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Comparison of non-coding intergenic region of mtDNA of the European honeybee *Apis mellifera* L.with a haplotype of Indian honeybee *Apis cerana indica* from Tamilnadu, Southern India

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Abstract

Non - coding intergenic region between tRNA and COII of mtDNA from a sample of *A.cerana indica* from Tamilnadu State, Southern India was sequenced and compared with its western counter part *Apis mellifera*. Results showed high amount of similarity.

Keywords: *Apis mellifera, Apis cerana*, haplotype, mtDNA, non-coding region.

INTRODUCTION

Biodiversity of the honeybees was first assessed using morphometrics based on an extensive study and multivariate analyses by Ruttner *et al.*, (1978). However the analysis of mtDNA has become a widely used approach in studying the biogeography of the honey bees (Cornuet *et al*., 1991) and has been shown to be a powerful tool in discriminating variations in species and subspecies of honey bees (Moritz *et al.*, 1986; Smith, 1988; Smith and Brown 1988; Hall and Muralidharan (1989). In recent years mt DNA analysis has been used to determine the level of inter- and intra specific genetic variations and population of differentiation of honey bees (Smith *et al*., 2000). MtDNA analysis is particularly good for determining stock structures among conspecific populations because effective population size estimated from mtDNA polymorphism is smaller than that of nuclear DNA (Birky *et al.*, 1989) and is more reliable to show effects of genetic drift and faster evolutionary changes than nuclear DNA (Ward and Grewe, 1994). In addition mtDNA of an individual within a particular colony of honey bees is identical. As a result one individual can represent the genetic pattern of the colony obviating effects of within colony variation (Garnery *et al*.,1995).

The Asian hive bee *Apis cerana* was calssified into four subspecies by Ruttner (1987, 1988) on the basis of morphometric variation and geographic distribution. It is closely related to western honey bee species *Apis mellifera*. The African and European bee races were studied by using mtDNA (Moritz *et al*, 1994). The complete sequence of mtDNA of *A.mellifera* was reported by Crozier and Crozier (1993). The molecular data on the evolutionary history of honey bee based on mtDNA analysis indicates that *A.cerana* is the close relative to *A. mellifera* (Garnery *et al.*, 1991and 1992). Variation in mtDNA of *A.cerana* was first reported by Smith (1991).

Mitochondrial haplotype diversity across the range of *Apis cerana* is suggestive of its biological diversity (Smith and Hagen, 1996; Smith *et al*; 2000, 2004; Hepburn *et al*., 2001a,b, Smith, 2002; Arias and Sheppard, 2005). In India, two races of *A. cerana*, (Hill and Plain) and often recognized. The analysis of mtDNA diversity in India was studied by Smith and Hagen (1996), Smith *et al.*, (2000) and Tanaka *et al*., (2001). Their studies were restricted only in few parts of northern India and there is no study in the Tamilnadu (southern India) so far. So a case study was carried out as a first step on this line to fill up this lacuna. We investigated of mtDNA from non-coding region between tRNA and CO II a sample *Apis cerana indica* collected from Tamilnadu, Southern India and compared it with its counterpart, the Western honey bee *A. mellifera* L.

MATERIALS AND METHODS

Sampling

Adult *A.cerana* workers honeybees were collected, killed by immersing in absolute ethanol and kept at –20°C until they were processed in the laboratory. One worker bee per colony was analyzed for mitochondrial DNA

DNA Isolation and Amplification

Genomic DNA was extracted from the thorax of *A. cerana* by following the method described by Smith and Hagen (1996) with slight modifications.One µL of this solution was used for the PCR amplification. The tRNA leu-COII region was amplified following Smith and Hagen, (1996).with the primers located at the 5′-end of the tRNA leu gene and located at the 5′-end of the COII gene in a total volume of 12.5 µL. The size of the PCR amplified products was determined after the electrophoretic

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separation on 1.5% agarose gels. Amplification primers: TCTATACCACGACGTTATIC 3090 (Cytochrome Oxidase I) and GATCAATATCATTGATGACC 3937 (Cytochrome Oxidase II) (Smith and Hagen, 1996).

DNA Sequencing

Amplified portion of PCR product from the representative *A.cerana* was sequenced on an automated DNA sequencer (Applied Biosystem) at the sequencing service (Chromous Biotech, Bangalore, South India). Nucleotide sequences obtained were aligned using Clustal W (Thompson *et.al*., 1994), where the 5′ and sequence of a 82 bp fragment implied from *A. mellifera* was a reference.

The sequences were compared using the bioinformtics tools BLAST and CLUSTALW.

RESULTS AND DISCUSSION

The non-coding intergenic region of mtDNA from the European honeybees *Apis mellifera* of 848 base pairs and *Apis cerana indica* of 744 bp were sequenced and data were given below.

Sequence data (848 bp): (*Apis mellifera* L.)

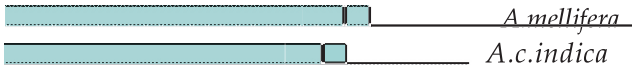
TCTATACCACGACGTTATTCAGACTATCCAGATTCTTATTACTGTTGAAATTCAATTTCATCTATAGGATCAATAA
TTTCATTAAATAGAATAATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATTATTATTTAAATT
CAACCAATCATCACTTGAATGATTAAATTTTTTACCACCTCTAGATCATTCACATTTAGAAATTCCATTATTAATTA
AAAATTTAAATTTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAACTTAAGATTCAAATAT
AAAGTATTTTTAAACTTTTATTAAAATTTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTTTAA
TAAAATAAATAATTAATTTTATTTTTATATTGAATTTTAAATTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAAT
AAATTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATTTA
TATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTTCATTTCATAATATAGTTATAATAATTATTA
TTATAATTTCAACATTAACTGTATATATTATTTTAGATTTATTTATAAACAAATTCTCAAATTTATTTTTATTAAAAAA
TCATAATATTGAAATTATTTGAACATTATTCCAATTATTATTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTA
TATTTAATTGATGAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATATTGATC

Sequence data (744 bp): (*Apis cerana indica*)

TCTATACCACGACGTTATTCAGATTATCCAGATTCTTATTATTGTTGAAATTCAATTTCATCATTAGGATCAATAA
TTTCATTAAATAGAATTATCTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCAAAACGATTAATATTATTCAA
ATTTAATCAATCATCTCTTGAATGATTAAATTTTTATCCACCATTAGATCACTCACATTTAGAAATTCCAATATTA
ATTAAAAACTTAAATATAAAATCAATTTTAGTTAAATTTTAATATGGCAGAATAGTGCATTGAATTTAAGATTCA
AAAATAAAGTTTTTAATTTACTTTTATTAAAAATTTAATAAATTATAATTATATTTAATTTTTAATATAATATACAA
TTTTATAATTTATTAAAATTAATAATTTAAAAATTTATTATTAAAATTTCTACATGATTCATATTTATATTTCAAG
AATCAAATTCATACTACGCTGATAATTTAATTTCATTCCATAATATAGTAATAATAATTATTATTATAATTTCTAC
ATTAACAGTTTATATTATTATAGATCTTTTTTTTAAATAAATTTTCTAATTTATTTTTATTAAAAAATCATAATATTG
AAATTATTTGAACAGTAATCCCAATTATTATTTTATTAATTATTTGTTTCCCATCATTAAAAATTTTATATTTAATT
GATGAAATTGTAAATCCATTTTTTTCTGTAAAATCAATTGGTCATCAATGATATTGATC

The alignment data showed that there was a gap or deleted region of ~105 bp within the region analyzed. The gap or deletion was confirmed bi-directionally. The BLAST shows that the sample sequence data *Apis cerana indica* matched with *Apis mellifera* sequence data between bases 1 and 338 as well as bases 521 and 848 (*Apis mellifera*). Thus, the region between 338 bp and 521 bp (183 bp in size) on *Apis mellifera* is replaced with region 338 bp and 417 bp (79 bp in size) on *Apis cerana indica*. There was 91 % identity between the two sequences.

BLAST data (sequence alignment of the honey bee species):

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TCTATACCACGACGTTATTCAGACTATCCAGATTCTTATTACTGTTGAAATTCAATTTCA
||||||||||||||||||||||| |||||||||||||||| ||||||||||||||||||
TCTATACCACGACGTTATTCAGATTATCCAGATTCTTATTATTGTTGAAATTCAATTTCA

TCTATAGGATCAATAATTTCATTAAATAGAATAATTTTTTTTAATTTTTATTATTTTAGAA
||  |||||||||||||||||||||||||||||| || ||||||||||||||||||||||||
TCATTAGGATCAATAATTTCATTAAATAGAATTATCTTTTTAATTTTTATTATTTTAGAA

AGATTAATTTCTAAACGAATATTATTATTTAAATTCAACCAATCATCACTTGAATGATTA
|||||||||||| |||||| || ||||||| ||||| || |||||||| ||||||||||||
AGATTAATTTCAAAACGATTAATATTATTCAAATTTAATCAATCATCTCTTGAATGATTA

AATTTTTTACCACCTCTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTA
|||||||  |||||  ||||||| |||||||||||||||||| |||||||||||||| |||
AATTTTTATCCACCATTAGATCACTCACATTTAGAAATTCCAATATTAATTAAAAACTTA

AATTTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAACTTAAGA
|||  |||||||||||||| |||||||||||||||||||||| |||||||||||| ||||||
AATATAAAATCAATTTTAGTTAAATTTTAATATGGCAGAAT-AGTGCATTGAATTTAAGA

TTCAAATATAAAGTATTT-TTAAACTTTTATTAAAATTT
|||||| ||||||| |||  |  ||||||||||||||||
TTCAAAAATAAAGTTTTTAATTTACTTTTATTAAAATTT


TATTAAAATTTCCACATGATTTATATTTATATTTCAAGAATCAAATTCATATTATGCTG
|||||||||||| |||||||| |||||||||||||||||||||||||||||| || ||||
TTATTAAAATTTCTACATGATTCATATTTATATTTCAAGAATCAAATTCATACTACGCTG

ATAATTTAATTTCATTTCATAATATAGTTATAATAATTATTATTATAATTTCAACATTAA
|||||||||||||||| |||||||||| |||||||||||||||||||||||| |||||||
ATAATTTAATTTCATTCCATAATATAGTAATAATAATTATTATTATAATTTCTACATTAA

CTGTATATATTATTTTAGATTTATTTATAAACAAATTCTCAAATTTATTTTTATTAAAAA
| || ||||||||| ||||| | ||| |||| ||||| || ||||||||||||||||||||
CAGTTTATATTATTATAGATCTTTTTTTAAATAAATTTTCTAATTTATTTTTATTAAAAA

ATCATAATATTGAAATTATTTGAACAATTATTCCAATTATTATTCTATTAATTATTTGTT
||||||||||||||||||||||||||| | || |||||||||||| ||||||||||||||
ATCATAATATTGAAATTATTTGAACAGTAATCCCAATTATTATTTTATTAATTATTTGTT

TTCCATCATTAAAAATTTTATATTTAATTGATGAAATTGTAAATCCTTTTTTTTCAATTA
| ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||  | |
TCCCATCATTAAAAATTTTATATTTAATTGATGAAATTGTAAATCCATTTTTTTCTGTAA

AATCAATTGGTCATCAATGATATTGATC 848  A. mellifera
|||||||||||||||||||||||||||||
AATCAATTGGTCATCAATGATATTGATC 744  A.c. indica
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Results of CLUSTALW analysis is given below, which also shows 91% identity between the sequences.

CLUSTAL W (1.83) multiple sequence alignment

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AM          TCTATACCACGACGTTATTCAGACTATCCAGATTCTTATTACTGTTGAAATTCAATTTCA 60
AC          TCTATACCACGACGTTATTCAGATTATCCAGATTCTTATTATTGTTGAAATTCAATTTCA 60
            ************************ ***************** ******************

AM          TCTATAGGATCAATAATTTCATTAAATAGAATAATTTTTTTTAATTTTTATTATTTTTAGAA 120
AC          TCATTAGGATCAATAATTTCATTAAATAGAATTATCTTTTTAATTTTTATTATTTTTAGAA 120
            **  ************************** ** ************************

AM          AGATTAATTTCTAAACGAATATTATTATTTAAATTCAACCAATCATCACTTGAATGATTA 180
AC          AGATTAATTTCAAAACGATTAATATTATTCAAATTTAATCAATCATCTCTTGAATGATTA 180
            *********** ****** ** ******* ***** ** ******** ************

AM          AATTTTTTACCACCTCTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTA 240
AC          AATTTTTATCCACCATTAGATCACTCACATTTAGAAATTCCAATTATTAATTAAAAACTTA 240
            *******  *****  ******* ****************** ************ ***

AM          AATTTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAACTTAAGA 300
AC          AATATAAAATCAATTTTAGTTAAATTTTAATATGGCAGAATA-GTGCATTGAATTTAAGA 299
            *** ************** ********************** ********** ******

AM          TTCAAATATAAAGTATTTTT-TAAACTTTTATTAAAATTTCCCCACTTAATTCATATTAAT 359
AC          TTCAAAAATAAAGTTTTTAATTTACTTTTATTAAAATTT---------------AATAAA 344
            ****** ******* *** *  ***************              * ***

AM          TTAAAAATAAATTAATAACAATTTTTAATAAAATAAATAATTAATTTTATTTTTATATTG 419
AC          TTATAATTATATT-----TAATTTTTAATATAATATACAATT---------------- 381
            *** ** ** ***      ********** **** * ****

AM          AATTTTAAATTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAAA 479
AC          --TTATAA-------------------------TTTATTAAAATTAATAATTTAA------ 409
              ** ***                         ***************** ****

AM          ATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATG 539
AC          ------------------------------AAATTTA--TTATTAAAATTTCTACATG 435
                                          *******  ************* *****

AM          ATTTATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTTCATTTCA 599
AC          ATTCATATTTATATTTCAAGAATCAAATTCATACTACGCTGATAATTTAATTTCATTCCA 495
            *** ****************************** ** ******************** **

AM          TAATATAGTTATAATAATTATTATTATAATTTCAACATTAACTGTATATATTATTTTAGA 659
AC          TAATATAGTAATAATAATTATTATTATAATTTCTACATTAACAGTTTATATTATTATAGA 555
            ********* ********************** ******** ** ********* ****

AM          TTTATTTATAAACAAATTCTCAAATTTATTTTTATTAAAAAATCATAATATTGAAATTAT 719
AC          TCTTTTTTTAAATAAATTTTCTAATTTATTTTTATTAAAAAATCATAATATTGAAATTAT 615
            * * *** **** ***** ** ************************************

AM          TTGAACATTA-TTCCAATTATTATTCTATTAATTATTTGTTTTCCATCATTAAAAATTTT 778
AC          TTGAACAGTAATCCCAATTATTATTTTATTAATTATTTGTTTTCCATCATTAAAAATTTT 675
            ******* ** * ************ ***************** ******************

AM          ATATTTAATTGATGAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATG 838
AC          ATATTTAATTGATGAAATTGTAAATCCATTTTTTTCTGTAAAATCAATTGGTCATCAATG 735
            **************************** ********  * ********************

AM          ATATTGATC 847
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AM = *Apis mellifera*, AC = *A.c. indica*

Morphological (Ruttner, 1988; Hepburn *et al*., 2001a; Radloff *et al*., 2005) and mitochondrial haplotype (Smith and Hagen, 1996; Smith *et al*., 2000, 2004; Hepburn *et al.,* 2001b; Smith, 2002) diversity across the range of *A.cerana* is suggestive of its biological diversity. The results of the present study is a new contribution to the mtDNA haplotype diversity of *A.c.indica.*

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