Journal of Apicultural Research and Bee World 48(1): 19-22 (2009)
DOI 10.3896/IBRA.1.48.1.05

ORIGINAL RESEARCH ARTICLE

Mitochondrial DNA support for genetic reserves of *Apis mellifera syriaca* in Jordan.

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Received 3 January 2008, accepted subject to revision 17 January 2008, accepted for publication 26 June 2008.

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Summary

We analyzed mitochondrial DNA variation in honey bee colonies in Jordan using *Dra*I restriction profiles of the COI-COII intergenic region. Seven different haplotypes were observed, three of which belonged to the C mitochondrial lineage. Samples displaying haplotypes of the C lineage were concentrated in the north of the country where frequent importations of European bees have occurred. We conclude that the C mitochondrial haplotypes found in the study reflect past or recent importations of non-native honey bees. No C haplotypes were detected in areas that have been identified as possible source populations for the conservation of original *A. m. syriaca* in the south of the sampling region, thus confirming the existence of comparatively pure genetic resources of this subspecies in Jordan.

El ADN mitocondrial apoya las reservas genéticas de *Apis mellifera syriaca* en Jordania.

Resumen

Hemos analizado la variación del ADN mitocondrial en colonias de abejas de Jordania usando la restricción con *Dra*I de la región intergénica COI-COII. Se han observado siete haplotipos diferentes, tres de los cuales pertenecen al linaje mitocondrial C. Las muestras que presentaron haplotipos del linaje C estaban concentradas en el norte del país donde se producen importaciones de abejas europeas. Concluimos que los haplotipos mitocondriales C encontrados en el estudio reflejan importaciones pasadas o recientes de abejas no-nativas. No se detectaron haplotipos C en zonas que han sido identificadas como posible fuente de poblaciones para la conservación de la original *A. m. syriaca* en el sur de la región de muestreo, lo que confirma la existencia de recursos genéticos relativamente puros de esta subespecie en Jordania.

Keywords: *Apis mellifera syriaca*, Jordan, genetic variability, mitochondrial DNA, *Dra*I restriction, conservation

Introduction

Apis mellifera syriaca is the native honey bee subspecies of Jordan, much of Syria, Lebanon, Palestine and Israel (Ruttner, 1988; 1992). Similar to other honey bee subspecies of the Mediterranean, it expresses behavioural adaptations to a regional climate with very high temperatures and nectar dearth in summer, including reduced brood rearing during the hottest months, increased swarming and frequent absconding (Ruttner, 1980; 1988; 1992; Sheppard *et al.*, 1997; Haddad and Fuchs, 2004).

Although they require extensive maintenance efforts due to their lack of adaptation to the prevailing environmental conditions, honey

bee strains from European sources have frequently been imported into the region (Brother Adam, 1954; Ftayeh *et al.*, 1994; Haddad and Fuchs, 2004). Together with a growing tendency among beekeepers to adopt migratory beekeeping practices, this increases concerns about hybridization of the native honey bees with strains of Italian (*A. m. ligustica*), and Carniolan (*A. m. carnica*) origin (Haddad and Fuchs, 2004). Recently, a project for the conservation of *A. m. syriaca* in Jordan was initiated with the aim of implementing breeding efforts to improve locally adapted strains and utilizing their advantageous traits for resident small-scale and medium-sized beekeeping operations (Haddad and Fuchs, 2004). 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 they also found comparatively isolated areas with a population of *A. m. syriaca* that was similar to the oldest reference samples available from this geographic region. Two regions were identified as potential source populations for conservation measures.

Mitochondrial DNA markers have been widely used to study genetic variation within *Apis mellifera*, but the majority of the available data pertain to variation among European and African honey bee subspecies (Garnery *et al.*, 1992; 1998; Franck *et al.*, 1998; 2000a; 2001; De la Rúa *et al.*, 2001). Comparatively few studies have been conducted on genetic variation of honey bee populations native to the Near and Middle East (Palmer *et al.* 2000; Franck *et al.*, 2000b; Kandemir *et al.*, 2006a).

Several honey bee mitochondrial lineages have been reported to co-occur in the Near East. The honey bees of most of Turkey, Iran and Cyprus belong to the mitochondrial C lineage present in the Eastern Mediterranean, while samples from southern Turkey (Palmer *et al.*, Kandemir *et al.*, 2006), Egypt and Syria (Arias and Sheppard, 1996) and Lebanon (Franck *et al.*, 2000) displayed different patterns of mitochondrial DNA. Clearly, the phylogeographic assignment and nomenclature of mitochondrial lineages found in the Near East bears implications for the phylogenetic history of *Apis mellifera*. In this paper we report mitochondrial DNA variation among honey bees of Jordan from areas with active traditional beekeeping, for the purpose of identifying geographic areas suitable for the conservation of *A. m. syriaca*.

Materials and methods

Due to climatic and vegetation constraints, historically beekeeping was only possible in the western part of Jordan. Only in the last few decades have beekeepers started beekeeping activities in the eastern part of Jordan, after the establishment of irrigated farms in the desert area. For the present study, honey bees were sampled from two areas in the northwest and a more southern location next to the Dead Sea, where traditional beekeeping with clay hives is still practiced to a large extent by the local beekeepers. In the region between these two areas, beekeeping with modern equipment, frequent migration and importation of foreign stock is common, so this area was excluded from the sampling. Sample locations and exact geographic coordinates were described in Haddad and Fuchs (2004). A total of 24 samples from this collection were analysed for this study, most of which had been previously classified using morphometric methods.

Total nucleic acids of one individual worker per sample were extracted with a phenol-chloroform method (Arias and Sheppard, 1996). The mitochondrial fragment containing the intergenic region between the $tRNA_{leu}$ gene and the second subunit of the cytochrome oxidase gene was PCR-amplified with the primer pair E2-H2 (Garnery *et al.*, 1993), using the experimental conditions described in Garnery *et al.* (1998) with modifications of Kandemir *et al.* (2006b). A 10 µl aliquot of each reaction was run on an agarose gel and stained with ethidium bromide to determine the length of the amplified fragment. The remaining 20 µl of each positive reaction were digested with the restriction enzyme *Dra*I at 37°C overnight. Restriction fragments were separated on 10% acrylamide gels, stained with ethidium bromide and photographed under UV illumination.

Results

Haplotypes of two different lineages were found in the samples from Jordan. Six samples showed haplotypes known from the mitochondrial C lineage, while 18 were of types previously reported from the Lebanon and Syria (Franck *et al.*, 2000b). Two of the C lineage haplotypes displayed the pattern C1. The remaining four C lineage samples expressed the pattern C2, with three of them being more specifically C2a and one C2b (Franck *et al.*, 2000b; Garnery *et al.*, 1993). The 18 non-C lineage haplotypes possessed fragment lengths of the undigested amplification products of ~640 bp, ~820 bp and ~1020 bp, corresponding to the presence of P_oQ, P_oQQ, and P_oQQQ sequence elements, respectively. *Dra*I digestion of these samples produced four different restriction patterns. Three of these haplotypes had been designated as O1a, O1', O1" from honey bees of Lebanon (Franck *et al.*, 2000b). We also found a previously unpublished haplotype, which was distinguished from the published O3 haplotype (Franck *et al.*, 2000b) by the presence of an additional Q element and tentatively termed O3'.

The geographic distribution of haplotypes is shown in Fig. 1. Table 1 summarizes the mitochondrial haplotype of each sample together with the morphological classification reported by Haddad and Fuchs (2004).

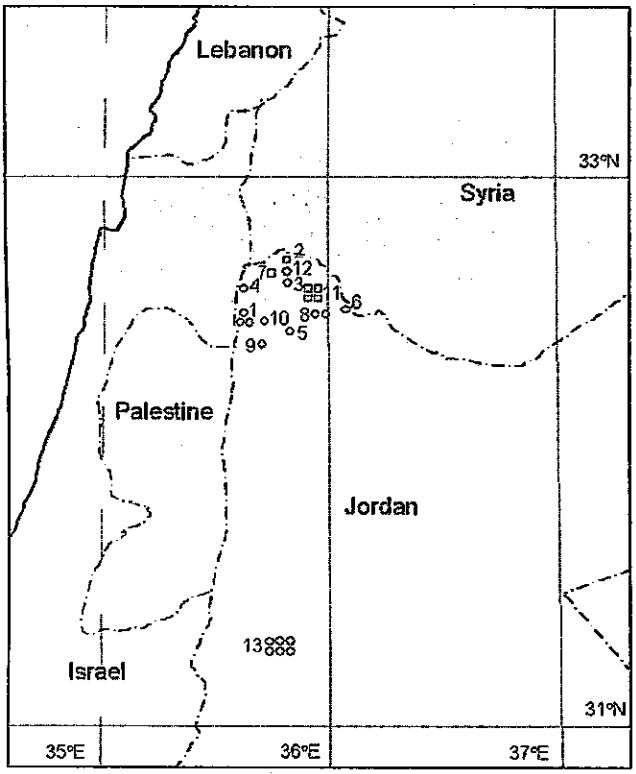

Fig. 1. Geographic positions of sampling locations in Jordan and mitochondrial haplotypes observed at each location. Each symbol corresponds to one colony. Circles represent the haplotype group typically found in *A. m. syriaca*, squares represent haplotypes associated with the mitochondrial lineage C. Numbers correspond to the location numbers in Table 1.

Discussion

Our results show that only limited introgression of mtDNA variants from the C lineage appeared in the *A. m. syriaca* populations of Jordan. The indigenous *A. m. syriaca* populations of Jordan can be characterized by the same mitochondrial haplotypes described from Lebanon (Franck *et al.*, 2000b) and known from Egypt (Franck *et al.*, 2001) and the Arabian Peninsula (Meixner *et al.*, unpublished data). Samples displaying haplotypes of the C lineage were limited to the northern sampling area of the country. Frequent importations of Italian bees are known to have occurred in this area, and the influence of *A. m. ligustica* was also detectable by morphometrical analysis (Haddad and Fuchs, 2004). Several samples displayed mtDNA patterns of the C lineage together with the morphology of *A. m. ligustica* (Table 1) and, thus, probably represent recent importations. Samples with C lineage mtDNA and the morphology of *A. m. syriaca* or, conversely, *A. m. ligustica* morphology and mtDNA patterns described by Franck *et al.* (2000b), were also observed, indicating some level of hybridization between native and imported stock. In contrast, no C haplotyopes or *A. m. ligustica* morphology were detected in areas that had been identified as possible source

Table 1. Mitochondrial haplotype and morphometrical classification of each sample. All morphometrical classifications were with P > 0.99. Location numbers refer to Fig. 1.

Loc No.	Location	*Haplotype*	*Morphological Classification*
1	Abu Ziyad	O3'	-----
1	Abu Ziyad	O1'	*A. m. syriaca*
1	Abu Ziyad	O1a	*A. m. syriaca*
2	Aen Alsaed	C2	*A. m. syriaca*
3	Ein At Trab	O3'	*A. m. ligustica*
4	Baqoorah	O1'	*A. m. syriaca*
5	Mazar	O1'	*A. m. syriaca*
6	Ramtha	O1"	*A. m. syriaca*
7	Malka	C2	——
8	Howwarah	O1a	*A. m. syriaca*
8	Howwarah	O1a	*A. m. syriaca*
9	Kofor Awan	O3'	*A. m. syriaca*
10	Dair Abi Sa'id	O1"	*A. m. ligustica*
11	Maro	C1	A. m. syriaca
11	Maro	C2	*A. m. ligustica*
11	Maro	C1	*A. m. syriaca*
11	Maro	C2	-----
12	Kofor Soom	O1a	-----
13	Wadi Ben Hammad	O1"	-----
13	Wadi Ben Hammad	O1"	*A. m. syriaca*
13	Wadi Ben Hammad	O1a	*A. m. syriaca*
13	Wadi Ben Hammad	O1a	*A. m. syriaca*
13	Wadi Ben Hammad	O1'	*A. m. syriaca*
13	Wadi Ben Hammad	O1a	——

populations of original *A. m. syriaca* (Wadi Ben Hammad, Abu Ziyad) based on morphometric analysis (Haddad and Fuchs, 2004), thus confirming the preservation of comparatively pure genetic resources of this subspecies in Jordan. Further fine-scale analysis of Jordanian honey bees using molecular and morphological tools will enable researchers to fully characterise the consequences of importation of non native stock and of the adoption of migratory beekeeping practices on the native honey bees of the region.

The results further contribute to the mtDNA characterisation and allocation of *A. m. syriaca* bees. Whilst the indigenous honey bees of the entire Near East belong to the same morphological lineage (Ruttner, 1988), a transition between two different mitochondrial lineages appears to run through this region, roughly following a west to east trajectory from southernmost Turkey to western Iran. The bees of the northern and eastern part, including Turkey, northern Syria, northern Iraq and the whole of Iran show the typical mitochondrial characteristics of the C lineage (Kandemir *et al.*, 2006a; Meixner *et al.*, unpublished data; Kandemir *et al.*, unpublished data), but from Lebanon through southern Syria to Egypt different mitochondrial patterns prevail (Arias and Sheppard, 1996; Franck *et al.*, 2000; 2001; Meixner *et al.*, unpublished data). The extent and nature of this transition zone, however, remains largely unknown due to insufficient available samples and few published data.

Acknowledgements

Financial support was granted to NH by DFG & GTZ of Germany and NCARE of Jordan.

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