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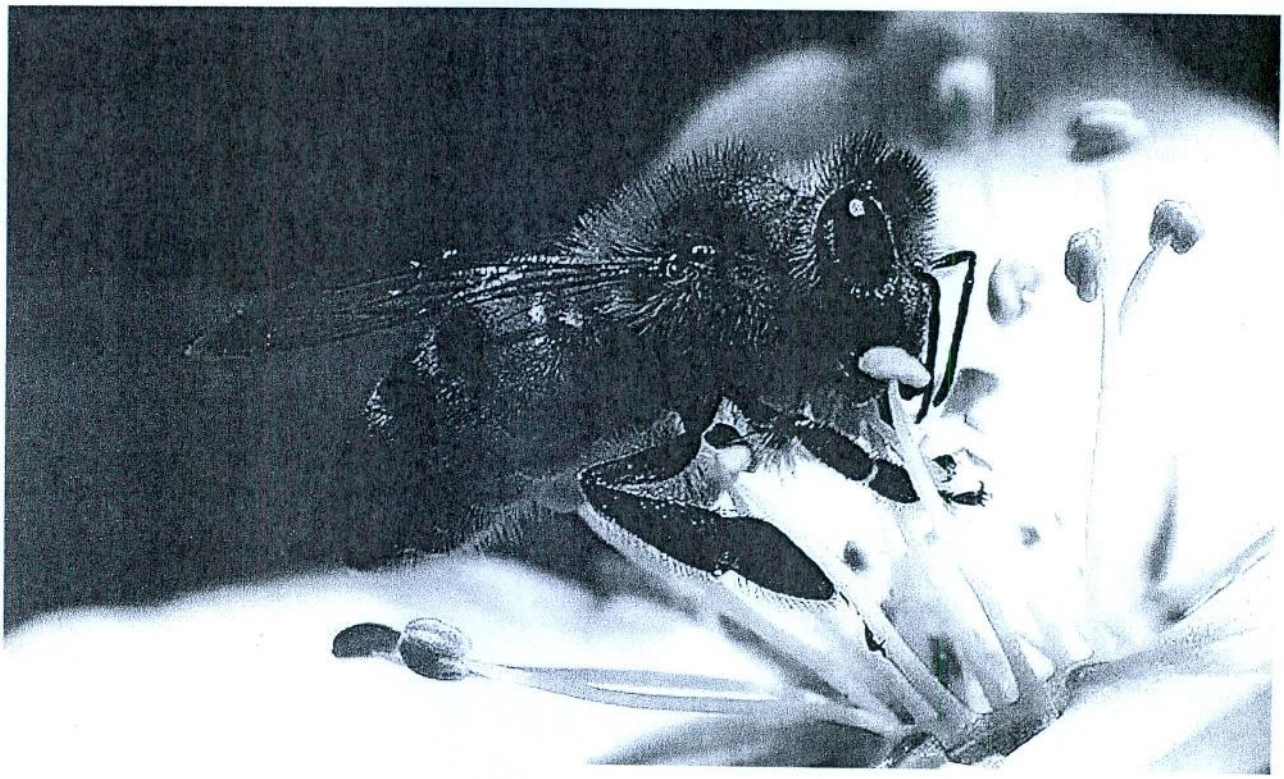
Genetic analyses of Syrian honeybee diversity '*Apis mellifera syriaca*'



PRESENTED BY:

Mr. Mohamed Alburaki
Student: Master II

SUPERVISORS:

Dr. Lionel Garnery
Laboratory of Evolution,
Genomes and Speciation (LEGS/CNRS) France.

Dr. Ali Alburaki
University of Damascus,
Faculty of Agriculture, Honeybee Lab, Syria.

Dr. Bassam Issa
University of Damascus,
Faculty of Agriculture, Syria.

Research carried out at:

Laboratory of Evolution, Genomes and Speciation (LEGS/CNRS), Bât 13, Avenue de la Terrasse 91198 Gif - sur -Yvette, France.

Declaration

I hereby declare that the research work described in this thesis entitled " **Genetic analyses of Syrian honeybee diversity *'Apis mellifera syriaca'*** " has not been, and is not actually submitted to obtain any other University degree, and that all experiments and results indicated are of my own realization under the direction of my scientific supervisors. Sources of any other data, methods or results included have been clearly mentioned in the text and in the reference list.

The candidate
Mohamed Alburaki

Supervisors Approval:

Dr. Lionel Garnery (CNRS, Gif- sur-Yvette, France)

Prof. Dr. Ali Alburaki (Univ. of Damascus, Faculty of Agriculture)

Prof. Dr. Bassam Issa (Univ. of Damascus, Faculty of Agriculture)

The board of examiners

Prof. Dr.	Prof. Dr.	Prof. Dr.
Issam Kassem	**Bassam Issa**	**Majd Jamal**
Damascus University	Damascus University	Damascus University

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ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
APS	Ammonium persulphate
Big Dye	Reaction mix
bp	Base Pair
BSA	Serum albumin bovine
CAPS	Cleaved Amplified Polymorphisms
COI	Cytochrome Oxidase I
COII	Cytochrome Oxidase II
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide triphosphate
*Dra*I	Restriction endonuclease
EDTA	Ethylene di-amine tetra acetic acid
ETB	Ethidium Bromide
Kb	Kilo base
*mt*DNA	Mitochondrial DNA
Nt	Nucleotide
PCR	Polymerase Chain Reaction
Pk	Proteinase K
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragments Length Polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
Rox	Internal lane standard
Rpm	Revolutions per minute
SSRs	Simple Sequences Repeats
TBE	Tris borate EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	N-tris (hydroxymethyl) amino ethane
tRNA	transfer RNA
UHQ	Ultra high quality
UV	Ultra violet
VNTRs	Variable Number of Tandem Repeats

ABSTRACT

Five hundred DNA samples of Syrian honeybee '*Apis mellifera syriaca*' have been collected from five different regions in Syria: these are the most important regions for beekeeping. To identify the genetic diversity and the different evolutionary lineages of Syrian honeybees (Ruttner, 1988), the DNA samples were analyzed by PCR-RFLP (CAPS) technique. The analyses were done by using two molecular markers: *mt*DNA and microsatellites loci (Garnery *et al.*, 1992). PCR techniques were employed to amplify the intergenic region COI-COII (<500-950> bp). The restriction enzyme *Dra*I was used for identification of the haplotypes and to characterize the restriction patterns of these amplified intergenic sequences. The restriction assay was analyzed on poly-acrylamide gels to compare and analyze the band patterns with the already existing *mt*DNA markers for the determination of different haplotypes. Four new haplotypes were found and sequenced. The microsatellite PCR primers: specifically designed to amplify the microsatellite regions (SSR) were used to estimate -using genomic DNA- the level of variation of Syrian honeybee populations, and their relationships with populations from other evolutionary lineages. The results obtained were compared with already existing data, to determine the genetic diversity of Syrian honeybee '*Apis mellifera syriaca*'.

Key words: '*Apis mellifera syriaca*', DNA, *mt*DNA, PCR, PCR-RFLP, CAPS, SSR, Microsatellites loci, Intergenic region COI-COII and haplotype.

RÉSUMÉ

Cinq cent échantillons d'abeilles syriennes '*Apis mellifera syriaca*' ont été prélevés de cinq différentes régions de la Syrie (un individu par colonie) et analysés en utilisant deux marqueurs moléculaires, l'ADN mitochondrial et les loci microsatellites (Garnery *et al.*, 1992). L'ADN total (ADN*mt* et ADN génomique) a été extrait en utilisant la méthode Chelex sur thorax. L'ADN*mt* a été analysé par PCR-RFLP (CAPS), une technique qui permet d'identifier les haplotypes et qui se compose de l'amplification de la région intergénique COI-COII (<500-950> pb) du génome mitochondrial par PCR suivi d'une digestion des fragments à l'aide de l'enzyme de restriction *Dra*I. Quatre nouveaux haplotypes ont été identifiés et séquencés. Des amorces spécifiquement conçues pour amplifier les régions microsatellites (SSR), au niveau de l'ADN génomique, ont été utilisées pour évaluer le niveau de variation génétique des populations d'abeilles syriennes et leurs rapports avec des populations d'autres lignées évolutives. Les résultats obtenus ont été comparés avec des données déjà existantes pour déterminer la diversité génétique de l'abeille syrienne '*Apis mellifera syriaca*'.

Mots clés: '*Apis mellifera syriaca*', ADN, ADN*mt*, PCR-RFLP, CAPS, SSR, Loci Microsatellites, Région intergénique COI-COII et haplotype.

1 INTRODUCTION

The honeybee, with its high level of social colonial organization, has been the most fascinating, the most appreciated and the most highly insect observed by the man. A lot of economic, nutritive and medicinal benefits are related to the hive products and honeybee itself such as honey, pollen, royal jelly, propolis and wax. Even the venom of honeybee sting is employed for the treatment of arthritis, multiple sclerosis and other auto-immune diseases. On the other hand, the importance of the domestic bee in agriculture is fundamental due to its indispensable role in crop and natural plant pollination. Many of the flowering plants depend completely on insects in general and bees in particular for their pollination and will produce more when bees are particularly numerous. The value of the role played by the honeybee in pollination of flowers far exceeds the economic returns from the hive.

A strong relationship exists between the social behaviour of honeybee and flowering plants. The flowering plants appeared on the earth approximately 100 to 150 million years ago. For million of years, bees were solitary, on the basis of morphological evidence. The attainment of sociality as evidenced in the genus '*Apis*' was apparently established about 30 million years ago (Culliney, 1983). This social insect, as most of other social bees, was characterized and classified under the order: '*Hymenoptera*', and super family '*Apoidae*', and was placed in family '*Apidae*', as '*Apis mellifera*' (*Apis*: bee, *mellifera*: carrier of honey) by Linnaeus in 1758. Its name came from the idea that honeybee takes honey from the plants and carries it to their hives. We understood only later that the honeybees themselves produce the honey and use it as their food.

1.1 Relationship between honeybee and flowers

Honeybees visit flowers in search of nectar which is a vital component for honey production by the bees. Flowering plants have optimized their flowers to increase the

chance of pollination. So when the bees visit them they unwittingly carry pollen from flower to flower, thus pollinating the plants and permitting them to reproduce. Plants most successful in attracting bees and getting them to make repeated visits will out-reproduce those which are less successful. Thus flowers must attract and reward a visitor insect.

1.2 General biology of honeybee

The developmental cycle of the honeybee is particular. It is a haplo-diploid cycle; the diploid queen is able to provide two individual types, either diploid individuals (workers or queens), possessing 32 chromosomes after fertilization of the egg, or haploid males containing 16 chromosomes, resulting from unfertilized egg (Figure 1).

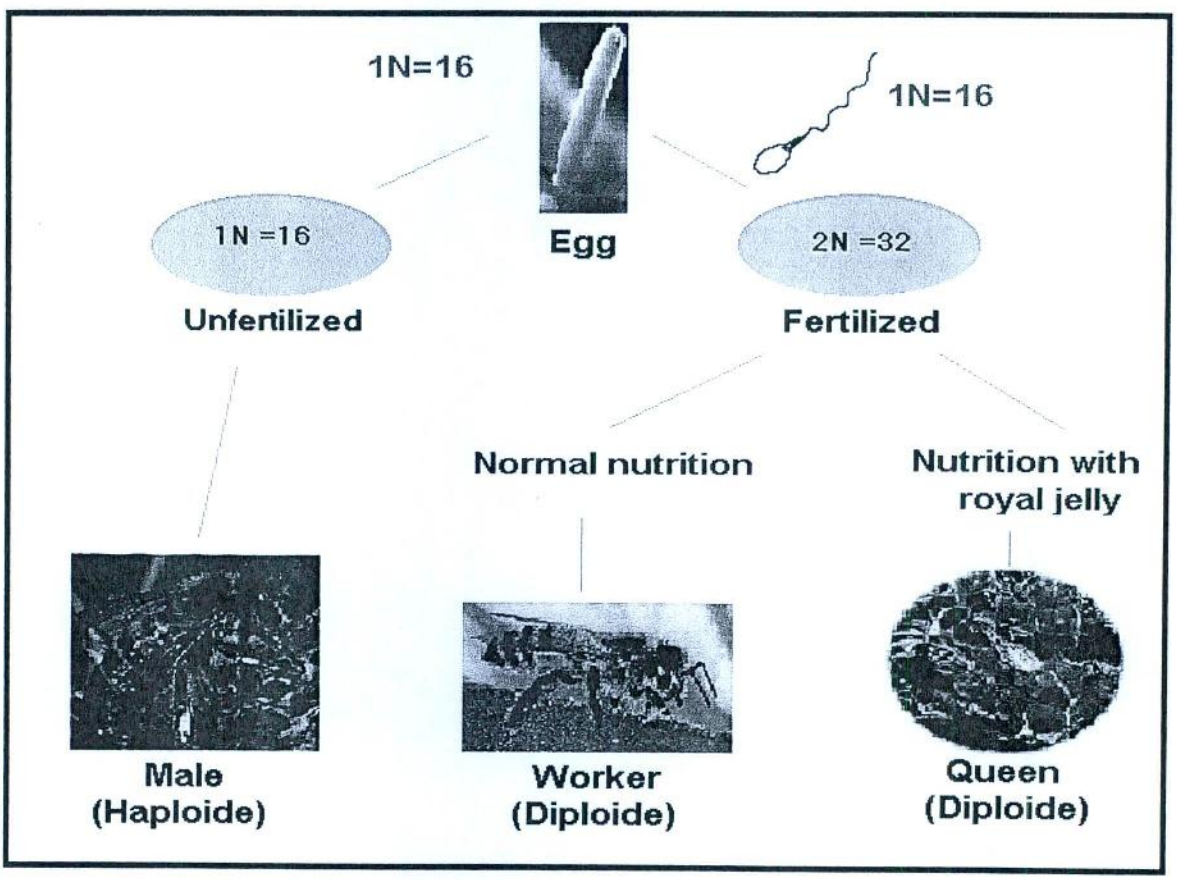

Fig.1: Differentiation of the eggs laid by the queen (Winston, 1993).

Fertilized eggs of the queen that produce a male larva (diploid male) are eaten by

the workers of the colony while fertilized ones that produce female larvae can develop into workers and or queens. Normal males are produced from unfertilized eggs.

The honeybee has, for a long time, been a biological reference and model for fundamental physiological and behavioural studies on insects. Scientists have conducted studies on morphometric and enzymatic characteristics of honeybee. Recently, the development of sophisticated techniques for the analysis of DNA polymorphism allowed us to better address questions of genetic diversity.

1.3 Evolutionary lineages of the domestic honeybee

Before being scattered all over the world by man, '*Apis mellifera*' was confined to Europe, Africa and Middle East. In its initial area of distribution, 26 geographic races or subspecies were recognized on the basis of morphological studies (Ruttner, 1988; Sheppard *et al.*, 1997; Sheppard and Meixner, 2003). The morphometrical studies thus allowed to group these subspecies in four evolutionary lineages (Ruttner, 1988): the African lineage (A) that group together the subspecies of Africa. The North Mediterranean lineage (C) that groups those of Central and Eastern Europe. The third one (M), the West Mediterranean lineage includes the subspecies of North Africa, North and West of Europe. The fourth one is the Oriental lineage (O) that groups the subspecies of Caucasus and Turkey (Figure 2).