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A Study of the Diversity in COI-COII Intergenic Region of Mitochondrial DNA in Different Persian Honeybee (A. Mellifera Meda)

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ABSTRACT

This study was the determination of genetic variation in COI- COII itergenic region of mtDNA in different persian haneybee (*A. Mellifera meda*) populations bye using PCR-RFLP and DNA sequencing methods and *DraI* and *HinfI* restrictyion enzymes. 92 worker honeybee collected from 15 different localities of Iran were used as material. *DraI* digestion of COI- COII itergenic region revealed 4 restriction fregment with 41, 47, 64 and 420 bp. İn all samples and no genetic variation was detected among the samples. Restriction with *HinfI* revealed two different haplotypes. İn 3 samples collected from Karaj, Ardabil and Hamedan *HinfI* digestion revealed 3 restriction fregment with 292,260 and 26 bp. (COI-II/ HinfI-C1) and the rest of samples revealed 4 restriction fregment with 292, 240, 26 and 20 bp.(COI-II/ HinfI-C2). According to *DraI* restriction Persian honeybee (*A. Mellifera meda*) was grouped as a member of C mtDNA lineage. The result of *HinfI* restriction shows that the Karaj, Ardabil and Hamedan populations are only different haplotype (COI-II/ HinfI-C1) from other populatipns. PCR-RFLP results were confirmed bye DNA sequencing.

KEYWORDS: COI-COII intergenic region, DNA sequence analysis، honey bee, mtDNA.

INTRODUCTION

One of the populated insects in the classification of animals is honeybee (Apis mellifera) which is spread all over the world beyond geography limitations. Corenet et.al [1] began their studies on the 63 samples of honeybees and they identified 4 various sizes (200, 250, 450 and 650 open pair) in the area between Cytochrome C Oxidase I and II Controller Genes. The bees of the longest part were under sequence setting and 2 different units were concluded (P: 54 open pairs containing 100% of A+T) and (Q: 196 open pairs containing 93% of A+T). Investigated samples showed various sizes of Q, PQ, PQQ and PQQQ. On the other hand, Q was divided into 3 different parts: Q1 (similar to 3' controller gene), Q2 (similar to tRNA controller gene (cintaining lucien)) and Q3 (similar to P). The sequence of P was observed in some and lacked in others. Unit P was observed in the size of 54 open pairs (P) in some samples and in other samples it was at the size of 69 open pairs (P0). The difference between P and P0 sequences was about 15 open pairs. Garnery et.al [2] studied the areas between controller genes of COI and COII related to honeybees' mitochondria genome. This area was proliferated through PCR and cut by Dra I limited enzyme. 21 haplotypes were identified in samoles containing 302 population of 12 bees' sub-groups. In part C, all the samples showed equal band population and were named C1 and part M showed 10 various haplotypes. Morits et.al [3] studied 102 samples from 29 different areas of Southern Africa honeybees. In this study areas between controller genes of COI and COII was proliferated through PCR and cut by Dra I limited enzyme and 4 parts of different sizes and 9 mitotypes were identified. The P0QQ showed the most frequency of 76%.

Özdile [4] carried out his study on 244 bee samples from 21 different areas of Turkey. Like previous researches, the area between controller genes of COI and COII of mitochondria genome was cut by Dra I limited enzyme and 5 different haplotypes were identified. 11 kinds of haplotype was identified in open sequence setting of this genetic area whose 7 kinds was related to C1 haplotype and 4 kinds to C2. In this study, haplotype C1 consisted of C1a, C1b, C1c, C1d, C1e, C1f, C1g and C2 consisted C2a, C2e, C2f and C2g. Haplotypes of C1a, C2a, C2e and C2g were reported in previous researches but haplotypes of C1b, C1c, C1d, C1e, C1f, C1g and C2f were identified for the first time.

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In this study, the main focus is on tha gathering of various samples of honey bees from all over Iran and in different population of Iranian bees the diversity of the area between controller genes of Cytochrome C Oxidase I and II mitochondria genome was identified by the indicator of RFLP on the bases of PRC technique.

MATERIALS AND METHODS

In this study the bees of 15 regions (Urumie, Ziveh, Tabriz, Qare Ziaodin, Zanjan, Ardebil, Karaj, Hamedan, Talesh and Ramsar) in Iran were sampled and about 92 mature worker bees were selected (by considering cost and budget) in order to continue the study.

The Increase of the Number of Area between Controller Genes of Cytochrome C Oxidase I and II through PRC

The isolation of genom DNA through Hall method [5-6] were done in laboratory condition and the part between controller genes of cytochrome C oxidase I and II is a place where is responsible for the control of tRNA (lucien) production. And the end of 5' controller gene COII is proliferated through Garnery et.al method and the sequence of utilized primares in this study is:

Primare F (Forward): 5' GGC AGA ATA AGT GCA TTG 3'

Primare R (Reverse): 5' CAA TAT CAT TGA TGA CC 3'

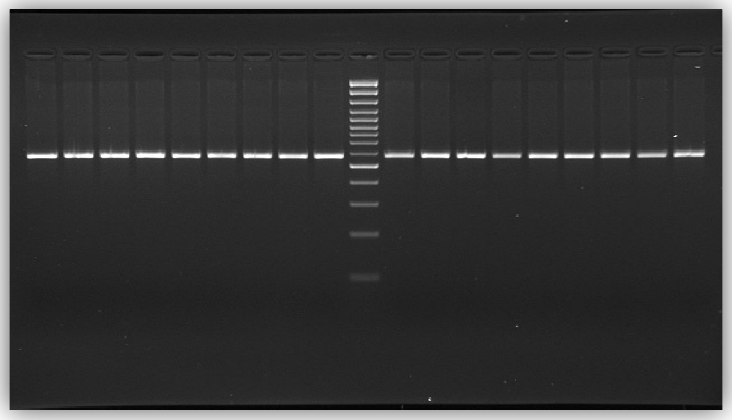


Fig1. The produced strips from gene area between controller genes of cytochrome C oxidase I, II in the length of 578 open strain pairs in 18 samples through polymerase chain reaction on agarose jelly 4%

The cut of COI-COII Intergenic Region through limited enzymes of Dra I and Hinf I

After proliferation of mentioned part through PCR, the production of this reaction were cut by limited enzymes of Dra I Hinf I under the temperature of 37 degree and the created samples were under electrophoresis on the metaphor agarose jelly 4% and poly acril amid jelly 10% and the differences of samples were observed in the infrared photography delicated to ethidium bromide of jelly.

Identification of the gene area sequence between controller genes of cytochrome C oxidase I and II

The automatic machine of genetic analyzer ABI PRISM 310 Avant was used in order to identify the open sequene related to the area between controller genes COI and COII in gathered samples of bees from different regions in Iran(area with the length of 515 open pairs in between 3380-3895 open pair).

RESULTS AND DISCUSSION

As considering the area between controller genes of cytochrome C oxidase I and II mitochonderia genome of bees gathered from 15 different regions of Iran, their genetic structure, haplotypes and the differences among their population were estimated. These estimation was carried out through utilizing lilited enzymes of Dra I, Hinf I, polymerase chain reaction technique, multi-form analyser on the bases of polymerase chain reaction (PCR-RFLP) and the sequence setting of nitric organic open sequence.

The results of cutting genetic areas between controller genes of cytochrome C oxidase I and II by limited enzyme of Dra I

In this study, the genetic area between controller genes of cytochrome C oxidase I and II was proliferated by polymerase chain reaction and then was cut by Dra I limited enzyme. This cutting caused 3 different points on all the samples. The length of these cuttings were 41, 47, 64 and 420 open pairs and there

were 4 strips on each sample on poly acril amid 10% jelly. This model were reported by Frank et.al [7] and on the bases of this report, Iranian honeybees are similar to genetics strain of C and hypotype C1.

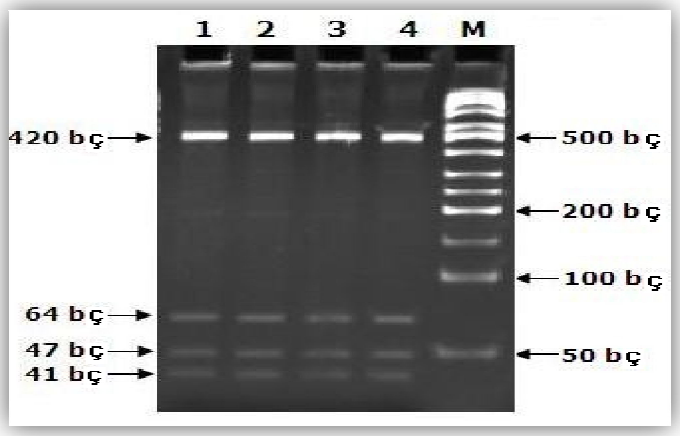


Fig2. The produced strips through Dra I limited enzyme cutting the area between controllers. Genes CO I and CO II of mitochondria genome on poly acril amid 10% jelly

In a study by Garnerry et.al [2] similar results were reached which was carried out on honey samples gathered from Astara region. Furthermore, other samples of Iranian honeybees were in C genetic part according to mitochondria genome.

Frank et.al [7-9] in 3 different researches on area between controller genes of C cytochrome oxidase I and II mitochondria genome of honeybees related to the races of A. m. Cypria, A. m. Armeniaca, A. m. Caucasica and A. m. anatolica observed that similar bands were created in mentioned genetic area by Dra I limited enzyme cutting and these bees were placed in C genetic place. On the other side, A. m. Syriaca were on O place according to mitochondria genome.

The results of cutting genetic areas between controller genes of cytochrome C oxidase I and II by limited enzyme of Hinf I

The productions of polymerase chain reaction were cut for genetic area by Hinf I limited enzyme and resulted in 2 different models of this enzyme. One of this models was observed in samples gathered from samples of Karaj, Ardebil and Hamedan. This genetic area was cut from 2 different points and parts of 26, 260 and 292 open pairs were created which show 3 strips on metaphor agarose 4% jelly. The second model was related to the samples gathered from different regions. This enzyme cut the gene from 3 various points and 4 part in the length of 20, 26, 242 and 292 open pairs were created. The cause of this variety on genetic area is the change of cytosine nucleotide by thymine nucleotide.

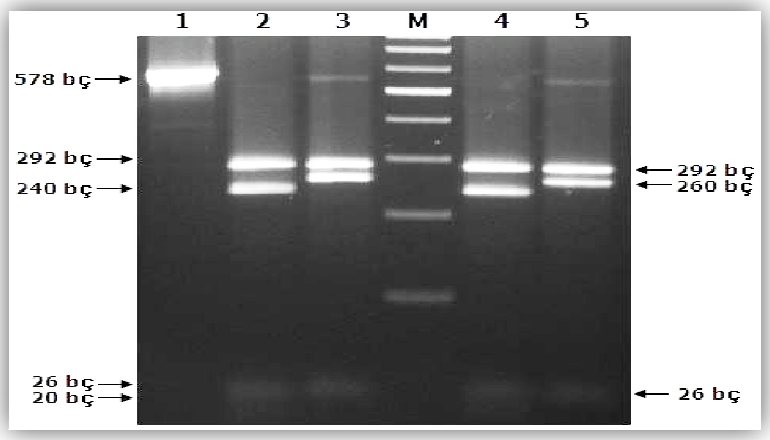


Fig 3. The produced strips through HinfI limited enzyme cutting the area between controller genes CO I and CO II of mitochondria genome on metaphor agarose4% jelly

Yılız study on bees in Turkey shows that there are 2 different haploides through cutting Hinf I [10]. But there was one kind of haplotype (COI-II/Hinf-C1) in one samples of Bulu and Yeghija and also other samples were of the second haplotype (COI-II/Hinf I-C2).
Supasat et.al [11] cut the genetic areas related to Thailand honeybees by Hinf I and identified 2 different haplitype (TahiA2 and TahiA1). There were 3 strip model of 26, 260 and 292 related to A. m. Ligustica and a number of A. m. Carnica in TahiA1. Also there were 4 strip models of 20, 26, 240 and 292 just related to A. m. Carnica in TahiA2.

According to mentioned studies, scarce haplotype (CO I-II/Hinf I-C1) and the most spreaded haplotype (CO I-II/Hinf I-C2) which showed similar result of all above researchers, the high number of haplotype CO I-II/Hinf I-C2 in Iran goes back to the import of queenbee (A. m. Carnica and A.m. caucasica) for 25 years.

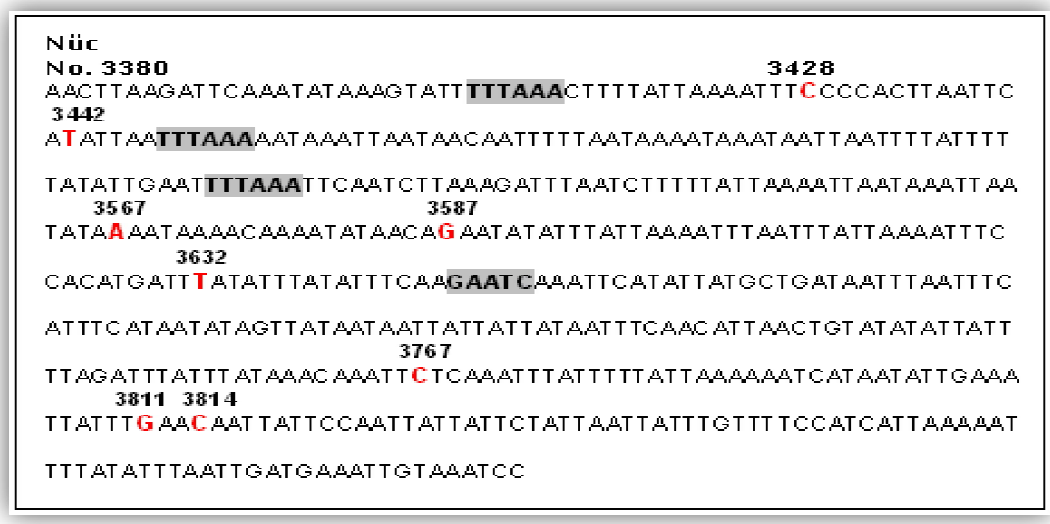
The results of sequence setting

In tested samples of genetic area related to mitichondria genome, it was observed a part of 515 open pair and it was compared to A. m. Ligustica . The different nucleotides are shown in table.1. On the bases of carried out studies, Iranian honeybees place in C part because of the various length and polymerase chain reaction.

Table1. The sequence of A. m. Ligustica bee as a control(-) shows the failed part and (.) same nucleotides

base number in Mitochondria genome			**3428**		**3442**		**3567**		**3587**		**3632**		**3767**		**3811**		**3814**
A. m ligustica		..	C	..	T	..	A	..	G	..	T	..	C	..	G	..	C
Iranian honey bee	254	..	.	..	-	..	.	..	A	..	C	..	T	..	.	..	.
	255	..	.	..	-	..	T	..	.	..	C	..	T	..	.	..	.
	1, 2, 4, 7, 8, 10	..	-	..	-	..	T	..	.	..	C	..	T	..	.	..	.
	5, 9	..	-	..	-	..	.	..	A	..	C	..	T	..	.	..	.
	3	..	-	..	-	..	.	..	.	..	C	..	T	..	.	..	.
	6	**..**	**-**	**..**	**-**	**..**	**T**	**..**	**.**	**..**	**C**	**..**	**T**	**..**	**C**	**..**	**G**

Table2. The nucleotide sequence of part 515 open pair between 3380-3859. TRNA contains lucien and there is a sequence of Q and the end of 5' CO II. The nucleotides are shown in different colors by the cutting of Dra I and Hinf I.



Conclusion

Iranian honeybee is classified in C genetic part through 3 different cutting points of Dra I limited enzyme in controller genes of cytochrome C oxidase I and II of mitichondria genome. There were 2 various haplotypes through utilizing Hinf I enzyme cutting genetic area between controller genes of cytochrome C oxidase I and II related to mitochondria genome. Haplotype of CO I- II/Hinf I-C1 is a scarce kind and it was observed in Karaj, Ardebil and Hamedan. The second haplotype of CO I- II/Hinf I-C2 is a spreaded kind which is found in the regions except than Karaj, Ardebil and Hamedan.

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