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GENETIC VARIATION OF DWARF HONEYBEE (*Apis florea* Fabricius) POPULATIONS DISTRIBUTED IN THE WESTERN PART OF IRAN BASED ON RAPD ANALYSIS

İran'ın Batısında Yayılan Cüce Balarısı (*Apis florea* Fabricius) Popülasyonlarında RAPD Analizine Dayalı Genetik Varyasyon

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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ABSTRACT

In this study, we investigated the genetic relationship and population differentiation within and among *Apis florea* populations sampled from three states in Iran, by using RAPD-PCR analysis. A total of 158 *A. florea* colonies from nine locations belonging to Ilam, Khuzestan and Bushehr states of Iran were evaluated. Of the 25 RAPD primers tested, 10 were identified with a total of 115 fragments. The populations included in this study showed high levels of genetic variation (H=0.21). According to genetic distance, the most genetically distant populations were Soush and Dezful, and the most similar populations were Sarollah and Musiyan. *A. florea* populations from three states grouped as only one big cluster on the tree based on Nei genetic distance. Isolation by distance test showed no significant relation between geographic and genetic distance. However, populations within each state showed higher similarities than the populations of other states.

Key words: ***Apis florea,* genetic variation, RAPD-PCR, Iran**

ÖZ

Bu çalışmada RAPD-PCR analizini kullanarak İran'daki üç eyaletten örneklenen *Apis florea* popülasyonları içindeki ve arasındaki genetik ilişkiyi ve popülasyon farklılaşmasını araştırdık. İran'ın İlam, Khuzestan ve Bushehr eyaletlerine ait dokuz lokasyondan toplam 158 *A. florea* kolonisi çalışma için değerlendirildi. Test edilen 25 primerden 10 tanesi toplamda 115 fragment ile belirlendi. Bu çalışmadaki popülasyonlar yüksek düzeyde genetik varyasyon göstermiştir (H=0.21). Genetik uzaklığa göre, genetik olarak en uzak popülasyonlar Soush ve Dezful; ve en yakın popülasyonlar Sarollah ve Musiyan'dır. Nei genetik uzaklığına bağlı olarak oluşturulan ağaçta üç eyaletteki *A. florea*popülasyonları sadece tek bir büyük küme olarak gruplanmıştır. Uzaklık ile izolasyon testi, coğrafik ve genetik uzaklık arasında anlamlı bir ilişki göstermemiştir. Ancak her eyaletteki popülasyonlar, diğer eyaletlerdeki popülasyonlardan daha çok benzerlik göstermiştir.

Anahtar kelimeler:*Apis florea,* genetik varyasyon, RAPD-PCR, İran

INTRODUCTION

The open nesting dwarf honeybee (*Apis florea* fabricius) is a prominent pollinator with a wide natural distribution extending from Southeast Asia to the Far East and occupies rainforests, savannas, subtropical steppes and semi-deserts in almost all places(Hepburn et al., 2005; Moradi and Kandemir, 2005). Recently, the distribution area of dwarf honeybees has been continuously expanding westwards, naturally and by means of anthropogenic factors (Mogga and Ruttner, 1988; Hepburn et al., 2005). It is now widespread in Iran, Pakistan, and Afghanistan (Otis, 1996), also in Iraq, Oman, and Yemen (Wongsiri et al., 1996) and has recently established colonies in Sudan, central Saudi Arabia (Hepburn et al., 2005) and around Aquba, Jordan (Haddad et al., 2008; Haddad et al., 2009). In Iran, *A. florea* can be found starting from Qhasr-e-Shirin in Kermanshah (Hashemi, 2004), to Ilam, Lorestan, Khuzestan, Bushehr, Fars, Hormuzgan, Kerman, Baluchestan, and Boyarahmad va Kohgiluye (Mossadegh, 1993; Ruttner et al., 1995). In addition, the distribution of *A. florea* may extend to west Azerbaijan state in northern Iran (Moradi and Kandemir, 2005). Although *A. florea* has a sympatric distribution with *A. mellifera,* there is no natural overlap between *A. florea* and *A. mellifera* in the world except in Iran. While *A. florea* extends along the Arabian Peninsula and into Africa, it has generated significantly better colonies that adapt to hot climatic conditions easily, and have established founder populations in differentgeographical areas (Haddad et al., 2009).

There are a number of investigations of geographic variation using morphometric approaches in *A. florea* populations (Mossadegh, 1993; Ruttner et al., 1995; Tahmasebi et al., 2002; Chaiyawong et al., 2004; Hepburn et al., 2005; Haddad et al., 2009; Kandemir et al., 2009; Özkan et al., 2009).Besides morphometry, two studies used a microsatellite approach to determine the mating frequency (Palmer and Oldroyd, 2001) or to study worker policing (Luke et al., 2001) in dwarf honeybees.There was no information about *A. florea* populations in Iran based on Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). Previously different subspecies of *A. mellifera* were subjected to RAPD studies. In some of these studies, inheritance pattern (Hunt and Page, 1992), QTL mapping (Hunt and Page, 1995), subspecies differentiation (Suazo et al., 1998) and population differentiation (Tunca and Kence, 2011) were studied by RAPDs. In the genus *Apis*, RAPD markers were used for distinguishing African and European honeybees (*Apis mellifera* L.) (Suazo et al., 1998). Besides its successful applications in many insect species, RAPDs are extensively used for generating genetic maps, understanding insect-plant and insect-pathogen interaction, determining resistance genes against insecticides, and identifying insect behavior (Jain et al., 2010). One of the most appealing application of RAPD in insect studies is that of genetic and geographic variations within and among related populations of insect species. RAPD became the most common molecular marker at the start of molecular studies because it is a simple and useful technique for investigating genetic diversity and measuring genetic differences within and among related species or populations (Welsh and McClelland, 1990; Jain et al., 2010) without the need of a priori knowledge about the genome of the study organism.

In this study, we used RAPD markers to examine nuclear DNA polymorphism in *A. florea* populations distributed in three states in Iran, and determined the genetic relationship and population differentiation within and among *A. florea* populations sampled from these three states.

MATERIAL AND METHOD

Sample Collection and DNA Isolation

The dwarf honeybee (*A. florea*) samples were collected in three consecutive years (2005-2007) from Bushehr and Khuzestan states along the coast of the Persian Gulf and from an inland state, Ilam (Table 1, Figure 1). A total of 158 *A. florea* colonies from nine locations were studied by using RAPD-PCR. Samples were preserved in 70% ethanol until the total nucleic acid extraction. DNA was isolated from individual bee thorax using the modified CTAB method of Doyle and Doyle (Doyle and Doyle, 1991). The purity and quantity of the total nucleic acid were determined spectrophotometrically (Agilent 2100 Bioanalyser NanoDrop ND-1000 Spectrophotometer).

RAPD-PCR Assay and Data Analysis

The PCR was run in 25µl of a reaction mixture containing 1µl of the DNA samples (200ng/µl); 2.5µl of 10X buffer with $(NH_4)_2$ SO_4 (Fermentas); 0.3µl of *Taq* DNA Polymerase (5u/µl, 500U Fermentas); 4µl of deoxynucleotide triphospate mix (100µM, 10µl of each nucleotide); 1.5µl of $MgCl_2$ (25mM); 1µl of 1pmol primers. Amplifications were performed us-

ing a Thermal Cycler (Techne TC-312). The PCR steps were as follows: 95°C for 1 min, 45 cycle of "94°C for 1 min, 36°C for 2 min, 72°C for 2 min" and 72°C for 15 min. Prescreening of 25 random oligo-nucleotide primers (UBC and Operon) revealed that 10 primers could be useful for further study and data collection (Table 2). The amplified fragments were separated on an agarose gel (1.5% w/v) at 70 volts for 2.5 hours. After electrophoresis, gel were stained with ethidium bromide and photographed under UV.

Table 1. Sampling locations for *Apis florea* in Iran.

State	Location	n	Coordinates
Ilam	1. Dehloran	15	32.41N 47.15E
	2. Sarollah	17	32.35N 47.22E
	3. Musiyan	12	32.32N 47.22E
	4. Dasht Abbas	29	32.29N 47.47E
	5. Ogahha	13	32.10N 47.41E
Khuzestan	6. Dezful	4	32.23N 48.23E
	7. Soush	11	32.11N 48.14E
	8. Ahvaz	25	31.17N 48.43E
Bushehr	9. Bushehr	32	28.59N 50.50E

(n: number of colonies)

Figure 1. Map indicating sampling locations in Iran. Numbers corresponds to the population names in Tables 1 and 3.

Table 2. List of the primer names, sequences and total # of bands obtained.

Primer	Sequence 5'→3'	Total # of bands
UBC-514	CGGTTAGACG	11
UBC-652	CCCAACACAC	10
UBC-691	AAACCAGGCG	13
UBC-694	GGTTTGGAGG	12
OPA-07	GAAACGGGTG	12
OPB-07	GGTGACGCAG	12
OPB-17	AGGGAACGAG	13
OPB-20	GGACCCTTAC	10
OPD-02	GGACCCAACC	9
OPD-13	GGGGTGACGA	13

The RAPD bands on the gel were scored as (1) or (0) for the presence or absence of the fragment, respectively. Only the most reliable and distinct bands were used to create a binary presence/absence data matrix. Monomorphic fragments and bands with low frequencies (that is less than 3/*n*, where *n* is the total number of scores in the data set) were excluded from the analysis (Lynch and Milligan, 1994). From the data matrix, population genetic parameters [percentage of polymorphic loci (P), observed number of alleles (N_a), effective number of alleles (Kimura and Crow, 1978) (N_e), Nei's (Nei, 1972) gene diversity (H) and Shannon's Information index (Lewontin, 1972) (I), genetic differentiation among subpopulations (G_{ST}) and gene flow (Nm)] were calculated by using Popgene version 1.32 software (Yeh et al., 2000). In addition, the expected heterozygosity of an individual in a population (H_S) and the expected heterozygosity of an individual in overall populations (H_T) were estimated in accordance with the Hardy-Weinberg expectations (Nei, 1987). The dendrogram of populations was also constructed based on Nei's (Nei, 1972) genetic distance with 1000 boot-strap values by using Population version 1.2.30 software (Langella, 1999). When population genetic parameters were estimated, *A. florea* populations were grouped with respect to nine populations. Similar genetic parameters were estimated, according to the geographic locations after grouping nine populations into three states. We also used Isolation by Distance (IBD) analysis to test if there is any significant correlation between geographic distance and genetic distance (Bohonak, 2002). The Mantel test was used to compare different distance matrices obtained from this study and the previous studies by using NTSYSpc software (Rohlf, 2005).

RESULTS AND DISCUSSION

In this study RAPD primers generating polymorphic banding patterns were utilized for estimating the population genetic parameters of *A. florea* populations. After screening 25 ten-base oligonucleotide primers, 10 primers were selected. Four of them were used in *Apis mellifera* previously (Suazo et al., 1998). All selected primers displayed intense and reproducible bands for further PCR amplification of the 158 samples from nine *A. florea* populations. A total of 115 polymorphic fragments were obtained by those 10 primers in the studied populations. The number of polymorphic RAPD fragments detected by each primer ranged from 9 to 13 (Table 2).

The proportion of polymorphic loci ranged between 6.09% in Dezful and 52.17% in Bushehr populations. The populations included in this study showed a low number of polymorphic loci compared to one population included in genetic study on *A. mellifera* (Tunca and Kence, 2011) where the lowest polymorphic band percentage is over 40. For all analyzed populations in the nine locations, the mean observed number of alleles (N_a) was 1.76. The range of observed number of alleles was between 1.08 in the Dezful and 1.52 in the Bushehr populations. The highest number of effective alleles (N_e) was observed in the Bushehr population (1.29), whereas the lowest N_e value was found in Dezful (1.05). When all populations in the nine locations were considered, the mean N_e value was 1.34. According to Nei (Nei, 1987), the calculation of proportion of polymorphic loci is not a good measure of genetic variation. A more appropriate measure of genetic variation is average heterozygosity or gene diversity. Nei's genetic diversity or heterozygosity (H) was the lowest in Dezful (H=0.03, I=0.04) and the highest in Bushehr (H=0.17, I=0.26). This means that the Bushehr population has a higher proportion of heterozygous genotypes than the other populations. For all populations, the mean observed heterozygosity was calculated as 0.21 (Table 3). The populations included in this study showed higher levels of genetic diversity (H=0.21, I=0.32) compared to the one done on *A. mellifera* (Tunca and Kence, 2011).

Table 3. Genetic diversity of nine *Apis florae* populations.

Location	Na	Ne	H	I	P	% of P
1-Dehloran	1.33±0.47	1.19±0.31	0.11±0.17	0.17±0.26	38	33.04
2-Sarollah	1.31±047	1.19±0.32	0.11±0.18	0.16±0.26	36	31.30
3-Musiyan	1.31±0.47	1.19±0.34	0.11±0.18	0.17±0.26	36	31.30
4-Dasht Abbas	1.44±050	1.22±0.32	0.14±0.18	0.21±0.26	51	44.35
5-Ogahha	1.35±0.48	1.24±0.37	0.14±0.20	0.20±0.28	37	32.17
6-Dezful	1.08±0.27	1.05±0.16	0.03±0.10	0.04±0.15	7	6.09
7-Soush	1.28±0.45	1.20±0.35	0.11±0.19	0.17±0.27	29	25.22
8-Ahvaz	1.50±0.50	1.24±0.31	0.15±0.18	0.23±0.26	51	44.35
9-Bushehr	1.52±0.50	1.29±036	0.17±0.19	0.26±0.28	60	52.17

Na: observed number of alleles, Ne: effective number of alleles, H: Nei's gene diversity, I: Shannon's Information index, P: number of polymorphic bands

The genetic distances between populations ranged from 0.46 (Soush-Dezful) to 0.06 (Sarollah-Musiyan). The most genetically distant populations were Soush and Dezful (genetic distance=0.463), whereas the most similar populations were Sarollah and Musiyan (genetic distance=0.059). According to these results, there was no affinity between genetic distance and geographic distribution (Table 4).

According to geographic distribution of populations based on states, populations from Khuzestan had the highest genetic diversity compared to populations from Bushehr and Ilam states. The value of percent polymorphic loci was similar in both Ilam and Khuzestan but lower in Bushehr state. A similar observation was done on the number of alleles (N_a). However,the population from Bushehr had the highest number of effective alleles (N_e) compared to populations from Ilam and Khuzestan (Table 5). The partition of total gene diversity was similar among geographic regions (H_T=0.17). The gene diversity within the Bushehr population was the highest (H_S=0.17), while it was the lowest in Khuzestan populations (H_S=0.10).

Table 4. Genetic distance between nine *Apis florae* populations.

Location	Dehloran	Sarollah	Musiyan	Dasht Abbas	Ogahha	Dezful	Soush	Ahvaz	Bushehr
Dehloran	-								
Sarollah	0.101	-							
Musiyan	0.084	0.059	-						
Dasht Abbas	0.094	0.061	0.104	-					
Ogahha	0.127	0.097	0.101	0.154	-				
Dezful	0.204	0.321	0.322	0.273	0.385	-			
Soush	0.178	0.133	0.135	0.134	0.185	0.463	-		
Ahvaz	0.150	0.221	0.211	0.203	0.256	0.140	0.321	-	
Bushehr	0.087	0.094	0.094	0.074	0.138	0.274	0.139	0.142	-

Genetic differentiation (G_{ST}) between populations was calculated for Ilam (G_{ST}=0.30) and Khuzestan (G_{ST}=0.40) populations. This indicates that Khuzestan populations have a greater population differentiation than Ilam populations (Table 5). The estimated gene flow (Nm) for Ilam and Khuzestan populations were 1.16 and 0.75, respectively, indicating that there is no genetic exchange between and within either populations. According to Wright (1969), the critical gene flow value is 0.5. When Nm values are below 1, it means that populations begin to differentiate due to genetic drift. Nm values below 0.5 indicate that populations will diverge extensively as a result of genetic drift (McDermott and McDonald, 1993). The Bushehr state had no genetic differentiation and gene flow was not calculated for Bushehr, due to having only one population (Table 5). Tunca et al. (2004) found a higher Nm value in their study of the eastern part of Turkey; they stated that there was no gene flow among *A. mellifera* populations in the Van region in Turkey (Nm=2.039).

Table 5. Genetic diversity *Apis florea* populations in three states of western part of Iran.

State	Na	Ne	H	I	H_T	H_S	G_{ST}	Nm	P	% of P
Bushehr	1.52 ±0.50	1.29 ±0.36	0.17 ±0.19	0.26 ±0.28	0.17 ±0.04	0.17 ±0.04	-	-	60	52.17
Ilam	1.61 ±0.49	0.28 ±0.35	0.17 ±0.19	0.26 ±0.27	0.17 ±0.04	0.12 ±0.02	0.30	1.16	70	60.87
Khuzestan	1.60 ±0.49	0.28 ±0.31	0.18 ±0.18	0.28 ±0.26	0.17 ±0.03	0.10 ±0.01	0.40	0.75	69	60.00

H_T: total gene diversity, H_S: gene diversity within populations, G_{ST}: genetic differentiation, Nm: gene flow, P: number of polymorphic bands

Nei's (1972) genetic distance values between populations were used to construct a dendrogram in order to examine the genetic relationship between nine *A. florea* populations (Figure 2 and Figure 3). According to Figure 2 only one big cluster were seen on the unrooted tree. This result was supported by low bootstrap values and Isolation by distance (IBD) analysis. Figure 3 demonstrated the genetic relationships among *A. florea* populations and the subpopulations in the western part of Iran. According to Figure 3, three populations (Bushehr, Ilam and Khuzestan) were approximately equal distance to each other. Within each state, for example in Khuzestan, Ahvaz and Dezful populations closer to each other than to the Soush population. In Ilam, there is no grouping among subpopulations. However, although there is a close resemblance among populations within state, when we carried out IBD analysis we did not find any correlation between geographic distance and the genetic distance (Figure 4). Similarly, Tunca and Kence (2011) did not find any correlation between geographic and genetic distance after Mantel test in Turkey.

Figure 2. Unrooted tree depicting the genetic relationships among nine *Apis florea* populations analyzed based on Nei's (1972) genetic distance. Numbers at branch points are bootstrap values.

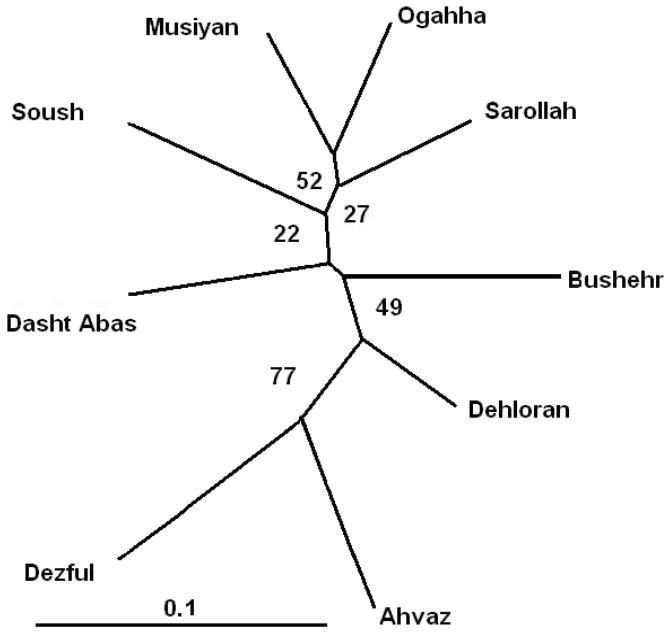


Figure 3. Unrooted tree depicting the genetic relationships among *Apis florea* populations analyzed based on Nei's (1972) genetic distance.

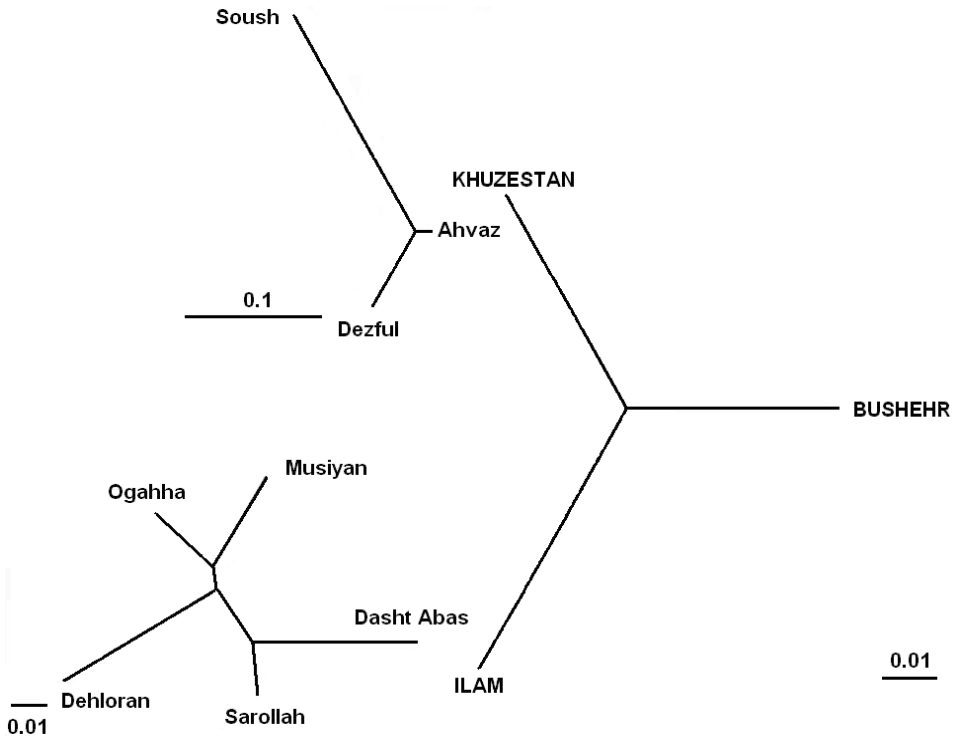
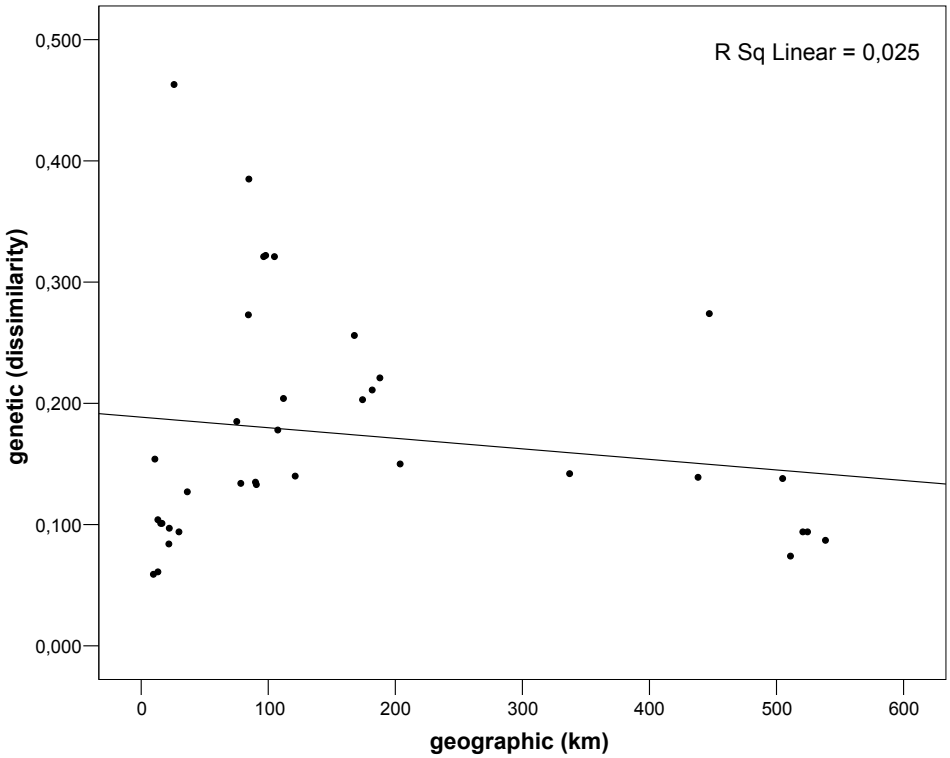


Figure 4. IBD analysis showing no significant correlation between geographic and genetic distance.



Clustering analysis showed that the genetic relationships among *A. florea* populations in three states based on RAPD data were not in agreement with the results obtained in previous studies using standard and geometric morphometry (Kandemir et al., 2009; Özkan et al., 2009). According to Kandemir et al. (2009) and Özkan et al. (2009) the colonies from Ilam, Khuzestan, and Bushehr showed a close grouping in multivariate statistical analysis of both landmark and standard morphometric characters. This similarity was not detected with RAPD genetic data and this was very well displayed when the distance matrices were compared (Table 6) with Mantel test. Both morphometric studies showed high correlation with each other but the correlations were low when compared to RAPD genetic distance matrix.

In conclusion, the extent of genetic variation in *A. florea* was investigated in its distribution area in three western states of Iran using RAPD-PCR analysis. The RAPD-PCR technique was proven to be extremely useful for differentiating geographically and genetically distinct populations in insects (Jain et al., 2010). Thus, we utilized this technique to find if there is such differentiation in these populations.

Table 6. Mantel test among population distances obtained from this study and previous morphometric studies (Standard and geometric). M; Standard Morphometry, GM; Geometric Morphometry, G; Genetic-this study.

	florea M	*florea* G	*florea* GM
florea M	-		
florea G	-0.018	-	
florea GM	0.481	0.146	-

We found that some populations are more diverse than others, some have no gene flow, and others have a considerable amount of gene flow, resulting in limited differentiation. Although this is the first study on genetic variation of *A. florea* species distributed in Iran based on molecular marker as RAPD-PCR, there are more markers left to continue to answer other raised questions, such as how much mitochondrial gene sequence variation found in these populations or how neutral markers differ in these populations. Thus, further genetic analysis, including mtDNA analysis, microsatellite analysis, will be necessary to determine the genetic relationship and population differentiation within and among *A. florea* populations in great detail. In a

previous study, it was shown that *A. florea* colonies have been established in Sudan, central Saudi Arabia (Hepburn et al., 2005), and around Aquba, Jordan (Haddad et al., 2008; Haddad et al., 2009). In order to determine the source of the founder populations in new distribution areas, aforementioned genetic analyses are needed to resolve the population structure and the origin of the *A. florea* populations.

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REFERENCES

Bohonak, A.J. 2002. IBD (Isolation By Distance): a program for analyses of isolation by distance. *Journal of Heredity*, 93: 153-154.

Chaiyawong, T., Deowanish, S., Wongsiri, S., Sylvester, H.A., Rinderer, T.E., De Guzman, L. 2004.Multivariate morphometric study of *Apis florea* in Thailand.*Journal of Apicultural Research*, 43: 123-127.

Doyle, J.J., Doyle, J.L. 1991. Isolation of plant DNA from fresh tissue.*Focus*, 12(1): 13-15.

Haddad, N., De Miranda, J.R., Bataena, A. 2008. Discovery of *Apis florea* in Aqaba, Jordan. *Journal of Apicultural Research*, 47: 173-174.

Haddad, N., Fuchs, S., Hepburn, H.R., Radloff, S.E. 2009. *Apis florea* in Jordan: source of the founder population. *Apidologie*, 40: 508-512.

Hashemi, M. 2004. *The complete guide to bee keeping*.2nd edn.730 p.

Hepburn, H.R., Radloff, S.E., Otis, G.W., Fuchs, S., Verma, L.R., Ken, T., Chaiyawong, T., Tahmasebi, G., Ebadi, R., Wongsiri, S. 2005. *Apis florea*: morphometrics, classification and biogeography. *Apidologie*, 36: 359-376.

Hunt, G.J., Page, R.E. 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theoretical and Applied Genetics*,85: 15-20.

Hunt, G.J., Page, R.E. 1995. Linkage map of the honey bee, *Apis mellifera,* based on RAPD markers. *Genetics*, 139: 1371-1382.

Jain, S.K., Neekhra, B., Pandey, D., Jain, K. 2010. RAPD marker system in insect study: A review. *IndianJournal of Biotechnology*, 9: 7-12.

Kandemir, İ., Moradi, M.G., Özden, B., Özkan, A. 2009. Wing geometry as a tool for studying the population structure of dwarf honey bees (*Apis florea* Fabricius 1876) in Iran.*Journal of Apicultural Research*, 48: 238-246.

Kimura, M., Crow, J.M. 1978.Effect of overall phenotypic selection on genetic change at individual loci.*Proceedings of the National Academy of Sciences of the United States of America*, 75: 6168-617.

Langella, O. 1999. Populations, version 1.2.30. A population genetic software. CNRS UPR9034, available from: http://bioinformatics.org/~tryphon/populations/.

Lewontin, R.C. 1972. The apportionment of human diversity.*Evolutionary Biology*, 6: 381-398.

Luke, A.H., Oldroyd, B.P., Wattanachaiyingcharoen, W., Barron, A.B., Nanork, P., Wongsiri, S. 2001.Worker policing in the bee *Apis florea*.*Behavioral Ecology and Sociobiology*, 49:509–513.

Lynch, M., Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers.*Molecular Ecology*, 3: 91-99.

McDermott, J.M., McDonald, B.A. 1993. Gene flow in plant photosystems.*AnnualReview of Phytopathology*, 31: 353-373.

Mogga, G.B., Ruttner, F. 1988. *Apis florea* in Africa; source of the founder population.*Bee World*, 69: 100-103.

Moradi, M.G., Kandemir, I. 2005. Observations on *Apis florea* "the Dwarf Honey Bee" in Iran.*American Bee Journal*, 145: 498-502.

Mossadegh, M.S. 1993. New geographical distribution line of *Apis florea* in Iran. In: Corner, L.J., Rinderer, T., Slyvester, H.A., Wongsiri, S. (eds.) *Asian apiculture*. Wicwas Press, Cheshire, Connecticut, USA, pp. 64-66.

Nei, M. 1972. Genetic distance between populations.*The American Naturalist*, 106: 283-292.

Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.

Otis, G.W. 1996. Distribution of recently recognized species of honeybees (Hymenoptera: Apidae: *Apis*) in Asia. *Journalof the Kansas EntomologicalSociety*, 69:311-333.

Özkan, A., Gharleko, M.M., Özden, B., Kandemir, İ. 2009. Multivariate morphometric study of *Apis florea* distributed in Iran.*Turkish Journal of Zoology*, 33: 93-102.

Palmer, K.A., Oldroyd, B.P. 2001. Mating frequency in *Apis florea* revisited (Hymenoptera, Apidae). *Insectes Sociaux*, 48: 40–43.

Rohlf, F.J. 2005. NTSYS-PC Numerical taxonomy and multivariate analysis system, version 2.20. Exeter Software, New York.

Ruttner, F., Mossadegh, M.S., Kauhausen-Keller, D. 1995.Distribution and variation of size of *Apis florea* F in Iran.*Apidologie*, 26: 477-486.

Suazo, A., McTiernan, R., Hall, H.G. 1998. Differences between African and European honey bees (*Apis mellifera* L.) in Random Amplified Polymorphic DNA (RAPD).*Journal of Heredity*, 89(1): 32-36.

Tahmasebi, G., Ebadi, R., Tajabadi, N., Akhondi, N., Faraji, S. 2002. The effect of geographical and climatological conditions on the morphometrical variation and separation of Iranian small honeybee (*Apis florea* F.) populations.*Journal of Science and Technology of Agriculture and Natural Resources*, 6: 176-185.

Tunca, R.I., Bilgen, G., Kence, M., Türkmut, L. 2004. Genetic analysis of honeybees of Van region in Turkey with RAPD method. *First European Conference on Apidology*, Udine, Italy, 19-23 September 2004, Proceeding book page 45.

Tunca, R.I., Kence, M. 2011. Genetic diversity of honey bee (*Apis mellifera* L.: Hymenoptera: Apidae) populations in Turkey revealed by RAPD markers. African Journal*of Agricultural*Research*,* 6: 6217-6225.

Welsh, J., McClelland, M. 1990. Fingerprinting genomics using PCR with arbitrary primers.*Nucleic Acids Research*, 18: 7213-7218.

Wongsiri, S., Lekprayoon, C., Thapa, R., Thirakupt, K., Rinderer, T.E., Sylvester, H.A., Oldrody, B.P., Booncham, U. 1996.Comparative biology of *Apis andreniformis* and *Apis florea* in Thailand.*Bee World*, 77: 23-35.

Wright, S. 1969. *Evolution and genetics of populations: The theory of gene frequencies*. Chicago: Univ. Chicago Press.

Yeh, F.C., Yang, R.C., Boyle, T. 2000. Popgene, version 1.32, Microsoft windows–based freeware for population genetic analysis.Center for International Forestry Research, University of Alberta, Edmonton, Alberta, Canada.

GENİŞLETİLMİŞ ÖZET

Giriş

Cüce Balarısı (*Apis florea* Fabricius) Güneydoğu Asya'dan Uzakdoğu'ya uzanan geniş doğal yayılış alanına sahip olup önemli bir tozlaştırıcıdır. Açık alanlarda yuva yaparak yağmur ormanları, savanlar, subtropik stepler ve yarı çöller gibi farklı bölgelere adapte olmaktadır. Son zamanlarda *A. florea*'nın yayılış alanı doğal yolla ve antropojenik faktörlerin etkisi ile sürekli olarak batıya doğru genişlemektedir. *A. florea*'nın doğal yayılış alanı içerisinde sahip olduğu coğrafik varyasyonu ortaya koymak için morfometrik yaklaşım ile yapılan çok sayıda çalışma bulunmaktadır. İran'daki *A. florea* popülasyonlarındaki genetik varyasyonu ortaya çıkartmak için RAPD-PCR metoduna dayalı herhangi bir bilgi bulunmamaktadır. Bu çalışmada İran'nın batısında bulunan üç eyaletteki *A. florea* popülasyonları arasında ve popülasyonlar içindeki genetik ilişki ve popülasyon farklılaşması RAPD-PCR analizi ile araştırılmıştır.

Materyal ve Metot

A. florea örnekleri2005-2007 yılları arasında Basra Körfezi kıyısındaki Bushehr ve Khuzestan eyaletleri ile iç kesimde yer alan Ilam eyeletinden toplandı. 9 lokasyondan toplam 158 koloniden örnekler RAPD-PCR analizi ile değerlendirildi. Nükleer DNA modifiye edilmiş CTAB metodu (Doyle and Doyle, 1991) kullanılarak işçi arıların toraksından izole edildikten sonra uygun bileşenler ve koşullar ile PCR işlemi gerçekleştirildi. Amplifiye olan parçalar agoroz jel elektroforezi üzerinde RAPD bantlarının olup olmamasına göre (1) veya (0) olarak değerlendirildi. Popgene 1.32 programı (Yeh et al., 2000) ile veri matrisinden popülasyon genetiği parametreleri [polimorfik lokus yüzdesi (P), gözlenen alellerin sayısı (N_a), etkili alel sayıları (N_e) (Kimura and Crow, 1978), gen çeşitliliği (H) (Nei, 1972), Shannon's Information index (I) (Lewontin, 1972), alt popülasyonlarda genetik farklılaşma (G_{ST}) ve gen akışı (Nm)] hesaplandı. Ayrıca Hardy-Weinberg'e göre (H_S) ve (H_T) değerleri hesaplandı (Nei, 1987). Population 1.2.30 programı (Langella, 1999) ile popülasyonlara ait dendrogram oluşturuldu. Coğrafik uzaklık ve genetik uzaklık arasında herhangi bir korelasyon olup olmadığını test etmek için Uzaklık

ile İzolasyon (IBD) analizi yapıldı (Bohonak, 2002). Bu çalışma ve önceki çalışmalardan elde edilen farklı uzaklık matrislerini karşılaştırmak için NTSYSpc programı (Rohlf, 2005) kullanılarak Mantel test yapıldı.

Sonuçlar ve Tartışma

Bu çalışmada 9 lokasyondan 158 örneğin DNA'sı PCR ile çoğaltıldıktan sonra 25 oligonükleotid primerinden, 4 tanesi daha önceden *Apis mellifera* için kullanılan, 10 tane primer seçilmiştir (Tablo 2). Tüm primerler için toplamda 115 polimorfik bant elde edilmiş olup her bir primer için polimorfik RAPD bantlarının sayısı 9 ile 13 arasında değişmektedir (Tablo 2).

Dokuz lokasyondaki ve 3 eyaletlerdeki popülasyonlar için RAPD-PCR analizine göre hesaplanan genetik parametreler Tablo 3 ve Tablo 5'te verilmiştir. Bu çalışmadaki populasyonlar yüksek seviyede genetik varyasyon (H=0.21) göstermektedir. Popülasyonlar arasındaki genetik uzaklık 0.46 (Soush-Dezful) ile 0.06 (Sarollah-Musiyan) değerleri arasında değişmektedir (Tablo 4). Genetik olarak en uzak populasyonlar Soush ve Dezful iken en yakın popülasyonlar Sarollah ve Musiyan popülasyonlarıdır. Bu sonuçlara göre genetik uzaklık ve coğrafik dağılım arasında herhangi bir ilişki bulunmamaktadır. Genetik uzaklığa göre oluşturulan dendrogramda, *A. florea* popülasyonları büyük bir küme şeklinde gruplanmıştır. Bu sonuç düşük bootstrap değerleri ve IBD testi ile desteklenmiştir.

RAPD verileri kullanılarak yapılan kümeleme analizindeki 3 eyaletteki *A. florea* popülasyonları arasındaki genetik ilişki standart morfometri ve geometrik morfometri kullanılarak yapılan önceki çalışmalardan (Kandemir et al., 2009: Özkan et al., 2009) elde edilen sonuçlar ile uyumlu bulunmamıştır. Mantel testi ile her üç analizden elde edilen uzaklık matrisleri karşılaştırıldığında, her iki morfometrik çalışmadan elde edilen matrisler birbirleri ile yüksek korelasyon gösterirken RAPD çalışması ile karşılaştırıldığında, korelasyon düşük bulunmuştur. IBD testine göre coğrafik ve genetik uzaklık arasında herhangi bir ilişki bulunamamıştır (r=-0.1597; P, 0.5780 1000 tekrarlama).

RAPD-PCR tekniğinin böceklerde coğrafik ve genetik olarak farklı olan popülasyonları ayırt etmek için son derece kullanışlı olduğu kanıtlanmıştır (Jain et al., 2010). Bu çalışmada da RAPD-PCR metoduna başvurarak doğal yayılış alanı içerisinde İran'ın üç batı eyaletinde *A. florea* popülasyonlarındaki genetik varyasyonun ölçüsü araştırılmıştır. Bazı popülasyonların diğerlerinden daha farklı olduğu, bazılarında hiç gen akışı olmadığı ve diğerlerinde önemli ölçüde gen akışı olduğu, farklılaşma olmadığı bulunmuştur. Bu çalışma RAPD-PCR metodu kullanılarak İran'da yayılış gösteren *A. florea* türündeki genetik varyasyon üzerine yapılmış ilk çalışma olmasına rağmen *A. florea* populasyonları içerisindeki ve arasındaki genetik ilişkileri ve popülasyon farklılaşmasını belirlemek için mtDNA analizleri ve mikrosatellit analizlerini içeren daha kapsamlı genetik analizler yapılması gerekmektedir.