

Genetic Structure of *Varroa* Mite Populations in *A. Mellifera syriaca*

¹Nizar Haddad N, Hussein Migdadi, ²Jay Evans J, Jeff Pettis J

¹National Center for Agricultural Research and Technology Transfer, Baqa' 19381 Jordan,

²United States Department of Agriculture, Agricultural Research Service, Bee Research Laboratory, Beltsville, Maryland 20705.

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ABSTRACT

Jordan is considered as a transcendent area between the African and the Asian countries. To provide a more precise picture of the genetic structure of varroa populations in Jordan compare with world wide picture, a total of 60 female mites belong to 10 *A.m ligustica* colonies collected from north of Jordan and 20 mites from *A.m syriaca* colonies collected from two locations; one from south where the bees are kept in traditional wooden hives and the second from the north in which bees are kept in a clay mode hives. These two traditional apiaries were found to be closest to the historical samples collected by Brother Adam in 1952. Using specific oligo nucleotide primers CO1F.F and CO1N.R and the amplified products were sequenced using Big Dye 2.0 end- terminal cycle sequencing and analyzed on an Applied Biosystems 3130 DNA analyzer. Sixteen varroa mites collected from several sites in Jordan were successfully sequenced and compared to those from Algeria, Bolivia, South Korea, Vietnam, China, Japan, Nepal and Sri Lanka. All Jordanian samples were matches with Korean haplotype of *V.destructor*. This is the first analysis of *V.destructor* types in Jordan parasitizing on *A.m.syriaca*. Further collection within *A.m.syriaca* can help confirm that the mites in this subspecies are as with *A.m ligustica* predominantly the Korean haplotype.

Keywords: Honeybee, *A. m. syriaca*, Varroa mites, Korean haplotype, PCR, sequencing.

Introduction

Beekeeping and hunting of wild honey have been important in the Middle East generally and in Jordan particularly for thousands of years[6]. The honeybee of the eastern Mediterranean, *A. mellifera syriaca*, is found in the regions of Syria, Lebanon, Israel, Gaza Strip and Jordan. First detailed descriptions were given by Buttell-Reepen[2], and its racial status was later confirmed by Ruttner based on samples collected by Brother Adam in[1].

Due to the high defensive behavior of the *A. mellifera syriaca*, many beekeepers started in the late seventies a world wide import of honey bee queens and bee colonies (MoA Jordan), but recent research confirmed that it is possible to find *A. mellifera syriaca* in some areas of Jordan kept in traditional bee hives[6]. In 1986 the beekeeping sector faced

major losses, with over 50% of the beehives dying. This happened one year after the first detection of the ectoparasitic varroa mite[9], but until now little information about honeybees in general and *Varroa* mites in particular are available from the Middle East.

The aim of this study was to gain insight into the genetic traits and invasion process of *V. destructor* at both local and worldwide scale and on the interaction between the Korea and Japan *Varroa* mites types in particular.

Material and methods

a. Populations

The studied populations consisted of a total of 60 female mites belonging to 10 *A.m. ligustica* from the

north of Jordan, where the bees are kept in modern bee hives and 20 mites from *A. mellifera syriaca* populations in 2 different locations, where the 1st location in the south where the bees are kept in traditional wooden hives while the 2nd location in the northern part of Jordan where the bees are kept in clay mode hives, these two traditional apiaries were conformed as the closest to the historical samples collected by Brother Adam in the 1952, using morphometric[6] and mitochondrial DNA methods[7].

b. DNA extraction and sequencing of *Varroa mites*.

Individual mites were dried in sterile microcentrifuge tubes, and then ground using disposable pestles. DNA was extracted by suspending these samples in 100 ul 10% Chelex-100 (Bio-Rad), followed by incubation at 95°C for 10 minutes. A 1:10 dilution of this suspension was used for PCR. using specific oligonucleotide primers CO1F.F and CO1N.R[3], sequences 5'-CAGATCGAAATTTTAATA C-3' and 5'-TACAGCTCCTATAGATAAAA-3', respectively). Reaction mixes consisted of 2 ul mite DNA extract, 2 U Taq DNA polymerase with recommended buffer (Boehringer), 1mM DNTP mix, 2mM MgCl₂, and 0.2 μM of each primer. PCR was carried out on an MJ-Research PTC-100 thermal cycler using 30 cycles of 93 ° C 1 min, 55 ° C 1 min, 72 ° C 1 min. Bands of an appropriate size were confirmed by agarose gel electrophoresis, then PCR products were purified directly (Gene Pure). Products were sequenced using Big Dye 2.0 end-terminal cycle sequencing (Applied Biosystems), followed by separation and analysis on an Applied Biosystems 3130 DNA Analysis machine. Sequencing was carried out in one direction from the 5' end using primer CO1F.F.

c. Sequence analyses

Sequences were checked and trimmed using the software program Sequencher (Gene Codes). Sequences were then aligned with each other and with known COI sequences for *Varroa* mites 7 samples from the Genbank database, one haplotype from Algeria[10], and nine samples from Bolivia, South America (Evans, J.D. and Hopkins, D., unpublished). Of the Bolivian samples, three (QB1, QB5, and QB9) fell into the 'Japan' haplotype, while the rest were of the more widespread 'Korean' haplotype.

Results and discussions

A total of 16 *Varroa* mites collected from several sites in Jordan in addition to those from Algeria, Bolivia, South Korea, Vietnam, China, Japan, Nepal and Sri Lanka were sequenced

successfully. The Jordanian samples were grouped in one cluster and were matches with the 'Korean' haplotype of *V. destructor*, which has historically been the more virulent of the two widespread *V. destructor* haplotypes. The sequenced mites were collected equally from the two bee subspecies. This is the first analysis of *V. destructor* types that are parasitizing *A. mellifera syriaca*. We took advantage of the sequence of the entire mitochondrial genome of *V. destructor*[3,8] to provide a more precise picture of the genetic structure of *Varroa* populations in Jordan in comparing with worldwide picture because of the unique geographical position of Jordan as a transcendent area between the African and the Asian continents. Further collections within *A. m. syriaca* can help confirm that the mites in this subspecies are, as with *ligustica*, predominantly the Korean haplotype. If so, any differences in mite resistance between *A. m. syriaca* and *A. m. ligustica* will likely reflect resistance traits of these two bee subspecies, rather than those of their mite parasites.

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