Journal of Wildlife and Biodiversity 2(2): 37-46 (2018)
by Arak University, Iran (http://jwb.araku.ac.ir/)
DOI: 10.22120/jwb.2018.86877.1026
Research Article

Genetic diversity evaluation of Persian honeybees (*Apis mellifera meda*) in North West of Iran, using microsatellite markers

Taghi Ghassemi-Khademi, Hassan Rajabi-Maham*, Shahrokh Pashaei-Rad

Department of Animal Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C., Evin, Tehran 1983963113, IR Iran. *email: h_rajabi@sbu.ac.ir

Received: 25 May 2018 / Revised: 18 June 2018 / Accepted: 20 June 2018 / Published online: 20 June 2018. Ministry of Sciences, Research and Technology, Arak University, Iran.

Abstract

Using eight microsatellite markers and PCR method, the polymorphisms of three Persian honeybee, *Apis mellifera meda,* populations in North West of Iran investigated and the genetic structure and diversity of populations explored. Total genomic DNA was extracted from thoraces of honeybees. PCR was conducted using eight pairs of microsatellite primers and its products were detected on 4% Nusieve/agarose. In total, seven, five and four microsatellite polymorphic loci were found in Ardabil, A. sharqi and A. gharbi populations, respectively and 42 alleles observed in 30 analyzed samples. A. sh honeybees showed high level of heterozygosity (0.563) and the lowest estimate was 0.438 for A. gh honeybees. Among three studied populations, Ardabil bees were containing maximum private alleles (17 alleles). Maximum genetic resemblance report was between Ardabil and A. sh corresponding to their geographical vicinity. In total, we detected low genetic divergence among honeybee populations of Iranian North West inferred from estimated total F_{ST}.

Keywords: *Apis mellifera meda*, microsatellite, genetic diversity, heterozygosity, northwestern Iran.

Introduction

In Middle East, the most important honeybee races are *Apis mellifera syriaca*, *A. m. adami*, *A. m. anatolica*, *A. m. cypria*, *A. m. caucasica*, *A. m. armeniaca* and *A. m. meda*. The last one, *Apis mellifera meda,* was first described by Skorikov (1929) based on the abdominal sternites shape and tongue length which has similarities with *A. m. ligustica*, *A. m. anatolica* and honeybees in the north of Iraq (Ghassemi-Khademi 2017a). The origin of Persian honeybee, *Apis mellifera meda*, is in Alburz Mounts and subsequently it extended toward west colonising the southeastern parts of Turkey then they passed to the north of Iraq and Syria. This subspecies is colonising one of the largest territory among all subspecies and show several sub-communities throughout Iran. Genetic variation extension in Persian honeybee populations proposes a beginning of its taxonomic radiation in this region (Ghassemi-Khademi 2017a).

Microsatellites are the most prevalent DNA markers recently using to assess animals population structure and diversity because of their unique characteristics as: random distribution overall the genome, high polymorphism, locus specificity and co-dominant heredity. In bees, microsatellites were characterized first in *Apis mellifera* and *Bombus terrestris* and then applied widely to study the population structure and diversity of *Apis mellifera* subspecies (Alhamlan 2007; DelaRua *et al*. 2002; Franck *et al*. 2000; Shaibi and Moritz 2010; Ting *et al*. 2008; Techer *et al*. 2015; Rahimi *et al.* 2014). Large samples (200-750 workers) are need in morphometry to determine genetic structure within an evolutionary lineage of honeybees, but 20-30

unrelated workers applying even 7 microsatellite loci are sufficient to reach the same level of resolution (Bodur 2005; Ghassemi-Khademi 2017a). There is no published report concerning the genetic diversity of honeybees in Azarbaijan part of Iran, whereas this region contains the majority of colonies and is the greatest center for apiculture and production of honey in Iran. The objective of this paper is to investigate the genetic variation of *A. m. meda* in northwest of Iran, using the microsatellite DNA markers. Consequently, we are interested in providing some theoretical basic for conservation and improvement apicultural industry in this region.

Material and methods

Sampling and DNA extraction

Adult honeybee workers were collected from 72 colonies placed in 26 different stations from northwest of Iran during July-August 2010. The sample sites and geographical distribution of collected colonies are shown in Figure 1. Samples are stored in absolute ethanol and kept at -20 ^{o}C until processing in the laboratory. Subsequently, 30 colonies were selected and a single honeybee worker per colony used for microsatellite analysis (N=30). The geographical coordinates and other information about selected colonies are given in Table 1. Total DNA was extracted from thoraces using the modified CTAB method (Asadi 2009). The quantity and quality of extracted DNA was assayed using Nanodrop spectrophotometer (ND1000, USA) and 1% agarose gel electrophoresis.

Microsatellite PCR amplification

The microsatellite DNA markers for eight microsatellite loci (Table 2) applied to explore the genotypes of each sample. The sequence of eight following primers were obtained from previous studies: FM2, ED1, DJ1, CD and IM A107, A113 and A76 (Ghassemi-Khademi 2017b). The conditions of polymerase chain reaction (PCR) amplification were 50 ng of genomic DNA, 3.0 µl of 10x buffer, 1.5 – 1.9 µl of 25 mmol/l $MgCl_2$ (Table 2), 1.5 µl of 10 mmol/µl dNTP, 1 µl of 10 pmol/µl each primer, 0.2 µl of 5 U/µl Taq DNA polymerase and final volume of PCR mix was 30 µl. Amplifications consisted of an initial 3 min of denaturation (94 °C) followed by 35 cycles of denaturation at 94 °C for 50 s, annealing at the optimal temperature (table 2) for 60 s, extension at 72 °C for 60 s and with a final extension at 72 °C for 10 min. After amplification, a 10 µl aliquot of the amplified microsatellite samples was combined with 3 µl of a loading buffer (0.4% bromo-phenol blue, 0.4% xylene cyanol and 5ml of glycerol) and was analyzed directly by electrophoresis through standard, horizontal and non-denaturing 4% agarose gels. The gels consisted of 3% NuSieve agarose (The high-resolution agarose)/ 1% Euromedex agarose. After electrophoresis, the gels were visualized by ethidium bromide staining. Allelic frequencies and number of alleles per locus were estimated by direct counting from observed genotypes.

Figure 1. Map of North West of Iran (Azarbaijan region) showing sampling locations of *Apis mellifera meda* (marked with black circles).

Table 1. Sampling locality, geographical coordinate and migration data of 30 analyzed honeybees selected from 30 apiaries in North West of Iran.

N	Province	Location	Coordinates	Altitude (Meters)	Direction of migration
1	Ardabil	Heiran	38° 24' 2N, 48° 36' 19E	896	Without migration
2	Ardabil	Namin, Dudaran	38° 24' 49N, 48° 30' 3E	1413	Without migration
3	Ardabil	Hir, Hesar	38° 4' 2N, 48° 25' 18E	1638	Without migration
4	Ardabil	Hir, Hesar	38° 4' 2N, 48° 25' 18E	1638	Without migration
5	Ardabil	Hir, Hir	38° 4' 43N, 48° 30' 18E	2006	North of Iran
6	Ardabil	Nir, Koraim	37° 57' 18N, 48° 14' 8E	1482	Without migration
7	Ardabil	Jafarabad	39° 38' 49N, 48° 2' 23E	32	North of Iran
8	Ardabil	Ardabil	38° 14' 59N, 48° 17' 36E	1338	Without migration
9	Ardabil	Sardabeh	38° 16' 60N, 48° 2' 9E	1987	North of Iran
10	Ardabil	Sardabeh	38° 16' 60N, 48° 2' 9E	1987	Without migration
11	Ardabil	Sarein, Shayeq	38° 8' 30N, 47° 59' 17E	1890	Without migration
12	Ardabil	Sarein, Shayeq	38° 8' 30N, 47° 59' 17E	1890	Without migration
13	Ardabil	Sarein, Hakim qeshlaqi	38° 8' 19N, 48° 10' 45E	1485	North of Iran
14	Ardabil	Meshgin, Lahrood	38° 30' 39N, 47° 49' 56E	1282	Without migration
15	Ardabil	Kosar, qaraqeshlaq	37° 46' 4N, 48° 18' 27E	1372	Without migration
16	Ardabil	Kosar, kerendeq	37° 47' 7N, 48° 22' 17E	2063	North of Iran
17	Ardabil	Khalkhal	37° 37' 8N, 48° 31' 33E	1770	Rarely to north of Iran
18	A. sh	Ahar	38° 28' 17N, 47° 3' 27E	1337	Inside of Province
19	A. sh	Sarab, Cenziq	37° 54' 55N, 47° 38' 59E	1677	Without migration
20	A. sh	Sarab, Cenziq	37° 54' 55N, 47° 38' 59E	1677	Inside of Province
21	A. sh	Hashtrood, Aliabad	37° 27' 16N, 47° 6' 3E	1648	Inside of Province
22	A. sh	Bostanabad, Anbardan	37° 51' 55N, 47° 56' 23E	1962	Inside of Province
23	A. sh	Azarshahr	37° 45' 51N, 46o 0' 2E	1442	Inside of Province
24	A. sh	Marand, Arbatan	38° 32' 11N, 45o 37' 40E	1160	Inside of Province
25	A. sh	Myaneh, Arbat	37° 25' 45N, 47o 47' 8E	1100	Without migration
26	A. gh	Khoi, Chavoshqoli	38° 30' 28N, 44° 52' 17E	1168	Without migration
27	A. gh	Salmas, Haftvan	38° 10' 3N, 44° 45' 23E	1392	South of Iran
28	A. gh	Urumia, Sero	37° 43' 39N, 44° 38' 34E	1634	South of Iran
29	A. gh	Bookan	36° 31' 16N, 46° 12' 32E	1371	Rarely to southwest of Iran
30	A. gh	Sardasht, Abbasabad	36° 11' 50N, 45° 25' 20E	1609	Without migration

Statistical analysis

As it is evident in Table 1, collected colonies from Ardabil and A. gharbi provinces don't have any migration or if they are migrant, their direction of migrations are toward the north of Iran (Ardabil) or south and southwest of Iran (A. gharbi). In addition, most of the collected colonies from A. sharqi had migrations inside of their province (except two colonies without migration). Therefore, we could to determine three honeybee populations based on direction of migration and lack of migration, and in this study, we compared these three populations genetically. Numbers and frequencies of alleles and private alleles were calculated with GenAlex 6 (Peakall and Smouse 2006). Observed heterozygosity (Ho) and expected heterozygosity (He) for polymorphic loci was

estimated using Arlequin ver. 3.5.1.2 program (Quintana-Murci *et al.* 2004). In addition, Significance of departures from Hardy-Weinberg Equilibrium (HWE) were tested using Hardy-Weinberg equilibrium (HWE) option of Arlequin program. The tests are performed by examining the null hypothesis that assumes random association of gametes as described by Guo and Thompson, 1992 (Nasidze *et al.* 2005). In addition, pairwise F_{ST} values were obtained using population comparisons option of Arlequin program. The F_{ST} levels between zero and 0.05 refer to a little genetic differentiation and between 0.05 and 0.15 indicate moderate level genetic differentiation (Al-Zahery *et al.* 2013). The inbreeding coefficients, F_{IS} and $F_{IT,}$ give deviations from Hardy-Weinberg equilibrium within subpopulations and within the total population, respectively. Positive values indicate a deficit and negative values indicate an excess of heterozygote individuals (Bodur 2005). F_{ST}, F_{IS} and F_{IT} measures of the total population composed of 3 honeybee populations for each of eight microsatellite loci were calculated using Genepop software (Aflakian *et al.* 2007a). Nei's original measures of genetic identity and distance (Aflakian *et al.* 2007b), the Shannon information index (i) were calculated. Population structure using genotype data was depicted using software STRUCTURE (Cardoso *et al.* 2013). Finally, tridimensional figure of differentiation three studied populations was depicted by Genetix software (Fraumene *et al.* 2006).

Table 2. Primer sequences (5'-3'), $MgCl_2$ concentrations (mmol/l) and TM temperatures (°C) used in this study.

Locus	Primer Sequence	$MgCl_2$	Tm
A76	F-GCCAATACTCTCG AACAATCG	1.5	58
	R- GTCCAATTCACATGTCGACATC		
A107	F-CCGT GGGAGGT TTATTGTCG	1.5	60
	R-CCTTCGTAACGGATGACACC		
A113	F-CTCGAATCGTGGCGTCC	1.7	60
	R-CCTGTATTTTGCAACCTCGC		
CD	F-CAGAAATATTTCCATATAC	1.7	54
	R-CTTTATCCACGCTTTGAGC		
DJ1	F-TTCGAGATTCTTCGATGGGGC	1.5	57
	R-AAGATTATTTCTTATCATTAAGC		
ED1	F-CAACAGCCGTGAACGCTATC	1.9	57
	R-TCATCGTGTACCAATAACG		
FM2	F-ATTCCCGGTATCATCTCTTG	1.8	56
	R-AATTCGTGGTTAAATTCAAAG		
IM	F-ACGCAAATGACAAGTATTAG	1.5	56
	R-GAGTGTATTTCGAAATCGATG		

Results

DNA isolation and genotyping

The CTAB method was more suitable and adequate method for DNA extraction from *Apis mellifera* tissues, among different methods. In addition, employing 4% agarose (3% NuSieve agarose/ 1% agarose), we separated clearly alleles of microsatellite markers with a size difference by at least 10%. Figure 2 shows the separation pattern of different alleles of the polymorphic microsatellite marker, IM, after performing electrophoresis on this gel.

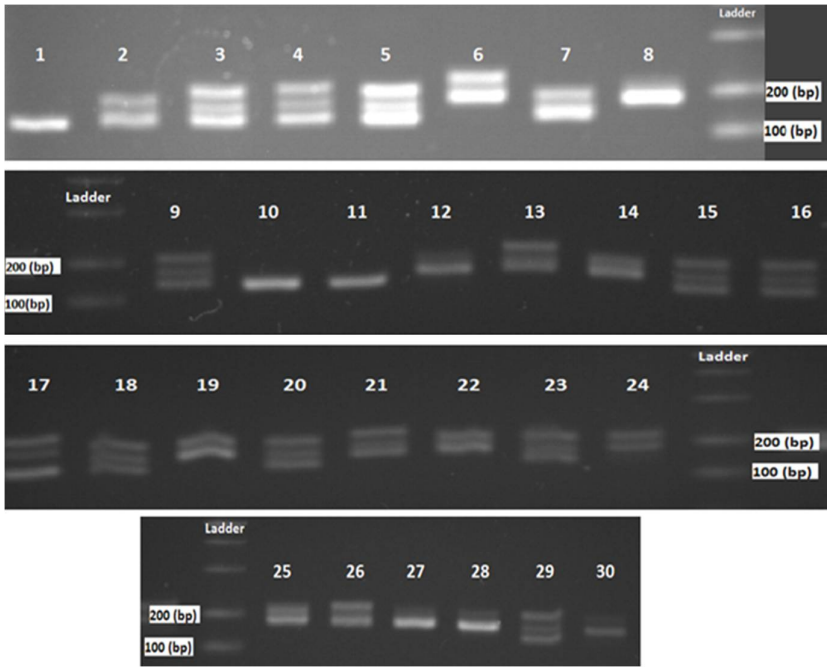

Figure 2. Gel electrophoresis banding pattern of 30 studied honeybees generated by polymorphic microsatellite marker, IM; Samples bands pattern from 1 to 30 and Ladder bands correspond to DNA Ladder marker

Genetic variation

A total of 42 alleles were detected in this study. Seven, five and four microsatellite loci were polymorphic in Ardabil, A. sharqi and A. gharbi, respectively. Ardabil and A. sharqi were containing 17 and one private alleles. The number of alleles per locus ranged from 1 (ED) to 11 (IM) and the average number of the observed alleles was 5.25. Highest and lowest number of effective alleles were 6.92 and 1 for A107 and ED loci, respectively (Table 3). Expected heterozygosity and Shannon information index (i) were containing maximum values for IM and A107 loci (Table 3). The low expected heterozygosity, the calculating polymorphic loci for each population, was obtained for A. gharbi population (0.438), while high value found in A. sharqi population (0.563). The observed maximum heterozygosity was seen in A. sharqi, too. Observed and expected heterozygosity values for each population have presented in table 4.

Table 3. Genetic diversity information obtained from eight microsatellite loci markers applied to all samples (S.D = Standard Deviation); Number of alleles (Na) and effective alleles (Ne), expected heterozygosity (He) and Shannon information index (I).

Locus	DJ	FM	ED	CD	A113	A76	IM	A107	Mean	S.D
Na	3	2	1	2	6	7	11	10	5.25	3.84
Ne	1.22	1.14	1	1.1	1.68	1.79	6.69	6.92	2.69	2.55
He	0.185	0.126	0	0.096	0.414	0.45	0.865	0.87	0.376	0.339
I	0.37	0.244	0	0.198	0.832	0.991	2.07	2.1	0.852	0.832

Table 4. Mean expected (He) and observed (Ho) heterozygosity estimated for polymorphic loci in each population.

	Ardabil	S.D	A. sharqi	S.D	A. gharbi	S.D
He	0.472	0.31	0.563	0.298	0.43	0.3
Ho	0.369	0.358	0.6	0.323	0.3	0.11

Genetic differentiation

Hardy-Weinberg test and population differentiation characters were calculated and represented in order to investigate genetic structure of populations. Four significant biases from the Hardy-Weinberg equilibrium were detected among 24 (8×3) locus per population combinations. Three deviations were detected at Ardabil population ($P<0.001$) and one deviation was in A. gharbi population ($P<0.05$). F_{ST}, F_{IS} and F_{IT} coefficients for the total population have given in table 5. Mean over loci F-statistics obtained were $F_{IT} = 0.193$, $F_{IS} = 0.166$ and $F_{ST} = 0.032$. In addition, estimates of F_{IS} value were 0.22, -0.07 and 0.34 in Ardabil, A. sharqi and A. gharbi, respectively and P-value was significant ($P < 0.05$) in both Ardabil and A. gharbi populations. AMOVA analysis showed variation among populations, individuals within populations and within individuals were 3.29, 16.08 and 80.63, respectively. Among three population pairs, the highest and lowest of pairwise F_{ST} value were found between A. sharqi and A. gharbi (0.08, P-value significant) and between Ardabil and A. sharqi (0.021, P-value non-significant), respectively (Table 6). In addition, differentiation among populations and individuals is depicted in Figure. 3. The population structure plot (Figure. 4) provided by STRUCTURE software, shows a relative homogeneity in A. sharqi population.

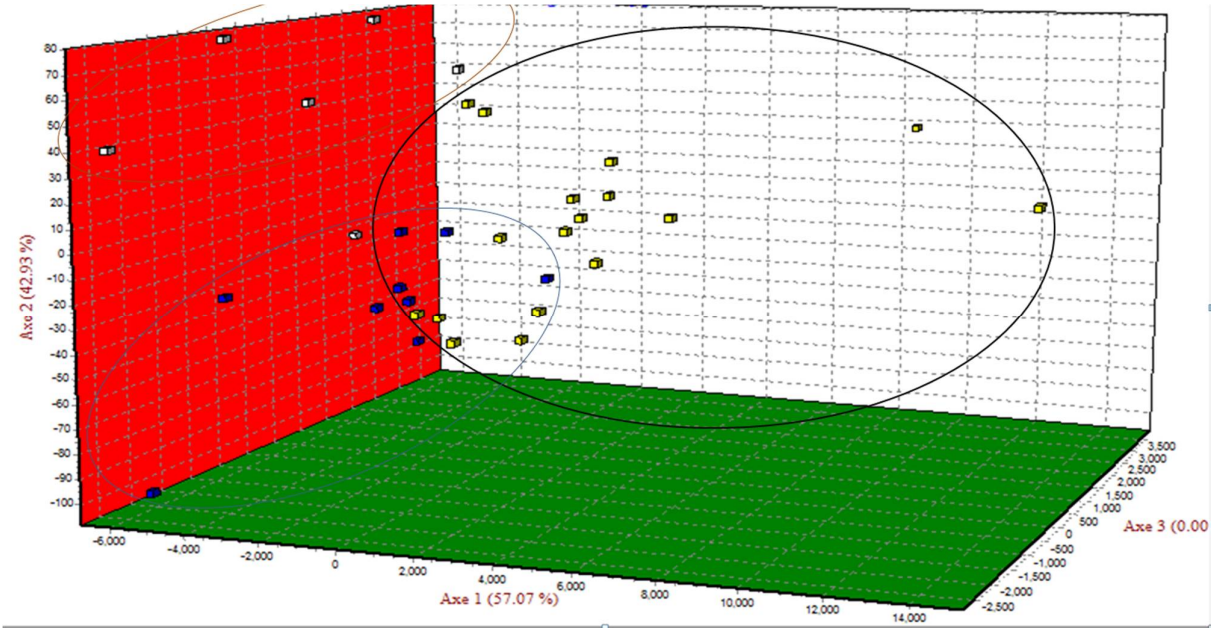

Figure 3. 3D graphic display of genetic differentiation containing all studied samples and corresponding populations.

Table 5. F statistics coefficients computed for each locus.

Locus	DJ	FM	ED	CD	A113	A76	IM	A107	Total
FST	0.016	-0.025		0.023	0.001	-0.007	0.096	0.016	0.032
FIS	0.821	1		-0.05	0.035	0.192	0.1	0.033	0.166
FIT	0.824	1		-0.026	0.036	0.187	0.186	0.049	0.193

Table 6. Estimates of pairwise F_{ST} value (above diagonal) and pairwise F_{ST} P-value (below diagonal) among three populations.

	Ardabil	A. sharqi	A. gharbi
Ardabil	****	0.021	0.059
A. sharqi	0.144±0.033	****	0.08
A. gharbi	0.072±0.018	0.018±0.012	****

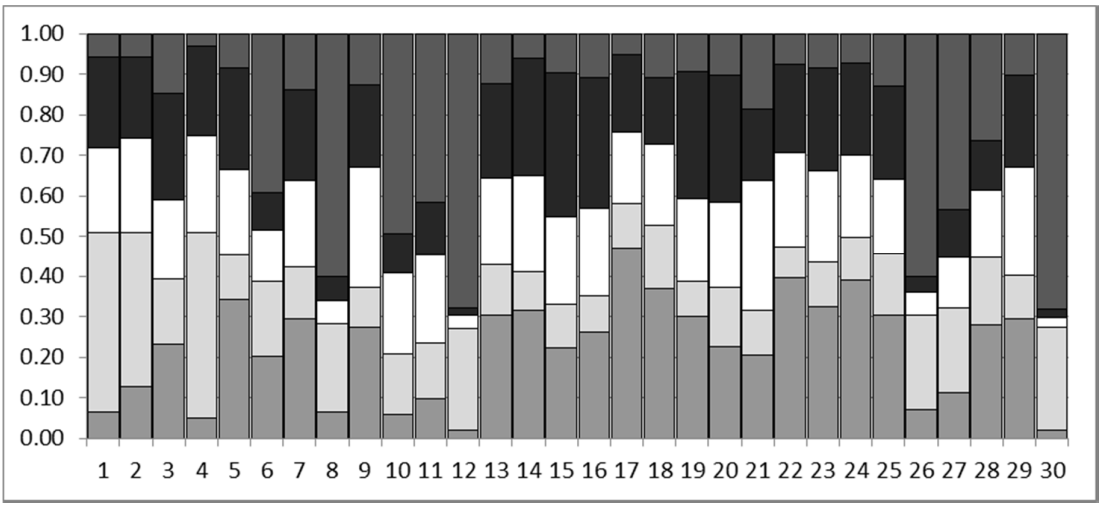

Figure 4. Population structure using genotype data (Notification: 1-17: Ardabil, 18-25: A. sharqi and 26-30: A. gharbi populations).

Discussion

Five microsatellite markers harboring high polymorphic microsatellite loci for *A. m. iberica* (Ghassemi-Khademi 2017b), but except one locus (IM), other loci have little or no variability in *A. m. meda* and it is one of the important tools to distinguish between two subspecies of honeybees. In this study, we introduce four microsatellite polymorphic loci (IM, A107, A113 and A76) for investigation of *A. m. meda* genetic variability. Effective number of alleles is a good span for the genetic variation, especially in conservation genetics study. Nevertheless, sometimes its effect on populations put more emphasis, even if the effective number of alleles is affected by sample size (Mielnik-Sikorska *et al.* 2013).

The average number of the alleles was 5.25 for the 8 microsatellite loci in this approach, which indicated that the sample size was rather suitable. Alleles that have been observed in only one population are called private alleles that are normally present in high frequency in isolated or protected honeybee populations. Bodur (2005), using nine microsatellite markers demonstrated that Ankara honeybee population have no private alleles and Ardahan honeybee population harbor maximum of private alleles (10 alleles) among 11 studied populations in Turkey (Bodur 2005). He believes that Ankara region is seriously

affected from migratory beekeeping, but Ardahan honeybees are under conservation in order to prevent gene flow. In this study, 13 private alleles were recognised in Ardabil population, because most of colonies in this province were collected from isolated, conservated and without migration apiaries. In other side, A. sharqi population showed just one private allele because collected colonies of this province have continuous migrations. Gene flow among collected colonies and other colonies in A. sharqi due to migratory beekeeping is major reason for this very low frequency of private alleles.

Mean expected heterozygosity, also called gene diversity, can reflect the variation of genetic structure (Malyarchuk *et al.* 2010). According to Ott definition in 2001, a polymorphic locus must have a heterozygosity of at least 0.10 on average (Sharma *et al.* 2010). The population of A. sharqi harbors moderate gene diversity (0.563) and showed no significant departure from Hardy-Weinberg equilibrium exact test in none of the polymorphic loci for this population whereas, a little lower gene diversity were seen in Ardabil (0.472) and A. gharbi (0.43) populations and at least one departure from HWE in polymorphic loci.

The collected colonies of former population have ordinary continuous migrations the two last populations not. High flow gene between collected colonies and other colonies due to migratory beekeeping is probably the major reason for rather high degree of heterozygosity in A. sharqi. Departure from HWE in two last populations may be due to a variety of causes: existence of migration in some of the collected colonies in both population, small population size, assortative mating system (including inbreeding), selection and existence of 'null alleles' (Irwin *et al.* 2010). Shaibi and Moritz demonstrated that genetic diversity of both Brak and the coastal intensive migratory beekeeping populations in Libya were significantly higher than that of Kufra population (a remote oasis in deep desert, with neither migratory nor intensive beekeeping operations) (Shaibi and Moritz 2010). In addition, another research showed the gene diversity about 0.44 for non-migratory colonies of *A. m. iberica* from southeastern Spain (DelaRua *et al.* 2002).

Our analysis of results showed a relative low F_{ST} (3.2 %) among the three studies *A. m. meda* populations. Existence of just one known subspecies of common honeybee (*A. m. meda*), geographic vicinity, cold and mountainous climate and high altitude (Table 1) of sampling sites are probably the causes of estimated low F_{ST}. The minimum value of pairwise F_{ST} observed between Ardabil and A. sharqi (0.021) in agreement with their geographic vicinity. Pairwise significant F_{ST} value between Ardabil and A. gharbi and between A. sharqi and A. gharbi were 0.059 and 0.08, respectively indicative of moderate level of genetic differentiation (Malyarchuk *et al.* 2010). Pairwise F_{ST} values that are reported between evolutionary lineages of *Apis mellifera* are generally higher than 0.1 (0.06-0.61) (Franck *et al.* 2001; Franck *et al.* 2000; Kumar *et al.* 2008). Within lineages, pairwise F_{ST} levels are generally lower than 0.1 for M and A lineages (Van Oven and Kayser 2009). This could be different for C lineage containing Persian honeybees, among which the reported F_{ST} was up to 0.24 (Franck *et al.* 2001; Franck *et al.* 2000; StatSoft 2011). The estimated F_{IS} values were high in both Ardabil (0.22) and A. gharbi (0.34) populations. A number of reasons probably have contributed to the deficit of the heterozygotes: selection (genetic hitchhiking effect) existence 'null alleles', small sample size where rare genotypes are likely to include in samples, existence of the Wahlund effect (Bodur 2005, Ghassemi-Khademi 2017b).

Conclusion

The present study is the rare studies about genetic diversity of *A. m. meda* in North West of Iran. The Azarbaijan region is the greatest pole of apiculture and production of honey in Iran. Therefore, to increase the accuracy of the

studies for this important region, additional markers and samples are required.

Acknowledgments

This study was supported by a grant from Shahid Beheshti University. The authors would like to thank the generous beekeepers of Iranian Azarbaijan region that kindly provided honeybee samples for this study.

References

Aflakian N., Houshmand M., Sharma S., Bamezai R.N.K. 2007a. Database: Maternal genepool of Arab population of Iran. from National Center for Biotechnology Information (NCBI), GenBank: EU239582-EU239560. http://www.ncbi.nlm.nih.gov/

Aflakian N., Houshmand M., Sharma S., Bamezai R.N.K. 2007b. Database: Maternal genepool of Phars population of Iran. from National Center for Biotechnology Information (NCBI), GenBank: EU239536-EU239655. http://www.ncbi.nlm.nih.gov/

Al-Zahery N., Saunier J., Ellingson K., Parson W., Parsons T., Irwin J. 2013. Characterization of mitochondrial DNA control region lineages in Iraq. International Journal of Legal Medicine 127(2): 373-375.

Alhamlan F.S. 2007. Evidence for genetic differences in the Africanized honeybee populations of south and north America. M.Sc thesis, Washington State University, Washington.

Asadi N. 2009. Laboratory manual on cytogenetics and molecular genetics in Animal science. ASRI Publication, Karaj, Iran.

Bodur, C. 2005. Genetic structure analysis of honeybee populations based on microsatellites Ph.D Thesis, Middle East Technical University Ankara.

Cardoso S., Valverde L., Alfonso-Sánchez M.A., Palencia-Madrid L., Elcoroaristizabal X., Algorta J., Catarino S., Arteta D., Herrera R.J., Zarrabeitia M.T. 2013. The expanded mtDNA phylogeny of the Franco-Cantabrian region upholds the pre-neolithic genetic substrate of Basques. PloS one 8(7): e67835.

DelaRua P., Galian J., Serrano J., Moritz R.F.A. 2002. Microsatellite analysis of non-migratory colonies of Apis mellifera iberica from south-eastern Spain. Journal of Zoological Systematics and Evolutionary Research 40: 164-168.

Franck P., Garnery L., Loiseau A., Oldroyd B.P., Hepburn H.R., Solignac M., Cornuet J.M. 2001. Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. Heredity 86: 420-430.

Franck P., Garnery L., Solignac M., Cornuet, J.M. 2000. Molecular confirmation of a fourth lineage in honeybees from the near east. Apidologie 31: 167-180.

Fraumene C., Belle E.M., Castrì L., Sanna S., Mancosu G., Cosso M., Marras F., Barbujani G., Pirastu M., Angius A. 2006. High resolution analysis and phylogenetic network construction using complete mtDNA sequences in Sardinian genetic isolates. Molecular biology and evolution 23(11): 2101-2111.

Ghassemi-Khademi T. 2017a. Taxonomy and comarative biology of world and Iranian honey bees (Volume 1). ACECR Publicatin, 400 Pages.

Ghassemi-Khademi T. 2017b. Introduction of appropriate RAPD and SSR markers to study the genetic structure of the Iranian honey bees (Apis mellifera meda). Aldrovandia 28(1): 119-121.

Irwin J.A., Ikramov A., Saunier J., Bodner M., Amory S., Röck A., O'Callaghan J., Nuritdinov A., Atakhodjaev S., Mukhamedov R. 2010. The mtDNA composition of Uzbekistan: a microcosm of Central Asian patterns. International Journal of Legal Medicine 124(3): 195-204.

Kumar S., Nei M., Dudley J., Tamura K. 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in bioinformatics 9(4): 299-306.

Malyarchuk B., Derenko M., Denisova G., Kravtsova O. 2010. Mitogenomic diversity in Tatars from the Volga-Ural region of Russia. Molecular biology and evolution 27(10): 2220-2226.

Mielnik-Sikorska M., Daca P., Malyarchuk B., Derenko M., Skonieczna K., Perkova M., Dobosz T., Grzybowski T. 2013. The history of Slavs inferred from complete mitochondrial genome sequences. Plos one 8(1): e54360.

Nasidze I., Quinque D., Ozturk M., Bendukidze N., Stoneking, M. 2005. MtDNA and Y-chromosome variation in Kurdish groups. Annals of human genetics 69(4): 401-412.

Quintana-Murci L., Chaix R., Wells R.S., Behar D.M., Sayar H., Scozzari R., Rengo C., Al-Zahery N., Semino O., Santachiara-Benerecetti A.S. 2004. Where west meets east: the complex mtDNA landscape of the southwest and Central Asian corridor. The American Journal of Human Genetics 74(5): 827-845.

Rahimi A., Mirmoayedi A., Kahrizi D., Abdolshahi R., Kazemi E., Yari K. 2014. Microsatellite genetic diversity of Apis mellifera meda skorikov. Molecular Biology Reports DOI 10.1007/s11033-014-3667-7.

Peakall R., Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295.

Shaibi T., Moritz R.F.A. 2010. 10,000 years in isolation? Honeybees (Apis mellifera) in Saharan oases. Conservation Genetics 11: 2085-2089.

Sharma V., Singh L., Thangaraj K., Nandan A., Sharma,V.K. 2010. Database: Phylogenomic study of a concealed Ladakh tribe of the Great Himalayas. from National Center for Biotechnology Information (NCBI), GenBank: HM036578-HM036529.

StatSoft I. 2011. STATISTICA, ver. 10: StatSoft.

Techer M.A., Clemencet J., Simiand C., Portlouis G., Reynaud B., Delatte H. 2015. Genetic diversity of the honeybee (Apis mellifera L.) populations in the Seychelles archipelago. Insect Conservation and Diversity 1: 14. doi: 10.1111/icad.12138.

Ting J., Ling Y., Min L., Wen-bin B., Guo-hong C. 2008. Study on genetic diversity of Apis cerana cerana and Apis mellifera ligustica in china with microsatellite markers. Research Journal of Animal Sciences 2(6): 178-182.

Van-Oven M., Kayser M. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Human mutation 30(2): E386-E394. http://www.phylotree.org/.