honey bee in this study

Primers	H_E	Shannon index (I)	average *Gst*	average gene flow (*Nm*)
A1	0.3	0.4		
A2	0.31	0.41		
A3	0.287	0.37		
A4	0.31	0.41		
A5	0.34	0.49		
A6	0.32	0.41		
A7	0.31	0.4		
A8	0.35	0.52		
A9	0.341	0.5		
A10	0.29	0.36		
Average	0.315	0.412		
Average *Gst*	0.384		0.085	
Average gene flow (*Nm*)				4.98

Table 4. Nei's (1978) unbiased measures of genetic distance

Population	Kafri	Kirkuk	Sulaymaniyah	Arbil	Dahuk
Kafri	***				
Kirkuk	0.033	***			
Sulaymaniyah	0.042	0.058	***		
Arbil	0.052	0.068	0.053	***	
Dahuk	0.048	0.081	0.050	0.065	***

Table 5. Analysis of molecular variance (AMOVA) for RAPD variation

Source of variation	df	Sum of squares	Estimated variance	Variance components (%)
Among populations	15	15.23	0.098	5
Within populations	85	154.96	1.69	95
Total	99	171.65	1.89	100

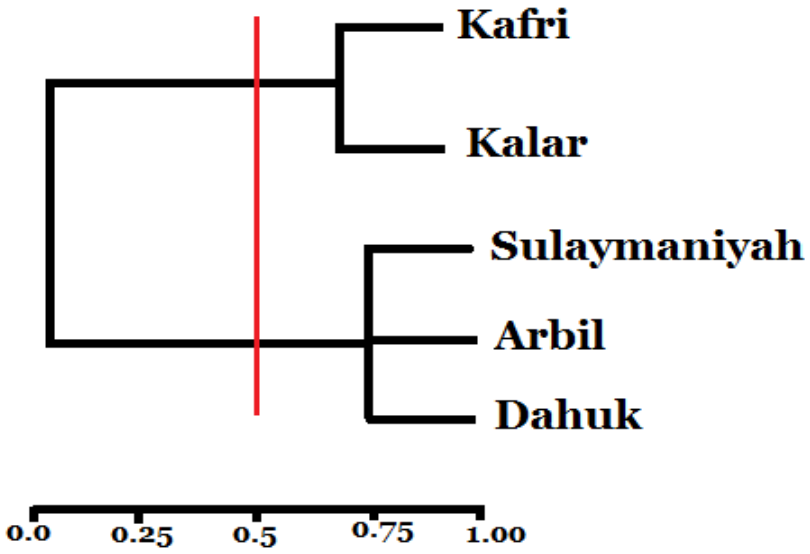

Fig. 3. Dendrogram generated based on UPGMA method between five honey bee populations Kurdistan region of Iraq

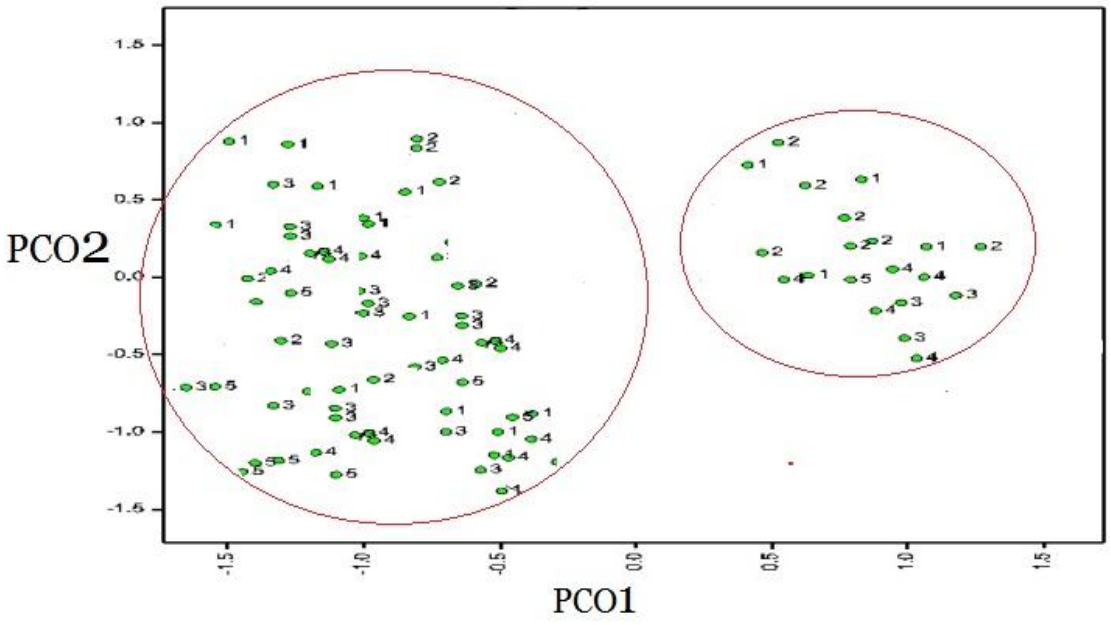

Fig. 4. Distribution of five populations accessions by 2-dimensional principal coordinate analysis (1-Dahuk, 2-Arbil, 3- Sulaymaniyah, 4-Kalar, and 5-Kafri)

4. DISCUSSION

There is one local subspecies of honey bee in the north and northeast Kurdistan region of Iraq, *Apis mellifera meda*, which during the past thousands years of Iraqian civilization has adapted itself with different climatic conditions and plant flora varieties of the Middle East. This subspecies has also acquired a specific resistance against diseases and pests which attack other honey bees [3,17,29,30]. The results of study revealed that the ability of honey bee to adapt to environmental changes depends significantly on the genetic diversity in the honey bee. Effective conservation of a vulnerable species depends mainly on the knowledge of patterns of genetic variation. In this study, we analysed the genetic diversity of five honey bee populations obtained from distinct geographic areas in Kurdistan region of Iraq. Several methods based on PCR have been developed for genetic analysis of *Apis mellifera* L. in neighbour countries such as Iran [5,6,29-31]. ISSR markers require no genomic structure information on the studied material, and the binding sites of ISSR markers are randomly distributed on the DNA, so their use is appropriate to the study of the genetic diversity [29]. Consequently, ISSR markers are one of the best methods for the assessment of genetic diversity among populations in species where little molecular genetic information is available. Although ISSR markers are sensitive to several reaction factors, it is quite useful when used with caution. Cluster analysis showed that studied honey bee populations formed two groups, one of these groups consisted of populations of Kalar and Kafri cities, while the other group comprised of populations of Dahuk, Arbil, and Sulaymaniyah. The results of UPGMA dendrogram indicated that the most genetic differentiation was between Kafri and Arbli honey bee populations. It was consistent with the geographic proximity of the populations. The results showed that the Kafri honey bee population had the lowest level of heterozygosity while the honey bees in the Arbil had not a low variability regarding a number of alleles per locus and heterozygosity. The average gene diversity (H_E) of 0.315 and mean Shannon indices (I) of 0.412 indicated that the genetic variation within populations are low. Pairwise Genetic distance estimates based on Nei's (1978) unbiased genetic distances (ranging from 0.033 to 0.081) was not highly significant concerning genetic diversity among various populations of *Apis mellifera* L. Analysis of molecular variance (AMOVA) showed that genetic variation within populations was higher than among populations. Genetic diversity within populations is highly essential for the adaptation to changing environments and, as a consequence, for long-term survival of a species.

The dendrogram based on UPGMA method exhibited the ability of the ISSR markers to detect the genetic variability between and within the honey bee populaion used in this study and to identify groups and subgroups with different levels of genetic distance. Also, the results of this study showed that it is possible to distinguish the honey bee races to carry out phylogenetic studies and to select lines for the highest genetic diversity using this type of marker. Finally, ISSR markers could be used in molecular marker-assisted breeding programs. Khemakhem et al. [32] and Shouhani et al. [33] indicated that ISSR can be useful as DNA-based molecular markers for studying genetic diversity and phylogenetic relationships of Mayetiola and honey bee haplotypes. This could be due to bees across the successive migrations are adjacent to the provinces although sampling performed from a beekeeper that never has moved out of the province. The bee colonies traded popular among beekeepers in every city and county together.

5. CONCLUSION

It can be concluded that the ISSR markers in this study could properly grouped the study populations based on their geographical distribution. The results of this study have revealed that genetic diversity of honey bee populations in Kurdistan region of Iraq is low, so a regular program for breeding is required. This study has provided new information about genetic diversity in selected local honeybee in Kurdistan region of Iraq and will be useful for selection, future local biodiversity conservation and controlled breeding programs.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Oldroyd BP, Nanork P. Conservation of Asian honey bees. Apidologie. 2009;40: 296–312.
2. Rahimi A, Miromayedi A, Kahrizi D, Abdolshahi R, Kazemi E, Yari KH. Microsatellite genetic diversity of *Apis mellifera meda* skorikov. Molecular Biology Reports. 2014;41:7755–7761.
3. Rahimi A. Study of the genetic diversity of Iranian honey bee (*Apis mellifera meda* Skorikow, 1829) populations using the mtDNA COI–COII intergenic region. Biologija. 2015;1-6.
4. McGregor S. Insect pollination of cultivated crop plants, Agricultural handbook. U.S. Government Printing Office, Washington, DC. 1976;496.
5. Ruttner F. Biogeography and taxonomy of honeybees. Springer, Berlin. 1988;284.
6. Hall HG, Smith DR. Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America, 15 December, University of Chicago, United States. 1991;4552-4558.
7. Arias MC, Sheppard WS. Molecular phylogenetics of honey bee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequence. Molecular Phylogenetic Evoluation. 1996;5:557–566.
8. Kauhausen-Keller D, Ruttner F, Keller R. Morphometric studies on the micro-taxonomy of the species *Apis mellifera* L. Apidologie. 1997;28:295–307.
9. Smith DR, Slaymaker A, Palmer M, Kaftanolu O. Turkish honeybees belong to the east Mediterranean mitochondrial lineage. Apidologie. 1997;28:269–274.
10. Palmer MR, Smith DR, Kaftanolu O. Turkish honeybees: Genetic variation and evidence for a fourth lineage of *Apis mellifera* mtDNA. Journal of Heredity. 2000;91:42–46.
11. Franck P, Garnery L, Solignac M, Cornuet JM. Molecular confirmation of a fourth lineage in honeybees from the near east. Apidologie. 2000;31:167–180.
12. Franck P, Garnery L, Loiseau A, Oldroyd BP, Hepburn HR, Solignac M, Cornuet JM. Genetic diversity of the honey bee in Africa: Microsatellite and mitochondrial data. Heredity. 2001;86:420–430.
13. Adl MBF, Gençer HV, Firatli Ç, Bahreini R. Morphometric characterization of Iranian (*Apis mellifera meda*), Central Anatolian (*Apis mellifera anatoliaca*) and Caucasian (*Apis mellifera caucasica*) honey bee populations. Journal of Apicultural Research. 2007;46:225–231.
14. Özdil F, Yildiz MA, Hall HG. Molecular characterization of Turkish honey bee populations (*Apis mellifera* L.) inferred from mitochondrial DNA RFLP and sequence results. Apidologie. 2009a;40:570–576.
15. Özdil F, Fakhri B, Meydan H, Yildiz MA, Hall HG. Mitochondrial DNA variation in the CoxI–CoxII intergenic region among Turkish and Iranian honey bees (*Apis mellifera* L.). Biochemical Genetics. 2009b;47:717–721.
16. Bouga M, Alaux C, Bienkowska M, Büchler R, Carreck NL, Cauia E, Chlebo R, Dahle B, Dallolio R, De La Rúa P, Gregorc A, Ivanova E, Kence A, Kence M, Kezic N, Kiprijanovska H, Kozmus P, Kryger P, Le Conte Y, Lodesani M, Murilhas AM, Siceanu A, Soland G, Uzunov A, Wilde J. A review of methods for discrimination of honey bee populations as applied to European beekeeping. Journal of Apicultural Research. 2011;50:51–84.
17. Ruttner F, Tassencourt L, Louvaux J. Biometrical statistical analysis of the geographic variability of *Apis mellifera* L. Apidologie. 1978;9(4):363-381.
18. Luque C, Legal L, Staudter H, Gers C, Wink M. ISSR (Inter Simple Sequence Repeats) as genetic markers in Noctuids (Lepidoptera). Hereditas. 2002;136:251-253.
19. Hundsdoerfer AK, Kitching IJ, Wink M. The phylogeny of the *Hyles euphorbiae* complex (Lepidoptera: Sphingidae): Molecular evidence from sequence data and ISSR-PCR fingerprints. Organisms Diversity & Evolution. 2005;5:173-198.
20. Radjabi R, Sarafrazi A, Tarang A, Kamali K, Tirgari S. Intraspecific biodiversity of Iranian local races of silkworm *Bombyx* by ISSR (Inter-Simple Sequence Repeat) molecular marker. World Journal of Zoolology. 2012;7(1):17-22.
21. Gupta M, Chyi YS, Romero-Severson J, Owen JL. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theoretical and Applied Genetics. 1994;89:998-1006.
22. Meyer W, Mitchell TG, Freedman EZ, Vilgays R. Hybridization probes for conventional DNA fingerprinting used as

single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. Journal of Clinical Microbiology. 1993;31:2274-2280.
23. Wu K, Jones R, Dannaeberger L, Scolnik PA. Detection of microsatellite polymorphisms without cloning. Nucleic Acids Research. 1994;22:3257-3258.
24. Zietkiewicz E, Rafalsk A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics. 1994;20: 176-183.
25. Aljanabi SM, Martinez I. Universal and rapid salt extraction of high quality genomic DNA for PCR–based techniques. Nucleic Acids Research. 1997;25:4692–4693.
26. Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. POPGENE, the Uswe-friendly Shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta; 1997.
27. Page RDM. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences. 1996;12:357–358.
28. Peakall R, Smouse PE. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 2006;6:288–295.
29. Rahimi A, Mirmoayedi A, Kahriz D, Zarei L, Jamali S. Genetic diversity of Iranian honey bee (*Apis mellifera meda* Skorikow, 1829) populations based on ISSR markers. Cellular and Molecular Biology. 2016; 62(4):53-58.
30. Rahimi A, Mirmoayedi A, Kahriz D, Zarei L, Jamali S. Morphological diversity and phylogenetic relationships study of Iranian subspecies honey bee (*Apis mellifera meda*) populations via morphological characteristics. Sociobiology. 2017;64(1): 33-41.
31. Royan M, Rahim G, Esmaeilkhanian S, Mirhoseini S, Ansari Z. A study on the genetic diversity of the *Apis mellifera meda* population in the south coast of the Caspian Sea using microsatellite markers. Journal Apiculture Reserach. 2007;46: 236–241.
32. Khemakhem MM, Marrakchi M, Makni H. Genetic diversity of *Mayetiola destructor* and *Mayetiola hordei* (Diptera: Cecidomyiidae) by inter-simple sequence repeats (ISSRs). African Journal of Biotechnology. 2005;4:601-606.
33. Shouhani H, Dousti A, Radjabi Zarei M. Application of ISSR to study the genetic diversity of honeybee (*Apis mellifera* L.) populations in some areas of Iran. Journal of BioScience and Biotechnology. 2014;3(2):127-131.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26339