

A Preliminary Study for the Application of ISSR Markers to Discriminate Honey Bee (*Apis mellifera* L.) Populations in Turkey

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Abstract

Potential of Inter Simple Sequence Repeats (ISSR) markers in analyzing and discriminating of Turkish honeybee populations was assessed in this study. Genomic DNA samples of workers belonging to four subspecies and two ecotypes of honey bees were amplified by PCR using 5 microsatellite primers. Principal component and population structure analyses showed that ISSR method successfully defined and discriminated four distinct Turkish honeybee subspecies while the two ecotypes of *Anatoliaca* subspecies were not distinctively separated from each other. This study showed that ISSR markers can be a simple and low-cost alternative with high resolution compared to other markers such as microsatellite and RAPD, in analyzing the genetic variations in Turkish honey bee subspecies.

Introduction

Anatolia's diverse climatic and geologic structure and its location as a bridge between Europe, Asia, and Africa led a considerable honey bee biodiversity in this region represented by five subspecies: *A. m. caucasica* at black sea region and northeastern Anatolia, *A. m. carnica* at Thrace region of north western Anatolia, *A. m. anatoliaca*, at western and central Anatolia, *A. m. meda* at southeastern Anatolia, and *A. m. syriaca* at Syria border region of southeastern Anatolia, (Ruttner, 1988; Kandemir, Kence, & Kence, 2000; 2005)

Honeybee diversity in Turkey was extensively studied using allozyme polymorphism, mitochondrial DNA, RAPD and microsatellite markers (Smith, Slaymaker, Palmer, & Kaftanoglu, 1997; Palmer, Smith, & Kaftanoglu, 2000; Bodur, Kence, & Kence, 2007; Tunca & Kence, 2011; Karabağ, Tunca, Sevim, & Doğaroğlu,

2020). Inter-SSR markers is based on amplification of genomic DNA segments flanked by the inversely oriented and closely spaced within microsatellite loci using microsatellite core unit bearing oligonucleotide primers. It combines the elements of microsatellite and RAPD methods (Wu, Jones, Danneberger, & Scolnik, 1994). ISSR has fewer time and material cost compared to microsatellite analysis as it does not require designing specific primers for the taxon to be analyzed (Zietkiewicz, Rafalski, & Labuda, 1994). It also produces more polymorphism compared to RAPD (Gupta, Chyi, Romerao-Severson, & Owen, 1994) ISSR was previously used to analyze the genetic differences in honeybee populations of Saudi Arabia (Al Otaibi, 2008) and Lithuania (Čeksteryte, Paplauskienė, Tamasauskienė, Pasakinskienė, & Mazeikiene, 2012). The aim of the study is to assess the utility of ISSR markers to discriminate some honeybee populations of different

subspecies and regions in Turkey.

Materials and Methods

Worker honeybees were sampled from hives of pure line native subspecies maintained in the common garden of METU Biology Department. Subspecies used in this study are: *A.m. caucasica* from Artvin province, *A.m. syriaca* from Hatay, *A.m. carnica* from Kırklareli and, Yiğilca and Muğla ecotypes of *A.m. anatoliaca*. Head and thorax parts honeybee samples which were frozen at -80°C were dissected out and homogenized in ice cold TE buffer. DNA extraction from the homogenates were performed using Fermentas DNA isolation kit according to manufacturer's instructions. DNA concentration and quality in samples were determined by Nanodrop 2000c spectrometer. Five of SSR primers belonging to a primer set prepared by Biotechnology Laboratory of University of British Columbia (Vancouver, Canada) were used in PCR amplification (Table 1). ISSR-PCRs were carried out in a Techne TC-5000 gradient thermal cycler and reaction mixtures were prepared in a total volume of 15 µL each containing 1X PCR buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl and 0.1% Triton X-100), 2 mM of MgCl₂, 200 µM of each of the dNTPs (dATP, dCTP, dGTP and dTTP), 0.25 µM of primer, 0.5 U a qDNA polymerase (MBI, Fermentas) and 25 ng of template DNA. Time and thermal profile of the PCR were as follows 2 min of initial denaturation at 94°C, followed by 34 cycles; each consisting of a denaturation segment of 20 s at 94°C, an annealing segment of 1 min at 54 to 56°C depending on the primer and an extension segment of 2 min at 72°C. PCR was terminated with a final extension period of 5 min at 72°C, and products were electrophoresed in 1.5% agarose gels along with one lane of size markers. Fragments were visualized on a UV transilluminator following staining with 0.4 µg/mL ethidium bromide solution (Figure 1).

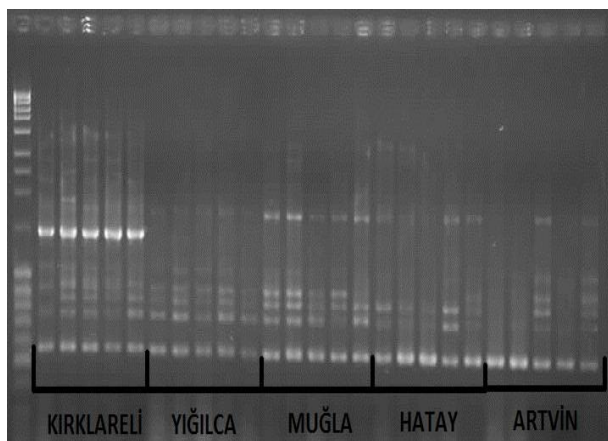


Figure 1. An example of PCR profiles of honey bees belonging to five populations.

Screening 150 honeybee samples with 5 SSR primers produced at 19 gel positions (loci). Each locus was treated as a separate character, and the presence or the absence of a PCR product at each position was scored as 1 and 0 respectively. Principal Component analysis (PCA) was performed by NTSYS-pc program to define and discriminate study samples. Population structure and possible hybridizations between samples were analyzed by Structure Software. Other genetic variation parameters were calculated by PopGene Program

Results

A total of 150 bee samples from 5 populations were screened with 5 primers in this study. In terms of genetic variation parameters (Table 1) populations showed a gradual increase with Artvin (*caucasica*) has the lowest while Kırklareli (*carnica*) had the highest scores (Table 2). The two- and three-dimensional graphics (Figures 2 and 3) of PCA analysis shows that the sample groups of four subspecies involved in our study, clearly separate from each other while Yiğilca and Muğla ecotypes of *anatoliaca* subspecies are generally grouped together. Population structure analysis (Figure 4) also show distinctive patterns for each subspecies although there is some degree of introgression between the populations. Yiğilca and Muğla ecotypes of *anatoliaca* honeybee also show remarkably similar structural patterns.

Discussion and Conclusion

Studies showed evidence of genetic homogenization and loss of genetic diversity in some areas of Europe. Dall'Olio, Marino, Lodesani, and Moritz (2007) reported high level of homogenization and decrease in previously reported genetic diversity in Italian peninsula. Canovas, de la Rua, Serrano, and Galian (2011) also found an increased level of homogenization in Iberian honeybee (*A. m. iberiensis*) colonies from Spain. Bouga et al. (2005 a,b) observed no genetic distinction in terms of mitochondrial and allozyme data between native *adami*, *macedonica* and *cecropia* subspecies in Greece. The main reasons for this phenomenon are migratory beekeeping and replacing the queens of native honeybee colonies with commercial breeds of other subspecies (especially *caucasica* in Turkey) and their hybrids (Kükrer, 2013). Therefore, a simple, fast and cost-effective method is needed for genetic screening for native honeybee subspecies to detect possible homogenization and introgression events.

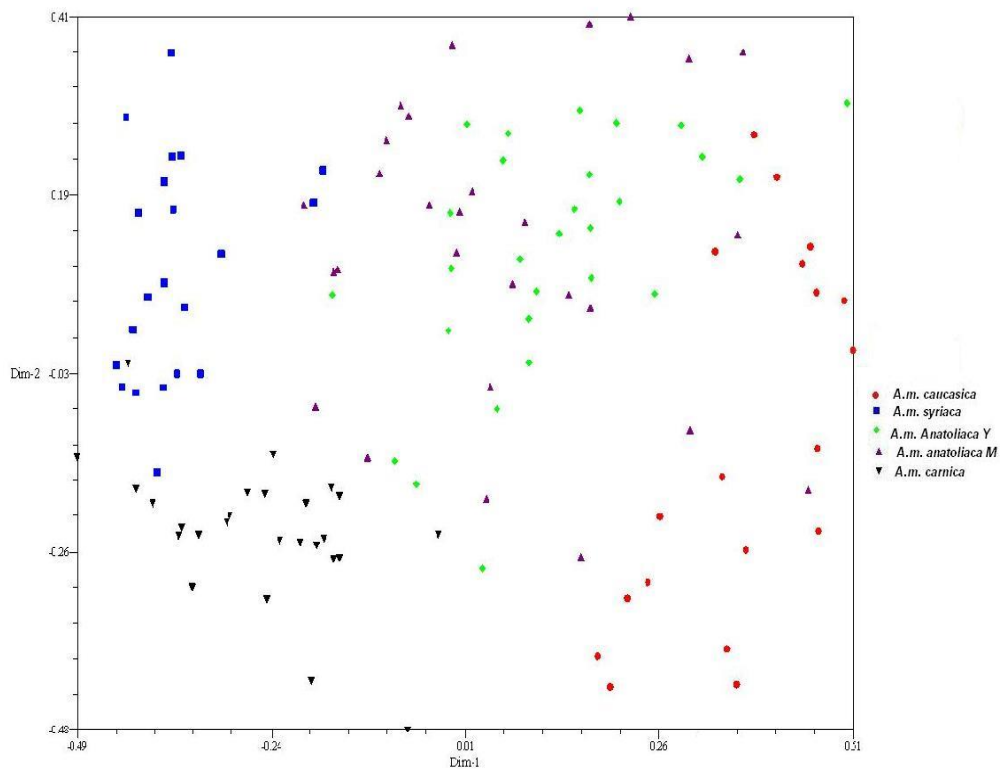
ISSR markers appeared to be an effective tool in analyzing and discriminating different honeybee populations and subspecies. Population structure analysis show distinct patterns between subspecies with some degree of hybridization. This is consistent with

Table 1. Primer sequences and numbers of gel positions scored.

Primers	Sequences 5'→3'	Total Bands	Polymorphic bands	Percentage of polymorphic Bands
UBC 818	CAC ACA CAC ACA CAC AG	3	2	66
UBC 840	CAC ACA CAC ACA CAC AG	5	4	80
UBC 847	CAC ACA CAC ACA CAC ARC	4	4	100
UBC 856	ACA CAC ACA CAC ACA CYA	5	4	80
UBC 899	CAT GGT GTT GGT CAT TGT TCC	2	2	100

Table 2. Genetic variation and polymorphism in different indigenous honeybee populations from Turkey. NPL number of polymorphic loci, PPL percentage of polymorphic loci, N_a observed number of alleles per locus, N_e effective number of alleles, H Nei's gene diversity / Shannon's Information index.

Populations	NPL	PPL	N_a	N_e	H	I
Artvin	10	50	1.500±0.513	1.250±0.361	0.146±0.196	0.223±0.280
Hatay	11	55	1.550±0.510	1.268±0.358	0.160±0.193	0.246±0.275
Kırklareli	12	60	1.600±0.502	1.393±0.389	0.228±0.207	0.337±0.297
Muğla	14	70	1.700±0.470	1.422±0.372	0.248±0.200	0.370±0.283
Yığılca	17	85	1.850±0.366	1.425±0.351	0.257±0.178	0.395±0.241

**Figure 2.** Two-dimensional principle coordinate analysis of 150 honey bees from 5 populations according to a distance matrix based on Jacard Index.

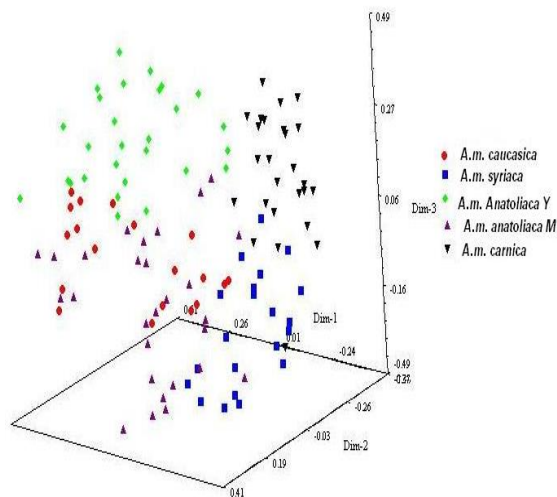


Figure 3: Three-dimensional principle coordinate analysis of 150 honey bees from 5 populations according to a distance matrix based on Jacard Index.

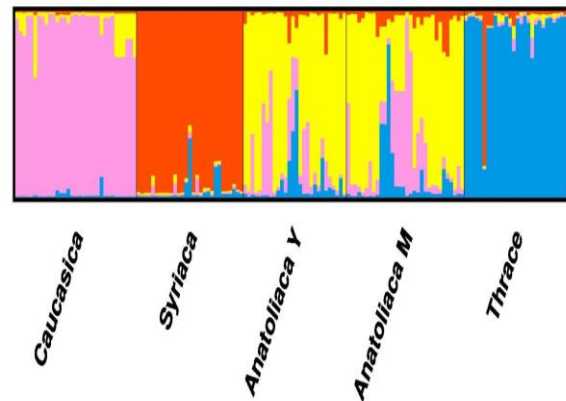


Figure 4: Structure analysis for five honey bee populations of Turkey.

previous studies which showed that honeybee populations in different regions of Anatolia still retain their distinctive genetic structures despite hybridization caused by migratory beekeeping (Tunca, 2009, Kükrcer, 2013). In addition to this, Yiğilca and Muğla ecotypes of *A. m. Anatoliaca* subspecies are revealed to be genetically quite similar even though they are adapted to different ecological conditions. In conclusion, ISSR method proved itself to be a simple and low-cost method capable of identifying and discriminating honeybee subspecies and populations of Turkey.

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