Genetica
DOI 10.1007/s10709-016-9897-y

CrossMark

Evaluation of *Apis mellifera syriaca* Levant region honeybee conservation using comparative genome hybridization

Nizar Jamal Haddad[1] · **Ahmed Batainh**[1] · **Deepti Saini**[2] · **Osama Migdadi**[3] · **Mohamed Aiyaz**[2] · **Rushiraj Manchiganti**[2] · **Venkatesh Krishnamurthy**[2] · **Banan Al-Shagour**[1] · **Mohammad Brake**[4] · **Lelania Bourgeois**[5] · **Lilia De Guzman**[5] · **Thomas Rinderer**[5] · **Zayed Mahoud Hamouri**[3]

Received: 11 July 2015 / Accepted: 21 March 2016
© Springer International Publishing Switzerland 2016

Abstract *Apis mellifera syriaca* is the native honeybee subspecies of Jordan and much of the Levant region. It expresses behavioral adaptations to a regional climate with very high temperatures, nectar dearth in summer, attacks of the Oriental wasp and is resistant to *Varroa* mites. The *A. m. syriaca* control reference sample (CRS) in this study was originally collected and stored since 2001 from "Wadi Ben Hammad", a remote valley in the southern region of Jordan. Morphometric and mitochondrial DNA markers of these honeybees had shown highest similarity to reference *A. m. syriaca* samples collected in 1952 by Brother Adam of samples collected from the Middle East. Samples 1–5 were collected from the National Center for Agricultural Research and Extension breeding apiary which was established for the conservation of *A. m. syriaca*. Our objective was to determine the success of an *A. m. syriaca* honey bee conservation program using genomic information from an array-based comparative genomic hybridization platform to evaluate genetic similarities to a historic reference collection (CRS). Our results had shown insignificant genomic differences between the current population in the conservation program and the CRS indicated that program is successfully conserving *A. m. syriaca.* Functional genomic variations were identified which are useful for conservation monitoring and may be useful for breeding programs designed to improve locally adapted strains of *A. m. syriaca.*

Keywords *Apis mellifera syriaca* · Array aCGH · SNP · *Varroa* resistance · Dry conditions

Introduction

Apis mellifera syriaca, is the native honeybee subspecies of Jordan and much of the Levant region (Ruttner 1988, 1992). Its behavioral manifestations include reduced brood rearing during the hottest months, an increasing swarming tendency, frequent absconding, and an ability to adapt egg laying to pollen availability and honey flow, keeping a hive clean, and a somewhat nervous temperament (Bodenheimer and Ben-Nerya 1937; Zakour et al. 2012; Ruttner 1992; Haddad and Fuchs 2004). It has an excessive production of swarm cells and, in particular, survival of virgin queens in the colonies until a mated queen has returned thus avoiding the risks of queen loss (Ruttner 1988). It is also adapted to withstand attacks from *Vespa orientalis* and *Merops orientalis* by ceasing flight activity when colonies are besieged (Blum 1956; Kalman 1973; Ruttner 1988; Haddad et al. 2005, 2007). Its colonies are usually free from the pollen beetle *Cryptophagus hexagonalis* in

Electronic supplementary material The online version of this article (doi:10.1007/s10709-016-9897-y) contains supplementary material, which is available to authorized users.

✉ Nizar Jamal Haddad
drnizarh@gmail.com; beeresearchdep@ncare.gov.jo

1 Bee Research Department, National Center for Agricultural Research and Extension, P.O.Box 639, Baqa' 19381, Jordan

2 Research and Development Unit, Genotypic Technology (P) Ltd, #259, Apurva, 2nd Floor, 4th Cross, R.M.V. 2nd Stage, 80 Feet Road, Bangalore 560 094, India

3 Jordanian Bee Research Station, National Center for Agricultural Research and Extension, Irbid, Jordan

4 Science Department, Science Faculty, Jerash University, Jerash, Jordan

5 Honey Bee Breeding, Genetics and Physiology Laboratory, ARS/USDA, 1157 Ben Hur Road, Baton Rouge, LA 70820, USA

comparison with *Apis m. ligustica* in the same apiaries and areas (Haddad et al. 2008). The genome of the *A.m. syriaca* has been sequenced as a prelude to identifying genes associated with their beneficial traits. (Haddad et al. 2015) Also, the mitochondrial genome of *A. m. syriaca* was recently reported (Haddad 2015).

Apis mellifera syriaca has the strongest hygienic behavior and the lowest *Varroa* infestation levels in comparison with *Apis m. anatoliaca, Apis m. carnica,* and *Apis m. caucasica.* These observations support the hypothesis that (sub) tropical bees have greater defenses against parasites (Kence et al. 2013). Wallberg et al. (2014) found that *A. m. syriaca* has gene flow between Africa and Asia, estimated to be about 18 %. This gene flow might have influenced both the strong defense behavior and the resistance of *A.m. syriaca* to *Varroa*. The samples which were used in the Wallberg et al. study are from the same apiary as the samples of the current study.

Conserving *A. m. syriaca* is an important priority for regional beekeeping. Much of the region's breeding programs of honeybees rely upon stocks imported from Europe, principally strains of Italian (*A. m. ligustica*), and Carniolan (*A. m. carnica*) origin (Haddad and Fuchs 2004) because they are well suited to management even though they lack local adaptability. While apiculture has benefited from using these European strains of honey bees, the presence of large numbers of European colonies in the region has the potential to jeopardize the genetic integrity of *A. m. syriaca* (Haddad and Fuchs 2004). *A. m. syriaca*, owing to its superior adaptations to its local environment and relatively higher resistance to *Varroa* has the potential to serve as a foundation for the development of a stock of honey bees with good beekeeping functionality provided its undesirable traits can be mitigated (i.e. limited honey production and strong defensive behavior). However, for *A. m. syriaca* to be maximized in stock development programs its preservation must first be assured since its definition as a clear sub-species is based on morphometric data. Haddad and Fuchs (2004) reported the influence of imported *A. m. ligustica* on honey bee populations in some regions of Jordan, but also found comparatively isolated areas (Wadi Ben Hammad and Abu Zead valley) with a population of A. *m. syriaca* from Jordan similar to reference samples collected in 1952 by Brother Adam and maintained at the Beekeeping Institute of the Polytechnische Gesellschaft in Oberursel, Germany. The Wadi Ben Hammad and Abu Zead populations were more similar to the 1952 samples than any other samples collected from the Levant region. Mitochondrial studies corresponded to these morphometric findings (Haddad et al. 2009).

The Wadi Ben Hammad and Abu Zead populations served as source populations for a project for the conservation of *A. m. syriaca* in Jordan, initiated in the year 2001. The aims of this project were to use selective breeding to improve the beekeeping functionality of this locally adapted strain for small-scale and medium-sized operations (Haddad and Fuchs 2004).

This program had three components. One population is maintained using instrumental insemination and is represented in this work by sample (1). A second population is selected for improved beekeeping characteristics and is maintained using natural mating in the un-isolated Maru research apiary where 1200–2000 queen bees cells are produced annually and distributed for free to the beekeepers in Jordan to increase numbers of *A. m. syriaca* drones in the drone congregation areas of the commercial beekeeping (samples 2 and 3). A third population is maintained without selection using natural mating in isolated areas of the Khanasry station (samples 4 and 5). Each sample represents one colony. The population at the Khansary station is intended to be a refuge population for the sub-species and is also valuable for determining changes in the selective breeding population.

To determine if our breeding populations in the program maintained their *A. m. syriaca* identity 11 years after their initiation, we obtained genome sequences of the *A. m. syriaca* control reference sample (CRS), collected in 2001, and compared it, using array comparative genomic hybridization (aCGH), with five *A. m. syriaca* samples from the Jordanian breeding program collected in 2012. The comparisons revealed genomic conservation during the past 11 years, thus confirming the existence of comparatively pure genetic resources of this subspecies in Jordan and the success of this conservation program.

Materials and methods

Sample collection

In 2012, five *A. m. syriaca* colonies were sampled (6 bees per colony) representing the three main components of the Jordanian breeding program. They are as follows: (1) artificial insemination lines, (2) and (3) *A. m. syriaca* drones from the natural mating lines in un-isolated Maru research apiary, (4) and (5) natural mating lines in isolated areas of Khanastry station. The control reference sample (CRS) was collected from Maro Station (Jordan) in 2001 when the breeding conservation program was started.

DNA extraction and evaluation

DNA was extracted from legs of individual honeybees (1 leg per bee, 6 bees per sample) by standard phenol chloroform procedures. Briefly, a leg was removed from each of the six

bees, pooled, crushed and then suspended in lysis buffer. 20 μL of Proteinase K 20 mg/mL (Qiagen, Cat. No. 19131) was added and the lysate was incubated at 56 °C for 2 h, followed by denaturation of Proteinase K at 65 °C for 15 min. 4 μL of RNase A 100 mg/mL (Qiagen, Cat. No. 19101) was added and incubated at room temperature for 30 min. Equal volume of Tris-equilibrated Phenol:Choloform:Isoamylalcohol (25:24:1) was added to the sample and vortexed. This solution was then transferred to a Phase lock Tube and centrifuged at $13000\times g$ for 12 min at room temperature. The aqueous phase was transferred to fresh 1.75 mL microfuge tube using cut tips and 1/10 volume of 3 M sodium acetate and 2.5 volume of cold absolute alcohol were added. The tubes were incubated at −80 °C for 30 min. The precipitated DNA was pelleted by centrifugation at $13{,}000\times g$ 30 min at 4 °C. DNA pellet was washed twice with 70 %. The supernatant was carefully aspirated and DNA pellet was dried using speed vacuum concentrator (45 °C for 2 min, Eppendorf Concentrator Plus, Eppendorf, Inc.) and then dissolved in 30 uL 1× TE pH. 8. The concentration and purity of the DNA extracted were evaluated using the Nanodrop Spectrophotometer (Thermo Scientific) and on 0.8 % agarose gel for integrity.

Microarray probe design

Genome-wide expression analysis of *Apis mellifera* was accomplished using Genotypic custom design and a comparative genome analysis on a microarray platform. The whole genome sequence of *Apis mellifera*, encompassing 16 chromosomes, was retrieved from the Assembled chromosome repository of NCBI (Amel 4.5). The complete genome sequence length was ~250 Mb (250,287,000 bp), which also contains repeat regions of about ~101 Mbp and gap regions of length ~21 Mbp. The sequence was masked for repeats and gaps, and final effective regions of length ~127 Mbp (127,987,805 bp) were considered for microarray design.

Probes were designed in the sense orientation with a distribution of ~60 bp spacing between each probe. The standardized protocol of probe design includes optimal GC %, melting temperature, removal of Poly A and palindromic segments. High quality probes were selected based on the above parameters and target specificity was checked using the BLAT alignment tool of UCSC genome browser. Probes having best alignment with the specific target regions were retained for array design. Final set of 416,484 probes which were feasible for Agilent's 2 × 480 K array format were considered for design. The probe group with all required annotations was uploaded in Agilent portal for AMADID generation.

Probe and microarray processing

Restriction digestion

A total of 2 μg DNA from each sample was digested with restriction enzymes Alu I (R0137S) and Rsa I (R0167S) at 37 °C for 2 h. The enzymes were heat inactivated at 65 °C for 20 min.

Sample labeling

Samples were labeled using the Agilent gDNA Labeling Kit (Part No: 5190-0449) following the kit-specified procedure. Briefly, 5 μl of random primers were added to 26 μL of each digested sample. The DNA was then denatured at 95 °C for 3 min followed by flash chilling at 4 °C for 5 min. 19 μL of labeling master mix containing Cy5-dUTP or Cy3-dUTP, dNTP, Buffer and Klenow, were added to the denatured DNA sample and incubated at 37 °C for 2 h, followed by enzyme heat inactivation at 65 °C for 10 min. DNA samples 1, 3, 4, and 5 were labeled with Cy3-dUTP and 2, 3, 5 and control with Cy5-dUTP.

Labeled DNA cleanup

The labeled samples were purified using Amicon Ultra columns, size 30 kDa (Catalog No.UFC503096). Samples were eluted with required volume with respect to array format.

Hybridization and wash

3 μg of each labeled Cy5-dUTP and Cy3-dUTP sample was combined and salmon sperm DNA (Catalog No: 1632-011), Agilent aCGH/CoC blocking agent (Part No: 5188-6416) and hybridization buffer (Part No: 5188-2460). The total hybridization volume was 260 μL. The labeled samples in above hybridization mix were denatured at 95 °C for 3 min and incubated at 37 °C for 30 min. The samples were hybridized onto the Agilent human 2 × 400 K CGH array (AMADID 35669) at 65 °C for 40 h. After hybridization, the slides were washed using aCGH Wash Buffer1 [Agilent Technologies, (Part No: 5188-5221/22) at room temperature for 5 min and aCGH Wash Buffer 2 (Agilent Technologies, Part No: 5188-5221/22)] at 37 °C for 1 min. The slides were then washed with Acetonitrile for 10 s. The microarray slide was scanned using an Agilent Microarray Scanner (Agilent Technologies, G2505C).

Data analysis

Feature extracted data from Agilent Feature Extraction Software were analyzed using Agilent GeneSpring GX Version 12 software. Two-color hybridizations were performed. Hybridization intensity values generated from feature extraction raw data were split based on channel type, Cy3 and Cy5. Normalization of the data was accomplished in GeneSpring GX using the 75th percentile shift and fold expression values were obtained in test samples with respect to the reference sample. Using the fold expression values, further analysis was carried out using the Agilent genomic workbench software (version 6.5) to identify the significant aberrations (amplification or deletion) in the five *A. m. syriaca* bee samples compared to CRS sample.

Functional analysis

DAVID database (http://david.abcc.ncifcrf.gov/) was used to retrieve homology based annotations for *Apis mellifera* with respect to the nearest homolog, *Drosophila melanogaste*r. Functional classification of the most significantly divergent genes was determined. Genes involved in varroa resistance and associated grooming behaviors (Navajas et al. 2008; Parker et al. 2012; Arechavaleta-Velasco et al. 2012; Boecking and Spivak 1999), were checked for variations. The microarray data discussed in this manuscript have been deposited in the NCBI Gene Expression Omnibus (GEO) under the GEO series accession number GSE43554.

Results

No significant differences were evident for comparisons of five contemporary samples of *A. syriaca* with the CRS collected in 2001. Overall, array comparisons showed low levels of variations in samples compared to the CRS, encompassing less than 2 % of all probes on the array. The number of amplifications exceeded the number of deletions in all samples.

Aberrations in *A. m. syriaca* bees compared to CRS sample

Table 1 shows the summary of the number of significant variations (amplification or deletion) in *A. m. syriaca* bees compared to the CRS. A small number of amplification and fewer deletions were observed in each of the 5 samples of *A. m. syriaca*. Table 2 shows percentage of variation in *A. m. syriaca* bees compared to the CRS. Total numbers of probes designed in the array are 416,477. Compared to this, 4427, 1150, 1474, 2195 and 5379 probes show variations (amplifications + deletions) for sample 1–5 respectively, as shown in Table 2a. This represents 1.06. 0.28, 0.35, 0.53 and 1.29 % of the total probes present on the array. The total number of probes that are common amongst the groups is 6934, which is 1.66 % of the total probes present on the array.

Table 1 Summary of number of aberrant regions in *A. m. syriaca* bees compared to the control reference sample (CRS)

Samples	Number of amplification	Number of deletion
1	381	11
2	233	14
3	379	3
4	353	20
5	400	100

Table 2b shows region wise variation in *A. m. syriaca* bees 1–5 compared to the CRS. *Apis mellifera* Genome size is ~236 Mb. Compared to this, 2,668,907, 523,230, 888,413, 973,977 and 3,377,395 bases show variations for sample 1–5 respectively, as shown in Table 2b. This represents 0.011, 0.002, 0.004, 0.004 and 0.014 % of the total genome size.

Chromosome wise genome wide alterations: amplification and deletions

Table 3 shows common amplification and deletions between the *A. m. syriaca* samples 1-5, distributed chromosome wise. The size of the amplified or deleted region and the number of probes covering that region are indicated.

Figure 1 shows chromosome level Genomic aberrations (amplifications and deletions) in all five *A. m. syriaca* bees compared to the CRS. Shown in the inner heat map are amplifications (red) and deletions (green) for each of the samples. Chromosome numbers are represented at periphery of chromosome ideograms with genomic coordinates in mega-bases and location of genes (blue).

Functions of the genes showing aberrations

For functional analyses, genes containing either amplifications or deletions were separated into functional categories which were primarily constitutive and not immunologically or behaviorally based. Figure 2 shows classification of aberrant genes based on function showing genes amplified and deleted. They fall mainly under the following categories: ATPases, cell adhesion, ion binding, kinases, localization genes, metabolic, nucleic acid binding

Table 2 Percentage of variation in *A. m. syriaca* bees compared to the CRS sample

Samples	Amplification	Deletion	Total	%
(a) Probe wise: Number of probes (aberrations) designed in the array: 416,477				
1	4187	240	4427	1.06
2	854	296	1150	0.28
3	1468	6	1474	0.35
4	1748	447	2195	0.53
5	3800	1579	5379	1.29
Common	5594	1340	6934	1.66

Samples	Amplification [bp]	Deletion [bp]	Total [bp]	%
(b) Coverage wise: *Apis mellifera* Genome size (~240 Mb)				
1	2,598,788	70,119	2,668,907	0.011
2	426,374	96,856	523,230	0.002
3	887,876	537	888,413	0.004
4	855,924	117,153	973,077	0.004
5	3,005,431	371,964	3,377,395	0.014
Common	3,690,585	394,269	4,084,854	0.0173

Table 3 Genome wide alterations common to all samples: amplification and deletions

Chromosome	Start pos.	End pos.	Size	No. of probes	*P* value	Amplification/deletion	Score
chr1	28,559,810	28,567,596	7787	11	0.000001	Amp	42.84
chr3	11,817,290	11,834,449	17,160	50	0.000002	Amp	34.18
chr3	11,744,496	11,747,226	2731	6	0.000000	Amp	59.03
chr3	11,744,786	11,747,226	2441	5	0.000001	Amp	40.40
chr5	5,586,491	5,589,130	2640	10	0.000002	Amp	33.17
chr5	13,883,567	13,895,491	11,925	8	0.000001	Amp	38.47
chr6	7,015,350	7,029,440	14,091	20	0.000002	Amp	37.40
chr7	7,542,136	7,548,149	6014	8	0.000002	Amp	35.18
chr8	7,551,481	7,552,980	1500	8	0.000002	Amp	34.73
chr9	9,038,161	9,039,120	960	5	0.000001	Amp	37.85
chr3	8,534,952	8,582,753	47,802	99	0.003726	Del	−5.01
chr3	10,823,948	10,850,262	26,315	87	0.003613	Del	−5.05
chr9	4,647,870	4,665,413	17,544	52	0.001222	Del	−6.80
chr9	4,909,808	4,926,659	16,852	52	0.001357	Del	−6.61
chr11	5,452,547	5,475,440	22,894	62	0.001683	Del	−6.24
chr11	5,458,811	5,475,440	16,630	54	0.002904	Del	−5.37
chr12	4,655,114	4,683,244	28,131	72	0.000962	Del	−7.25
chr12	4,651,574	4,664,129	12,556	70	0.001069	Del	−7.05
chr12	4,655,114	4,664,129	9016	60	0.000897	Del	−7.38
chr13	9,269,494	9,278,913	9420	54	0.017390	Del	−3.14

genes, pyrophosphatase activity, signal transduction, transcription, transferases, transmembrane, transport and Ubiquitin pathways. The most prevalent categories of functional amplifications were metabolic, localization, signal transduction and transcription. Deletions followed a similar pattern in that those with metabolic, signal transduction, transcription and pyrophosphatase activity were the most prevalent.

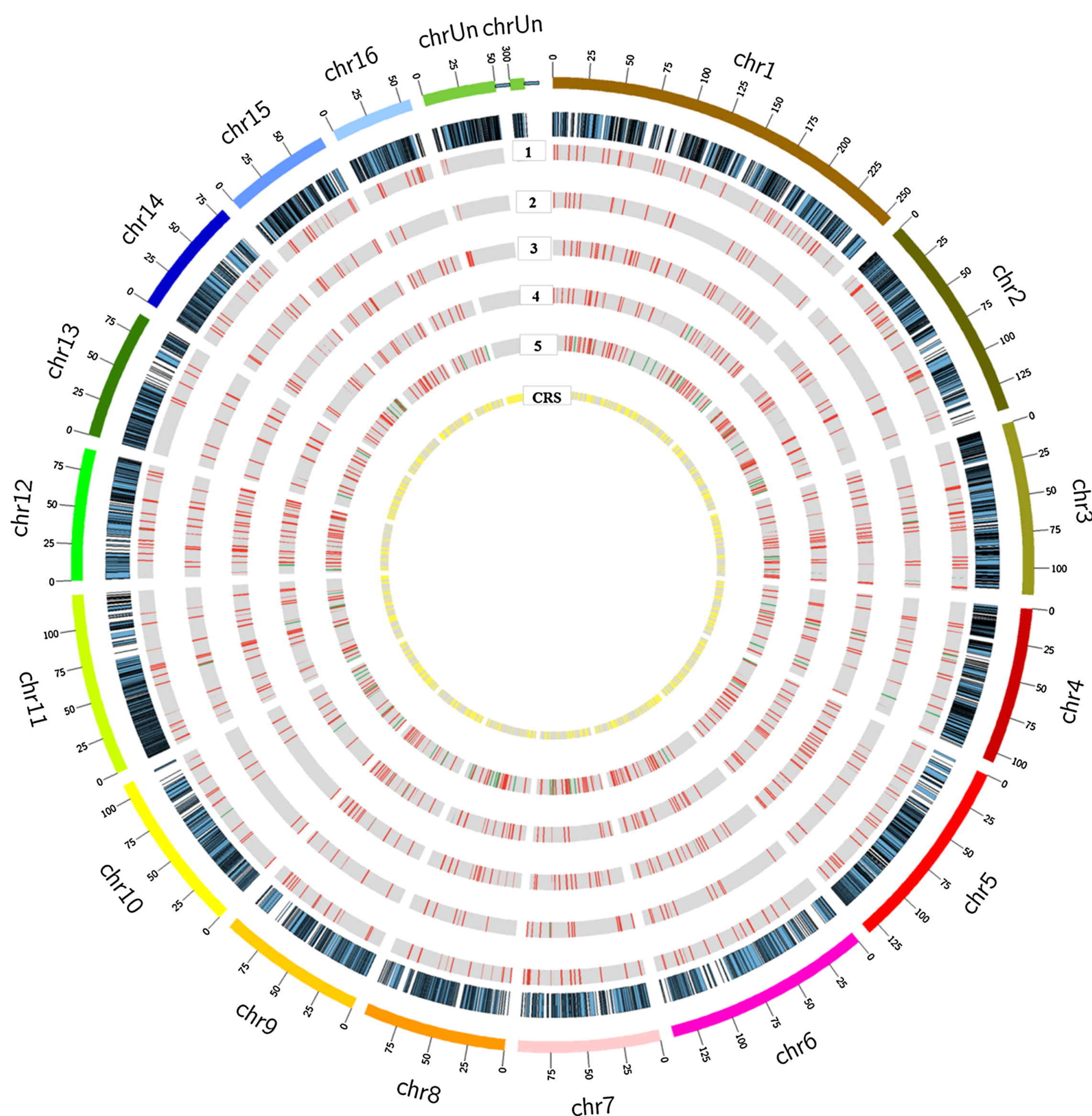

Fig. 1 Overview of genomic aberrations (amplifications and deletions) in all *A. m. syriaca* samples as compared to the control reference sample (CRS). Shown in the *inner* heat map are amplifications (*red*) and deletions (*green*) for each of the samples. Chromosome numbers are represented at the periphery of chromosome ideograms with genomic coordinates in mega-bases and location of genes (*blue*)

Discussion

Mostly due to development policies which have largely ignored well-adapted local stocks in developing countries, in the Middle East and other parts of the world, some indigenous honeybee stocks are at risk of becoming extinct (Ftayeh et al. 1994; Haddad and Fuchs 2004). Methodologies are not yet adequate for enhancing the diversity of honeybee genetic resources. Instead, introduction of "improved", exotic stocks as imports is the focus. Hence, to ensure survival and promote conservation, selection programs that improve efficiency and behavior in endangered breeds, whilst maintaining their environmental adaptation are started.

The predominant imported breeder lines are derived from *A. m. ligustica* (Blum 1956). Despite frequent importation of Italian bees, minimal influence of *A. m. ligustica* was detectable by morphometric analysis (Haddad and Fuchs 2004), thus confirming the preservation of comparatively pure genetic resources of the syriaca subspecies in some areas of Jordan. The samples collected in Jordan showed closest similarity to *A. m. syriaca*, as represented by reference samples collected in 1952 by Brother Adam in about the same region, than to any other

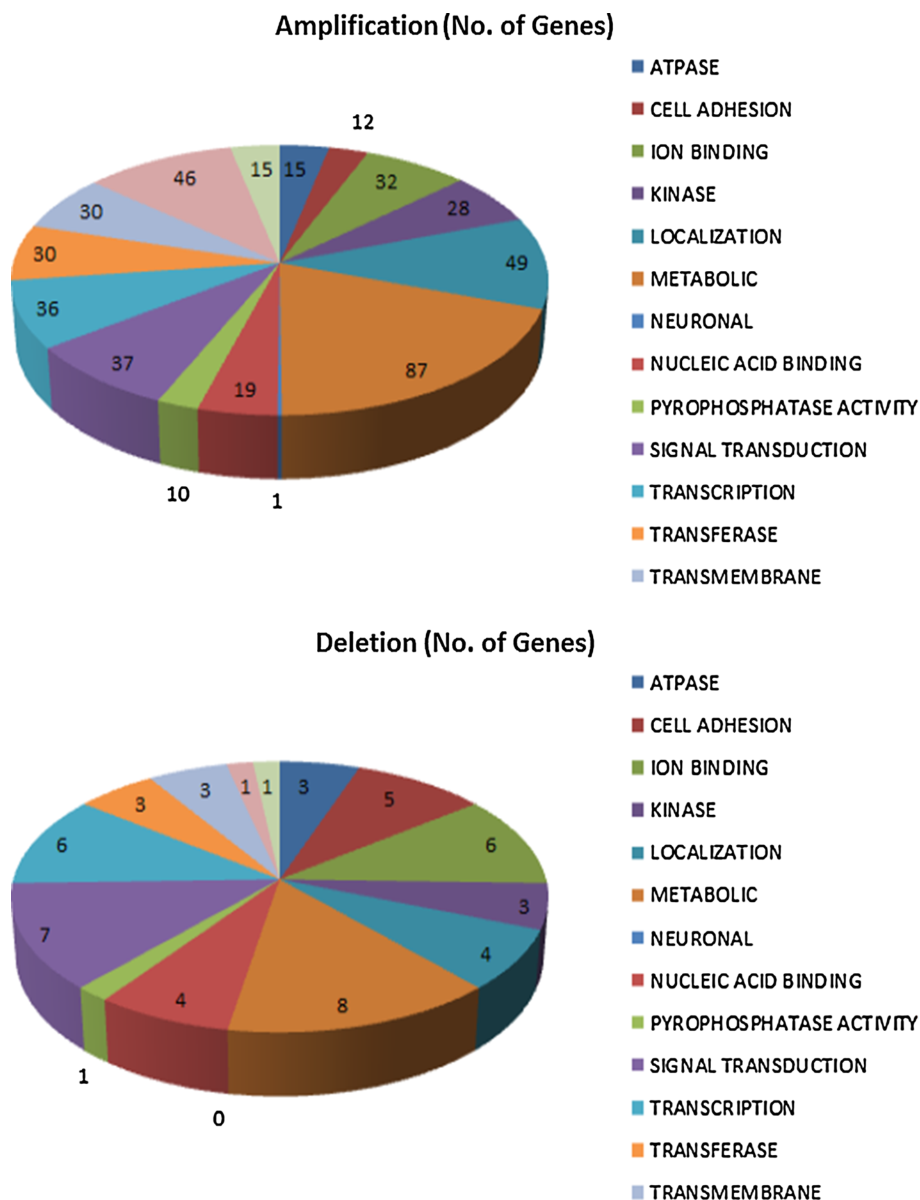

Fig. 2 Functional classification of genes containing amplifications or deletions for all *A. m. syriaca* samples as compared to the control reference sample (CRS)

subspecies. The slight change which has taken place in comparison to the reference samples of 1952 may likely be due to importation of foreign strains.

Array Comparative genomic hybridization (aCGH) technique is most widely used to analyze the genetic variations in chromosomal regions or sub chromosomal regions. It helps in measuring amplifications/ deletions, copy-number gains and losses and has been used to screen different types of genetic variation in humans, model species and some non-model organisms (Locke et al. 2003; Panova et al. 2014) to compare closely related genomes and ecotype studies. Large-scale genomic rearrangements are a major force of evolutionary change. These genomic changes are commonly referred to as aberration and include variations arising due to chromosomal/ gene duplications and deletions. These events provide a source of genetic material for mutation, drift, and selection to act upon, making new evolutionary opportunities possible. Genomic aberrations play a dominant role in the evolution of complexity and diversity, reduce probability of extinction and contribute to conservation of species. This hybridization method has also proved to be key source for genome analysis of *Apis mellifera* (Tsuruda et al. 2012). Fine-scale analysis of the honeybees using this molecular tool will enable researchers to characterize the consequences of importing of non-native stock and of the adoption of migratory keeping practices on the native honeybees of the region (Spotter et al. 2012).

The Jordanian breeding program is achieving its goal of preservation of *A. m. syriaca*. Samples 1–5 have

insignificant differences from the Control Reference Sample (CRS). The aCGH study of the samples 1-5, taken from the conservation program for *A. m. syriaca*, show a few amplifications and fewer deletions observed in the bees as shown in Table 3, compared to the CRS. This very small percentage of change, based on all the probes present on the array, is shown in Table 2. These very minor differences indicate that all the contemporary stocks represented by samples 1–5 retain the genetic identity of *A. m. syriaca*. The small changes are distributed over all the chromosomes as shown in Fig. 1. Functional classification of the genes showing aberration (Fig. 2) classifies the genes into mainly constitutive functional categories and are not directly involved in immunity, behavior or pathogen resistance including resistance to *Varroa* infestation (Navajas et al. 2008; Parker et al. 2012; Arechavaleta-Velasco et al. 2012; Boecking and Spivak 1999). These insignificant differences might be due to a small amount of genetic drift or reflect variance in the population. On the other hand it confirms the efficiency of the "beekeepers–researchers" participatory conservation approach by providing them with free queens cells of the targets lines of honey bees in the conservation program.

This study demonstrates that preserving subspecies with breeding programs can be done by either natural matings in areas isolated from other stocks or by using instrumental insemination. The conservation of the genetic integrity of the *A. m. syriaca* stock that was open-mated in an area with a mixture of honey bee stocks, but principally *A. m. ligustica*, in the general area was surprising. This is congruent with the observations of (Haddad and Fuchs 2004) who found little evidence of hybridization with *A. m. ligustica* in a morphological analysis of this open mated stock of *A. m. syriaca*. Clearly there is an isolating mechanism that prevents most hybrid matings. It may be that the *A. m. syriaca* apiary is isolated in its micro-habitat or that behavioral difference in flight time or flight ranges of queens and drones underlie this apparent isolation. Regardless of the cause, our experience with open mating *A. m. syriaca* should not be taken as a model for similar programs that intend to preserve other sub-species as our experience with open-mated stock maybe unique to our apiary conditions or to the behavior of *A. m. syriaca* itself.

The existence of viable populations of *A. m. syriaca* provides an important resource for honey bee breeding programs. Selective breeding of *A. m. syriaca* is providing a stock having varroa resistant, useful adaptations to dry lands and hot summers and possible future climatic changes which are expected to be more hot and dry in the Middle East and North Africa Region, as well it may improve beekeeping utility by virtue of being less sting prone, less likely to swarm and more productive in honey production. If lack of genetic variance for desirable traits stalls selection progress, out-crosses with European honey bees and reselection from the out-crossed population for the desired traits may result in a commercial stock having numerous desirable traits including those associated with being adapted to harsh and dry hot seasons.

The way forward is to strengthen the conservation breeding programs with local honeybees instead of imported queens. That would help maintain the population's natural diversity. It would also contribute to preventing the collapse of colonies, optimize sustainable productivity, and make it possible to maintain continual adaptation to environmental changes. Though more than half a century of foreign import has passed, it might not be too late to conserve pure *A. m. syriaca* to serve as origin for further investigations, preservation in a research apiary and breeding purposes.

Acknowledgements This research paper is part of the project entitled "Evaluation and Development of *Apis mellifera syriaca* with Useful keeping Traits for the Middle East Region." And the project entitled "Impact of the Varroa destructor infestation and its viral load on honeybee colonies in the Middle East and North Africa" funded by USAID grants numbers: TA-MOU-09-M29-075 and TA-MOU-11-M32-035.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

References

Arechavaleta-Velasco M, Alcala-Escamilla K, Robles-Rios C, Tsuruda J, Hunt G (2012) Fine-scale linkage mapping reveals a small set of candidate genes influencing honey bee grooming behavior in response to *Varroa mites*. PLoS ONE 7(11):e47269

Blum R (1956) The bees in the Near East. In: Proceedings of the International Beekeepers Congress, vol 16. Apimondia, Wien, p 28

Bodenheimer S, Ben-Nerya A (1937) One-year studies on the biology of the honey-bee in Palestine. Ann Appl Biol 24(2):385–403

Boecking O, Spivak M (1999) Behavioral defenses of honeybees against *Varroa jacobsoni* Oud. Apidologie 30(2–3):141–158

Ftayeh A, Meixner M, Fuchs S (1994) Morphometrical investigation in Syrian honeybees. Apidologie 25:396–401

Haddad N (2015) Mitochondrial genome of the Levant region honeybee, *Apis mellifera syriaca* (Hymenoptera: Apidae). Mitochondrial DNA 30:1–2. doi:10.3109/19401736.2014.1003846

Haddad N, Fuchs S (2004) Honeybee agrobiodiversity: a project in conservation of *Apis mellifera syriaca* in Jordan. Uludag J 2(3):116–120

Haddad N, Fuchs S, Haddaden J, Kopelke JP (2005) Recording of *Sphecophaga vesparum* Curtis, a natural enemy of the *Vespa orientalis* in the northern part of Jordan. Middle East J Zool 35(9):114–116

Haddad N, Evans J, Pettis J, Migdali H (2007) Genetic structure of varroa mite populations in *A. mellifera syriaca*. Adv Environ Biol 1(1):1–3

Haddad N, Esser J, Neumann P (2008) Association of *Cryptophagus hexagonalis* (Coleoptera: Cryptophagidae) with honey bee colonies (*Apis mellifera*). J Apic Res Bee World 47(3):190–191

Haddad N, Meixner M, Fuchs S, Migdadi H, Sheppard W (2009) Mitochondrial DNA variation in honeybees from Jordan. J Apic Res World 48(1):19–22

Haddad N, Batainh A, Migdadi O, Deepti S, Venkatesh K, Sriram P, Alhamuri Z (2015) Next generation sequencing of *Apis mellifera syriaca* identifies genes for Varroa resistance and beneficial bee keeping traits. Insect Sci. doi:10.1111/1744-7917.12205

Kalman C (1973) Our formerly very aggressive bee. In: Proceedings of the international beekeepers congress, vol 24. Apimondia, Bukarest, pp 306–307

Kence M, Oskay D, Giray T, Kence A (2013) Honeybee colonies from different races show variation in defenses against the varroa mite in a 'common garden'. Entomol Exp Appl 149(1):36–43. doi:10.1111/eea.12109

Locke DP, Segraves R, Carbone L, Archidiacono N, Albertson DG, Pinkel D, Eichler EE (2003) Large-scale variation among human and great ape genomes determined by array comparative genomic hybridization. Genome Res 13(3):347–357

Navajas M, Migeon A, Alaux C, Martin-Magniette ML, Robinson GE, Evans JD, Cros-Arteil S, Crauser D, Le Conte Y (2008) Differential gene expression of the honeybee *Apis mellifera* associated with *Varroa destructor* infection. BMC Genom 9:301

Panova M, Johansson T, Canbäck B, Bentzer J, Rosenblad MA, Johannesson K, Tunlid A, André C (2014) Species and gene divergence in *Littorina* snails detected by array comparative genomic hybridization. BMC Genom 15:687

Parker R, Guarna MM, Melathopoulos A, Moon KM, White R, Huxter E, Pernal S, Foster L (2012) Correlation of proteome-wide changes with social immunity behaviors provides insight into resistance to the parasitic mite, *Varroa destructor*, in the honeybee (*Apis mellifera*). Genome Biol 13:R81

Ruttner F (1988) Biogeography and taxonomy of honeybees. Springer, Berlin, pp 284–295

Ruttner F (1992) Naturgeschichte der Honigbienen. Ehrenwirth, München, p 357

Spotter A, Gupta P, Nurnberg G, Reinsch N, Beinefeld K (2012) Development of a 44 K SNP assay focussing on the analysis of a varroa-specific defence behaviour in honey bees (*Apis mellifera carnica*). Mol Ecol Res 12:323–332

Tsuruda JM, Harris JW, Bourgeois L, Danka RG, Hunt GJ (2012) High-resolution linkage analyses to identify genes that influence varroa sensitive hygiene behavior in honeybees. PLoS ONE 7(11):e48276

Wallberg A, Han F, Wellhagen G, Dahle B, Kawata M, Haddad N, Simões Z, Allsopp M, Kandemir I, De la Rúa P, Pirk C, Webster M (2014) A worldwide survey of genome sequence variation provides insight into the evolutionary history of the honeybee *Apis mellifera*. Nat Genet 46:1081–1088. doi:10.1038/ng.3077

Zakour MK, Ehrhardt K, Bienefeld K (2012) First estimate of genetic parameters for the Syrian honeybee *Apis mellifera syriaca*. INRA, DIB and Springer, Versailles, New York