

## The honeybees (*Apis mellifera* L) of Libya

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### ABSTRACT

We investigated honeybee populations of *A. mellifera* in Saharan and coastal locations in Libya to fill the North Africa gap of biogeography and distribution of honeybees, morphologically and using mtDNA analysis. It was found that Libyan honeybees are different, morphologically and genetically, from adjacent subspecies; and majority of Libyan bees (92%) belongs to oriental evolutionary lineage (O). As well as, it was found local impact of imported European honeybees. Further studies may to name the Libyan bees as a separate subspecies.

**Keywords:** *Apis mellifera*, Libya, morphometry, lineages, Subspecies

### INTRODUCTION

*Apis mellifera* is endemic to Africa, Europe and parts of western Asia ranging from Kirgizia in the east to the most western limits of Europe; from southern tip of Africa to the northern limits in Europe in south Scandinavia (Ruttner, 1988; Sheppard & Meixner, 2003). In this huge distribution range, *A. mellifera* can be found in a vast range of habitats ranging from desert to rain forests and from mountainous regions to plains. (Smith, 1961). Because of this variety of habitats, climatic conditions, and floras as well as separations factors, it is not surprising that *A. mellifera* has split into numerous subspecies (races) about 0.3-1.3 myr ago (Ruttner, 1988; Cornuet & Garnery, 1991b; Arias & Sheppard, 1996). Around 29 subspecies are currently recognized based on morphometric analyses (Ruttner, 1988; Engel, 1999; Sheppard & Meixner, 2003). Each race is characterized with a set of distinctive characteristics probably as a result of local adaptation to the various regions (Louveaux *et al.*, 1966).

Apiculture is an important part of human culture and the relationship between humankind and honeybees is probably as old as man himself. Prehistoric cave paintings indicate that the interest of humankind for honey already existed in the Paleolithic period. About 4000 years ago, Egyptians used clay pots to keep bees for honey production but also to harvest other bee products including propolis and wax (Crane, 1999).

North Africa experienced consecutive cycles of aridity and moistness. The divergences between honeybee subspecies from northern and southern sides of the Sahara may have occurred during the late Pleistocene (~ 15 000 years BP) when the Sahelian zone became a desert while the northwest of Africa characterized by Mediterranean-like vegetation with most favourable conditions for honeybees. About ten thousand years ago, the conditions in North and Central Africa became less arid and were much moister than at present. During that period, the Sahara desert disappeared (Lezine, 1989; Ritchie, 1994) which allowed for a population expansion and possible gene flow between the honeybees of North Africa and the Sahel. About 7,500 years ago aridity returned (Gasse & van Campo, 1994; Alley *et*

*al.*, 1997) and the conditions across North-, Central- and East-Africa became much drier than before culminating in an arid phase about 3,800 y.a. (Petit-Maire & Guo, 1996). Since then, the region was characterized by huge deserts creating today subspecies: *A. m. intermissa* along Mediterranean coast from Morocco through Algeria (Barour *et al.*, 2005) to Tunisia (Lebdi-Grissa, 1991a, b), *A. m. sahariensis* in the Saharan oases and the valleys along the northern edge of Sahara south of the Atlas mountain ridge (Hepburn & Radloff, 1996), and *A. m. lamarckii* along the Nile Valley in Egypt (Ruttner, 1988).

Sixteen identified subspecies of *A. mellifera* are distributed around Mediterranean Basin (Garnery *et al.*, 1993; Franck *et al.*, 2000, 2001), representing four (A, C, M and O) out of the five characterized evolutionary lineages (Garnery *et al.*, 1993; Arias & Sheppard, 1996; Franck *et al.*, 2000a, 2001; Miguel *et al.*, 2007; Cánovas *et al.*, 2008):

1. Lineage A in the southern part of the Iberian Peninsula, Northwestern Africa, Sicily and the Aegean Islands.
2. Lineage M from the northern part of the Iberian Peninsula to Northern Europe.
3. Lineage C in continental Europe south and east of the Alpine ridge.
4. Lineage O in the eastern Mediterranean including the Near East and Egypt

Libya is geographically located in North Africa between Egypt in the east, where *A. m. lamarckii* is endemic, and the other North African countries in the west, where the subspecies *A. m. intermissa* and *sahariensis*. Therefore, Libya provides the missing link in this west-east transition. According to unpublished report (Al Mahjoob *et al.*, 1999), about 125 000 managed colonies have been estimated in Libya in 1999. Unfortunately, little attention has been paid to study the honeybees of Libya, (El Banby, 1977; Mohaned *et al.*, 1982; Kosheim, 1998; Hussein, 2000 a, b). The main regions dealing with modern beekeeping located along the Mediterranean coast, as well as, there are scattered apicultural activities in the Saharan oases.

Ruttner (1988) alleged, based on morphometric analyses of adjacent countries, that the Libyan honeybees belong to *A. m. intermissa*, in spite El Banby (1977) concluded that bees from northeast Libya belong neither to *A. m. intermissa* nor to *A. m. lamarckii*.

Moreover, a morphometrical analysis of Libyan bees from coastal and desert locations showed that they are a unique distinguishable ecotype, distinct from both the adjacent *A. m. intermissa* and *A. m. lamarckii*.

In this work, I discussed the situation of Libyan bees.

## MATERIALS AND METHODS

### ***Morphometric analysis***

#### ***Sampling***

Samples were collected from four locations in Libya (Kufra, Baida, Brak and Surt) (Fig. 1). 37 characters were measured of ten workers of each colony (Ruttner *et al.*, 1978): 16 measurements of length, 7 of coloration, 3 of pilosity and 11 wing angles. The measurements were achieved using the facilities of the Institut für Bienenkunde, Oberursel, Germany (Meixner, 1994).

#### ***Statistical analysis***

The means, standard deviation and standard error of the individual workers values within every colony were computed. As well, reference samples from data base of Institut für Bienenkunde, Oberursel were used, these including five African subspecies adjacent or within the North African desert belt (*A. m. intermissa*, *A.m.*

*sahariensis*, *A. m. lamarckii*, *A. m. jemenitica* and *A. m. litorea*), and European subspecies *A. m. ligustica*, which has been imported to the country.

The general similarities were assessed by submitting the data to a principal component analysis (PCA) using colony means of 37 characters was used to select the more powerful characters in discriminating the samples by using discriminant analysis (DA). Last, morphometric distances were calculated on Z-normalized measurements and distance data were submitted to cluster analysis (CA). All statistical analyses were performed using the SPSS 15.0 statistical software.

#### **MtDNA analysis**

##### **Sampling**

105 samples were used in the mtDNA analysis: Al Aziziyah, Tripoli, Al Qasabat, Zlitan, Surt, Benghazi, Al Baida, Marzuq, Brak, Ejdabia and Kufra (Fig. 1).

##### **DNA extraction, PCR amplification, RFLP analysis and sequencing**

The total DNA was obtained by extracting DNA from muscles using Chelex protocol (Walsh *et al.*, 1991). The mtDNA region including the tRNA<sup>leu</sup> gene, the cox1-cox2 intergenic region and the 5' end of the cox2 sub-unit gene was amplified using the primers E2 and H2 (Garnery *et al.*, 1993). This intergenic region shows length and sequence variation related to the honeybee evolutionary lineages. It is composed of two types of sequences, P and Q. The sequence P can be absent (lineage C) or present in four different forms: P (lineage M), P0 (African lineage), P1 (African Atlantic sublineage, De la Rúa *et al.*, 1998, 2001, 2006) and P2 (lineage Y, Franck *et al.*, 2001).

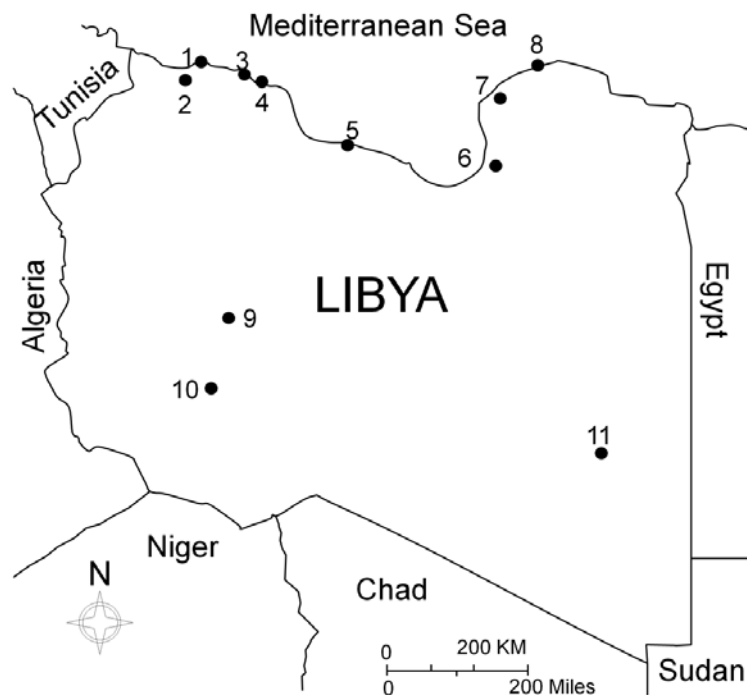


Fig. 1: Map of Libya showing the sampled locations: 1) Tripoli, 2) Al Aziziyah, 3) Al Qasabat, 4) Zlitan, 5) Surt, 6) Ejdabia, 7) Benghazi, 8) Al Baida, 9) Brak, 10) Marzuq and 11) Kufra.

The amplicon size was determined by run the PCR product of each sample on a 1.5% agarose gel, stained with ethidium bromide and photographed over a UV light screen. Then 10 µl of the PCR product were digested with *Dra I* and separated on 8% polyacrylamide to reveal RFLPs. At least one sample of each RFLP pattern was directly sequenced using the same primers as for the amplification.

## RESULTS

The Libyan bees showed morphological uniqueness, differed from all reference samples. They allocated separately from all reference samples with post-hoc probabilities of  $P > 0.9995$  when the data submitted to DA (Fig. 2). The Euclidian distance of all local group centroids was smallest to the centroid of *A. m. sahariensis*.

In fig. 3 the samples of Brak, Surt and Al-Baida incorporated into a cluster with *A. m. sahariensis* while Kufra samples were separated from them in another position.

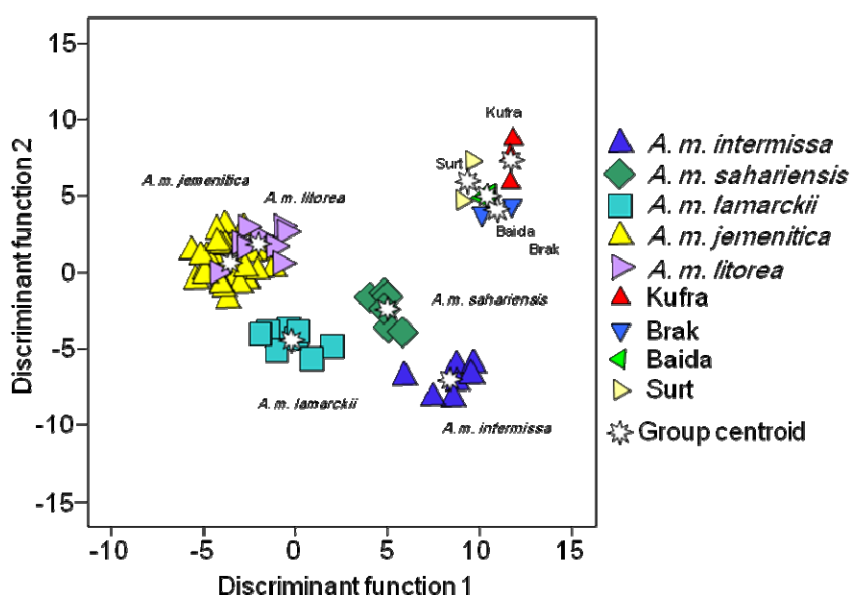


Fig. 2: Discriminant function sample scores of bee samples from four locations from Libya (small symbols) and of African reference groups (big symbols), together with their group centroids.

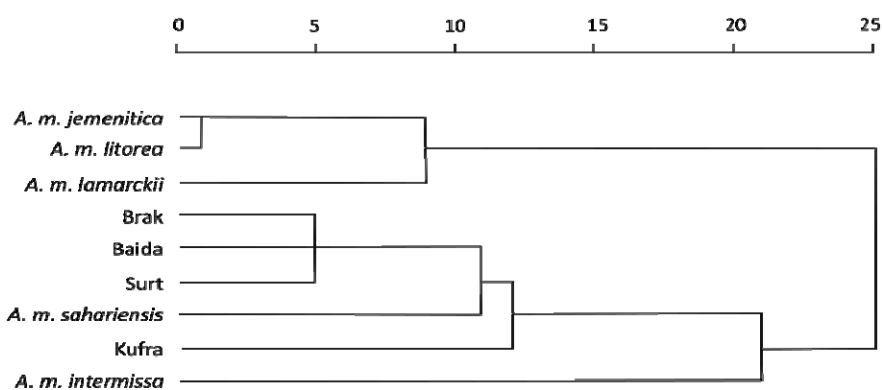


Fig. 3: Dendrogram of a cluster analysis based on squared Euclidian distances of the z-normalized character means of the colony samples of the four Libyan locations and of the African references samples.

105 were analyzed by analyzing the mtDNA region including the tRNA<sup>leu</sup> gene, the COI-COII intergenic region and 5' end of COII sub-unit gene. According to the total size of PCR products the majority was to the pattern P0QQQ (47.6%), which spreads across the country, followed by P0QQ (30.5%) then P0Q (10.5%), the pattern P0QQQQ (8.6%) and PQ represented the lowest percentage. The pattern P0QQQ occurred in almost all locations while P0QQQQ exists only in one location (Kufra). Some location characterized by only one pattern Zlitan and Baida; or by 2 patterns

Ejdabia, Benghazi, Al Qasabat and kufra; or three patterns Tripoli, Surt, Marzuq and Brak. But no location included all patterns (Table 1).

Table 1: Location of the studied Libyan honeybee populations, haplotypes and sample size (n).

Location	Latitude	Longitude	n	A1	A8	M3	O4	O5	O5'	O5''
Al Aziziyah	N32°31'	E13°00'	8	1		1	1	2	3	
Tripoli	N32°53'	E13°10'	17				3	5	9	
Al Qasabat	N32°35'	E14°02'	5					3	2	
Zlitan	N32°22'	E14°35'	4		4					
Surt	N31°12'	E16°35'	10				1	2	7	
Benghazi	N32°07'	E20°04'	4					3	1	
Al Baida	N32°44'	E21°37'	7					7		
Marzuq	N25°55'	E13°55'	7			2		2	3	
Brak	N27°32'	E14°14'	12				1	6	5	
Ejdabia	N30°06'	E20°50'	8					2	6	
Kufra	N24°11'	E23°18'	23						14	9
Total			105	1	4	3	6	32	50	9

The digestion of the PCR product by restriction enzyme *DraI* showed that 7 different haplotypes, PQ amplicon corresponds to the pattern M3, which originating in west Europe, while P0Q including the African patterns A1 and A8 and the oriental haplotype O4. The Amplicon P0QQ includes the haplotype O5, P0QQQ includes O5' and P0QQQQ includes O5''.

## DISCUSSION

About 50 000 units (colonies, package bees and queens), of commercial honeybees of Italian race and 3600 colony of Carniolan honeybees, have been imported to Libya in 1970's- 1990's (Al Mahjoob *et al.*, 1999). Nevertheless the principal component analysis, using reference samples of subspecies from data base, showed that the Libyan samples were beyond from the subspecies of central and west Mediterranean region that used in the analysis. This might mean there is no morphometric impact of European races on the honeybees of Libya. Since the same story has happened in Tunisia, where Lebdigrissa *et al.*, (1991a, b) compared European sample with Tunisian honeybees and they found that the former did not have any significant effect on Tunisian bees, although the beekeepers in all the Maghreb tried frequently to import *ligustica*, *macedonica*, *mellifera*, *carnica* and *caucasica* (Hicheri & Bouderbala, 1969; Second, 1974; Lebdigrissa *et al.*, 1991a). Hepburn and Radloff (1998) mentioned the same failure happened in Libya. As well, in South Africa, there were attempts to establish *ligustica* queens into *scutellata* colonies, all have failed (Hepburn & Radloff, 1998).

El Banby (1977) mentioned, in not detailed study, that the bees of Al Jabel Akhdar (Eastern Libya) more similar to *A. m. lamarckii* and not *intermissa*, as well, a hybrid between *carnica* and *lamarckii* produced a commercial line called "Queens Wadi" marketed in Libya

(Simonthomas & Simonthomas, 1980). Although both Al Baida, which is in Al Jabel Akhdar, and Kufra are located near the eastern borders of Libya where *A. m. lamarckii* is found in Egypt, it was apparent, in the discriminant analysis, the position of those locations was far from *A. m. lamarckii*. That might mean there was no impact of this race on neighboring populations of bees in Libya.

*A. m. intermissa* is widespread in North Africa; in Algeria (Barour, 2005), Tunisia (Lebdigrissa, 1991 a, b) and Morocco (Hepburn & Radloff, 1996). On the other hand, Libya has borders extend 450, 950 km with Tunisia and Algeria respectively, however, the cluster of this subspecies to Libyan bees cluster is not as near as that of *A. m. sahariensis*.

Regarding mtDNA analysis, most samples, 97 out of 105 samples analyzed, belong to O lineage, different from any named haplotype belonging to the oriental lineage. This lineage has been reported in *A. m. syriaca* from Lebanon, in *A. m. lamarckii* from Egypt (Franck *et al.*, 2000b, 2001) and in one colony of *A. m. litorea* from Somalia (Franck *et al.*, 2001). It's clear that all the samples of our study, which belong to lineage O, showed unique haplotypes to Libyan bees. That might mean Libya colonized by a relic indigenous race 15,000-8000 years B.P. in the "Holocene Pluvial". The occurrence of the similar haplotypes in all locations supports this fact and indicates that the areas, where the samples collected, were connected but they separated from each other under the powers of desertification and environment changes. Of particular interest are those honeybee samples from the remote Sahara oasis Kufra, which morphologically resemble *A. m. sahariensis*. These honeybees show a unique "private" haplotype (O5') suggesting that these oases might act as refugia colonized by relic populations from an indigenous race spread all over Libya 15000-8000 years B.P. during the "Holocene Pluvial".

It is logic to find the Libyan honeybees distant (morphometrically) from the reference samples even from *A. m. lamarckii*, which belong to haplotype O1c, was remote from Libyan honeybees, since the mtDNA analysis confirmed the morphomeric findings. North African honeybees' races (*A. m. sahariensis* & *A. m. intermissa*) exhibit these haplotypes A1, A2, A3, A4, A8, A9, A10 and A13 (Garnery *et al.*, 1995). The Haplotypes A1 and A8 were detected from colonies that were brought from Algeria (Almahjoob, personal communication).

Although Franck *et al.* (2001) found no evidence of imports of European honeybees to northern Africa, the M3 haplotype was identified in Marzuq and Al Aziziyah, indicating traces recent imports of queens by beekeepers. Interestingly the haplotype M3 detected at low frequency in Libya is typical for Italian *A. m. ligustica* populations (Franck *et al.*, 2000a).

According to the morphometric analyses and mtDNA analysis, the honeybees of Libya are beyond from adjacent subspecies, with it is own unique status. More comprehensive study is needed to confirm this status, which may lead to name the Libyan bees as a separate subspecies.

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#### ARABIC SUMMERY

### نحل العسل فى ليبيا

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تم فى هذا البحث تقصى عشائر من النحل مورفولوجياً، وباستخدام mtDNA فى المناطق الساحلية والواحات الصحراوية بليبيا، وذلك لسد الفجوة فى توزيع نحل العسل فى منطقة شمال أفريقيا. تبين أن نحل العسل فى ليبيا مختلف عن سلالات المناطق المجاورة مورفولوجياً وجينياً. كانت معظم العينات (92%) تنتمى للخط التطوري الشرقي (O lineage). علاوة على ذلك، تبين وجود تأثير محلي للنحل المستورد من أوروبا على النحل المحلي. دراسات مستقبلية قد تؤدي إلى تسمية النحل الليبي كسلالة لوحدها.