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Population structure of North African honey bees is influenced by both biological and anthropogenic factors

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Abstract Honey bee diversity is under threat due to anthropogenic factors as the use of pesticides and the replacement of local colonies to recover from colony losses. To assess the effect of these activities on the genetic diversity and structure of North African honey bee colonies, we studied colonies from the north (Tellian) and the south (Saharan) regions in Algeria, by determining their mitochondrial haplotype and the variation of ten microsatellite loci. Particular haplotypes have been found with a high frequency in each region that may constitute subspecies-specific markers for *Apis mellifera intermissa* (haplotype A8 at the north) and *A. m. sahariensis* (haplotype A9 at the south). Moreover, the presence of the haplotype A8 in some Saharan colonies may reflect recent introductions of *A. m. intermissa*. Structure analysis suggests that a natural differentiation between honey bee populations from Saharan and Tellian regions still exists despite increased colony movements (migration, queen purchases, etc.) during the last decades. One apiary established for the conservation of *A. m. sahariensis* showed no indication of maternal introgression since all the colonies bear the same haplotype A9. Furthermore, Hardy–Weinberg equilibrium observed in this population indicates that this apiary is appropriate for conservation programs of *A. m. sahariensis.*

Keywords *Apis mellifera intermissa* · *Apis mellifera sahariensis* · Mitocondrial DNA · Microsatellite DNA · Genetic structure · Conservation · Algeria

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Introduction

The ability of the honey bee *Apis mellifera* Linnaeus, 1758 to adapt to a great variety of environments resulted in the evolution of numerous subspecies that deserves morphological and population analyses (Hepburn and Radloff 1998). Honey bee populations of northwestern Africa are currently regarded as members of two subspecies, *A. m. intermissa* Buttel-Reepen, 1906 and *A. m. sahariensis* Baldensperger, 1924 (Hepburn and Radloff 1998), although the larger black honey bee from the Rif region was described as a third one named *A. m. major* by Ruttner (1975), which was not supported by the aforementioned authors. These subspecies show substantial morphometric differences but introgression and hybridization between morphoclusters have been revealed by pheromonal and allozyme data (Hepburn and Radloff 1996), as well as with mitochondrial and microsatellite data (De la Rúa et al. 2007).

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Apis mellifera intermissa extends across Morocco, Tunis and Algeria along the Mediterranean coast (Cornuet et al. 1988; Grissa et al. 1990). This subspecies is prone to swarming, shows an aggressive behaviour and an abundant use of propolis (Ruttner 1988). On the other hand, *A. m. sahariensis* is found to the south of the Atlas Mountains and bordering the Sahara to southwest Algeria (Baldensperger 1924). This somewhat smaller and yellowish-reddish honey bee is characterized by a moderate swarming tendency, a restricted number of queen cells, an immediate elimination of virgins during the swarming process, a little use of propolis and a weak defence reaction (Haccour 1960; Ruttner 1988).

The diversity of the honey bee is suffering over its natural range a number of negative effects derived from human practices (e.g., inappropriate use of pesticides and herbicides, De la Rúa et al. 2009). These practices have produced a decrease of the plant diversity that reduces the food sources for the bees. Furthermore, locust control campaigns in the Sahara during 1965, 1987 and 2003 decimated insect populations, including those of the Saharan honey bee, that were replaced by honey bees from the northern region. It is therefore postulated that the gene pool of south Algerian honey bee populations has been substantially altered by the introduction of colonies from north Algeria. For this reason there is a need to molecularly characterize the honey bee populations from the Algerian Sahara, as there are only few studies based on the northern populations from the Tellian region (Garnery et al. 1993; Franck et al. 2001; Achou 2007; Loucif-Ayad 2009).

To this aim we have studied a representative sampling of Algerian honey bee populations, first by determining their mitochondrial haplotype, and second by studying the population genetics and structure through the analysis of the variation of ten microsatellite loci. These molecular markers have been widely used to investigate different aspects of population genetics, parasitism, reproductive biology, or lineage differentiation (reviewed in De la Rúa et al. 2009). We test here the impact of honey bee queen introduction by looking for molecular markers corresponding total or partially to both northern and southern Algerian honey bee populations. In this way we shall be able to detect evidence of introgression from north populations into Saharan locations.

Materials and methods

Sampling and DNA extraction

Adult honey bee workers were sampled from 92 colonies in Algeria during 2008–2009 (Fig. 1; Table 1 in supp. mat.) at 14 different localities of the Tellian region (42 colonies in the coastal area, the Tellian Atlas Mountains and the highlands) and 7 localities of the Saharan region (50 colonies between the steppe and Saharan areas). These colonies have been grouped into six populations in relation to the following biogeographic areas (following Quezel and Santa 1962–1963; Barry et al. 1974): East and West Coast and Plateau in the Tellian region, and Steppe, Saharan Steppe and Desert in the Saharan region. The 12 colonies from Steppe belonged to one isolated apiary established with conservation purposes.

Samples were preserved in absolute ethanol and kept at −20 °C until they were processed in the laboratory. Total DNA was extracted from the third right leg of the worker

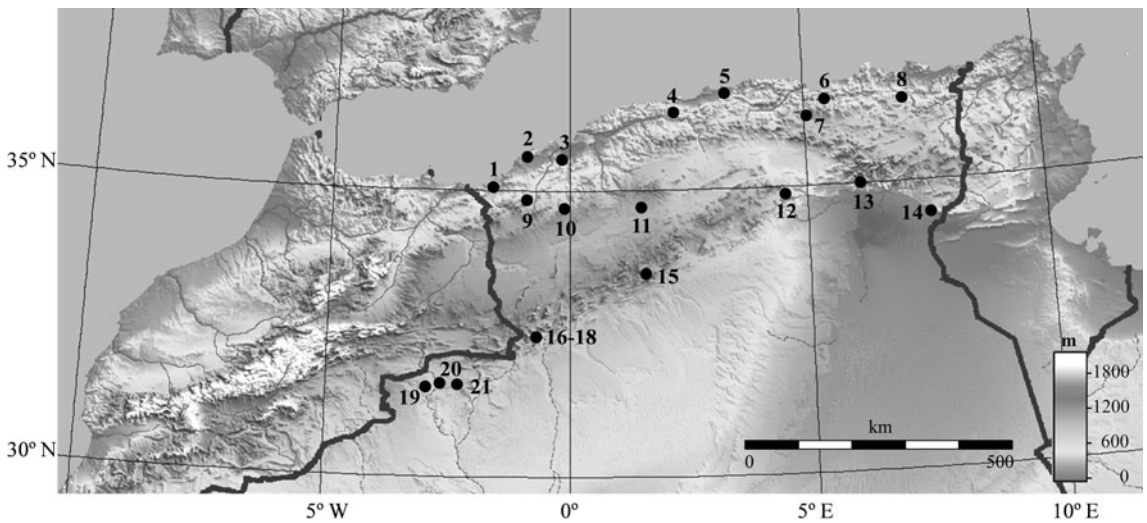

Fig. 1 Map of Algeria with the sampled locations. Sampling sites names corresponding to the numbers are shown in Supplementary material (Table 1)

bees using a 5 % Chelex solution (Walsh et al. 1991). A single honey bee worker per colony was used for mtDNA analysis and population genetic analysis with microsatellite markers (N = 92), while five honey bee workers per colony (N = 460) were used for population structure analysis with microsatellite markers.

Mitochondrial DNA analyses

The mitochondrial molecule of *Apis* subspecies has an intergenic region, located between the $tRNA^{leu}$ gene and the second subunit of the cytochrome oxidase gene (or cox2, following the nomenclature proposed for the mitochondrial genes by Boore 2001) that shows sequence and length polymorphisms (Cornuet and Garnery 1991). The nucleotide sequence of this region shows a combination of A-T rich units (P and Q elements) that characterizes the major evolutionary lineages (groups of subspecies) of *A. mellifera*. The P element can be present in different forms: P_0 (67 bp) and P_1 (51 bp) in African subspecies or P (54 bp) in west European subspecies such *A. m. mellifera*. The Q element, whose size varies between 192 and 237 bp, can be repeated in tandem one to four times in these subspecies. East European (as *A. m. ligustica* and *A. m. carnica*) and Near East subspecies have no P element and only one Q sequence in their intergenic region.

The mtDNA intergenic region was PCR amplified with the primers E2 (5′-GGCAGAATAAGTGCATTG-3′) located at the 5′ end of the gene $tRNA^{leu}$, and H2 (5′-CAATATCATTGATGACC-3′) located close to the 5′ end of the gene cox2 (Garnery et al. 1993), in a total volume of 25 µL. Annealing temperature was set at 48 °C following the conditions described by Garnery et al. (1993). To determine the amplicon size (= size of the intergenic region plus 3′- $tRNA^{leu}$ and 5′ cox2 segments), aliquots of 5 µL of each sample were electrophoresed on 1.5 % agarose gels stained with ethidium bromide and photographed over a UV light screen. The remaining 20 µL of the each amplicon were digested by the *Dra*I restriction enzyme (with recognition site 5′-TTTAAA-3′) at 37 °C overnight. Restriction fragments were visualized in 4 % NuSieve® agarose stained with ethidium bromide and photographed under UV light.

Haplotype determination was done taken into account the size of the amplicons and the restriction patterns of each sample. Haplotype frequency and gene diversity were calculated using GenAlEx software (Peakall and Smouse 2006). Fisher's exact test and a correspondence analysis were carried out to examine the possible association (contingence) between the haplotype frequency and the geographic region, using the ARLEQUIN v. 3.1 software (Excoffier et al. 2005) and the SPSS biostatistics software package v.19 (IBM® SPSS ® Statistics 2010).

Microsatellite genotyping and analyses

A total of 10 polymorphic microsatellite loci (plex 1: A7, Ap43, Ap55 and B124 and plex 2: A8, A79, A88, Ac11, Ap224 and Ap274; Estoup et al. 1995; Garnery et al. 1998; Solignac et al. 2003) were screened with two different multiplex PCRs. PCR reactions were carried out in 10 µL total volume containing 50 mM KCl, 10 mM Tris HCl (pH 9.0), 1.2 mM MgCl2, 0.3 µM of each dNTP, 0.8 µM of each primer, 1.5 units of Taq polymerase (Biotools) and 2 µL of DNA extract. Annealing temperature was set at 54 °C (plex 1) and at 50 °C for 20 cycles and 55 °C for another 20 cycles (plex 2). PCR products were visualized by capillary electrophoresis carried out on a ABI-3730 (Applied Biosystems) and sized with an internal size-standard (Servei Central de Suport a la Investigació Experimental, University of Valencia, Spain). Alleles were subsequently scored using GeneMapper® v3.7 software (Applied Biosystems).

Population genetic parameters were calculated with GenAlex v.6 (Peakall and Smouse 2006). Genetic diversity within populations was evaluated by computing allele frequencies, observed (Ho) and expected (He) heterozygosity. Hardy–Weinberg equilibrium was tested with Genepop (Raymond and Rousset 1995) and ARLEQUIN v. 3.1 (Excoffier et al. 2005) was used for calculating *F* statistics. *T* and ANOVA were used to test whether population parameters are equal between the groups with the SPSS biostatistics software package v.19 (IBM® SPSS ® Statistics 2010).

A Bayesian model-based clustering method STRUCTURE v 2.2 (Pritchard et al. 2000) was used for inferring population structure and assignment of individuals to populations. This method is probabilistically based on multilocus genotypes and estimates the posterior probability for a given number of K genetic populations. An admixture model assuming correlated allele frequencies was used. The results were based on simulations of 80000 burn-in steps and 1000000 MCMC (Markov Chain Monte Carlo algorithm) iterations. Five runs for each K value (K = 1 − 10) were used to estimate the most likely value of K. The number of populations was inferred from the value of ΔK as described in Evanno et al. (2005).

Results

Mitochondrial DNA

Four different haplotypes were found based on the size of the amplified intergenic fragment and the *Dra*I restriction fragment patterns. Inferred haplotypes belong to the African evolutionary lineage as they bear the P_0 sequence in the

mitochondrial intergenic region. These haplotypes have one (A1 and A8) and two (A2 and A9) repeats of the Q sequence (Garnery et al. 1995; De la Rúa et al. 1998). All of them have been observed in both Tellian and Saharan regions (Table 1). A8 haplotype was more frequent in the localities from the Tellian region (overall frequency = 0.50) whereas A9 was more frequent in the Saharan region (overall frequency = 0.70). Haplotype A2 was present in the Plateau (0.11) and the Desert populations (0.05). Mean gene diversity (D) values were 0.645 and 0.470 in Tellian and Saharan regions, respectively. Details of the geographical distribution of these haplotypes are given in Supplementary material (Table 1).

According to Fisher's test and corresponding analysis, A1 and A8 haplotypes were significantly associated with the Tellian colonies ($P < 0.0001$), and A9 haplotype with the Saharan ones ($P < 0.0001$).

Microsatellite data

The mean number of alleles ranged from 6.2 ± 0.7 to 8.0 ± 1.1 in West Coast and Saharan steppe populations, respectively. In the whole Saharan region the number of alleles was higher (10.2 ± 1.5) than in the Tellian region (9.9 ± 1.5) (Table 2). Gene diversity measured as expected heterozygosity (He) varied between 0.699 ± 0.054 in the East coast population and 0.752 ± 0.029 in the Desert population. Total values were higher in Saharan than in Tellian region but were not significantly different (*T* test, $P = 0.0523$). There was a significant effect among all populations on the gene diversity (ANOVA, $P < 0.01$), resulting from the differences among Tellian populations (ANOVA, $P < 0.05$), especially between Plateau and East and West Coast (*T* test, $P < 0.05$). There was no significant difference in the gene diversity among Saharan populations (ANOVA, $P = 0.115$).

The honey bee populations from the Saharan region showed an overall higher frequency of private alleles compared to the Tellian localities (2.5 ± 0.6), mainly due to the high value observed in the Saharan steppe (0.80 ± 0.33) but not significantly different (*T* test, $P = 0.0597$). However, there was a significant effect among all populations on the frequency of private alleles (ANOVA, $P < 0.001$), consequential from the differences among Saharan populations (ANOVA, $P < 0.001$). There was no significant difference in the frequency of private alleles among Tellian populations (ANOVA, $P = 0.108$).

Populations from both regions showed significant deviation from Hardy–Weinberg equilibrium due to the presence of an excess of heterozygotes except in Steppe ($P = 0.068$).

The analyses of population differentiation revealed a significant overall F_{ST} value of 0.05 between Tellian and Saharan regions. West Coast and Desert comparison scored the highest value (0.076). Interpopulation F_{ST} values were not significant between East coast and Plateau populations in the Tellian region, neither in any pairwise comparison within the Saharan region (Table 3). The results of the STRUCTURE analysis showed that the highest posterior probability corresponded to a model assuming two different populations: the Tellian and Saharan regions (Fig. 2). Cluster-1 was composed by 88 % of the honey bees from Tellian populations whereas cluster-2 was integrated by 92 % of honey bees from Saharan populations. However, when the model assumed three populations (K = 3), the clustering revealed one partition into two groups of the populations from Saharan region, separating the Steppe and Saharan steppe populations from the Desert population.

Discussion

As expected from previous results (Garnery et al. 1993; Garnery et al. 1995; Franck et al. 2001) this study has shown that naturally distributed honey bees in Algeria correspond to the African evolutionary lineage. Furthermore no signs of introduced honey bee stock have been detected in Algeria, in spite of known introductions in

Table 1 Haplotype frequency in each population and region and mean gene diversity (*D*)

Region	Population	N	A1	A2	A8	A9	*D*
Tellian	West Coast (1–4)	12	0.33		0.33	0.33	0.667
	East Coast (5–8)	12	0.33		0.42	0.25	0.653
	Plateau (9–14)	18	0.11	0.11	0.67	0.11	0.519
	Total	42	0.24	0.05	0.50	0.21	0.645
Saharan	Steppe (15)	12				1	0.000
	Saharan Steppe (16–18)	19	0.37			0.63	0.465
	Desert (19–21)	19	0.05	0.05	0.32	0.58	0.560
	Total	50	0.16	0.02	0.12	0.70	0.470

Numbers in brackets correspond to the localities shown in Fig. 1

Table 2 Summary statistics for the ten microsatellite *loci* in analysed populations from Tellian and Saharan regions showing number of honey bee workers genotyped (N), mean number of alleles (Na), mean number of private alleles (Pa), observed (Ho) and expected (He) heterozygosity and level of significant of the tests for Hardy–Weinberg equilibrium (HWE); (mean ± SD)

Region	Population	N	Na	Pa	Ho	He	HWE
Tellian	West Coast	12	6.2 ± 0.7	0.5 ± 0.2	0.675 ± 0.079	0.705 ± 0.047	*
	East Coast	12	6.6 ± 1.0	0.4 ± 0.2	0.599 ± 0.074	0.699 ± 0.054	***
	Plateau	18	7.4 ± 0.8	0.6 ± 0.3	0.639 ± 0.050	0.738 ± 0.033	***
	Total	42	9.9 ± 1.5	2.2 ± 0.9	0.638 ± 0.055	0.744 ± 0.041	***
Saharan	Steppe	12	7.0 ± 0.9	0.4 ± 0.2	0.633 ± 0.066	0.722 ± 0.043	ns
	Saharan Steppe	19	8.0 ± 1.1	0.8 ± 0.3	0.700 ± 0.068	0.741 ± 0.043	***
	Desert	19	7.7 ± 1.0	0.5 ± 0.2	0.642 ± 0.060	0.752 ± 0.029	***
	Total	50	10.2 ± 1.5	2.5 ± 0.6	0.662 ± 0.058	0.760 ± 0.037	***

ns not significant

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3 Differentiation between populations by pairwise F_{ST} values

	West Coast	East Coast	Plateau	Steppe	Saharan Steppe	Desert
West Coast	–	*	*	***	***	***
East Coast	0.0256	–	ns	***	***	***
Plateau	0.0225	0.0082	–	***	***	***
Steppe	0.0548	0.0490	0.0599	–	ns	ns
Saharan Steppe	0.0542	0.0463	0.0527	0.0000	–	ns
Desert	0.0761	0.0506	0.0567	0.0153	0.0117	–

ns not significant

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

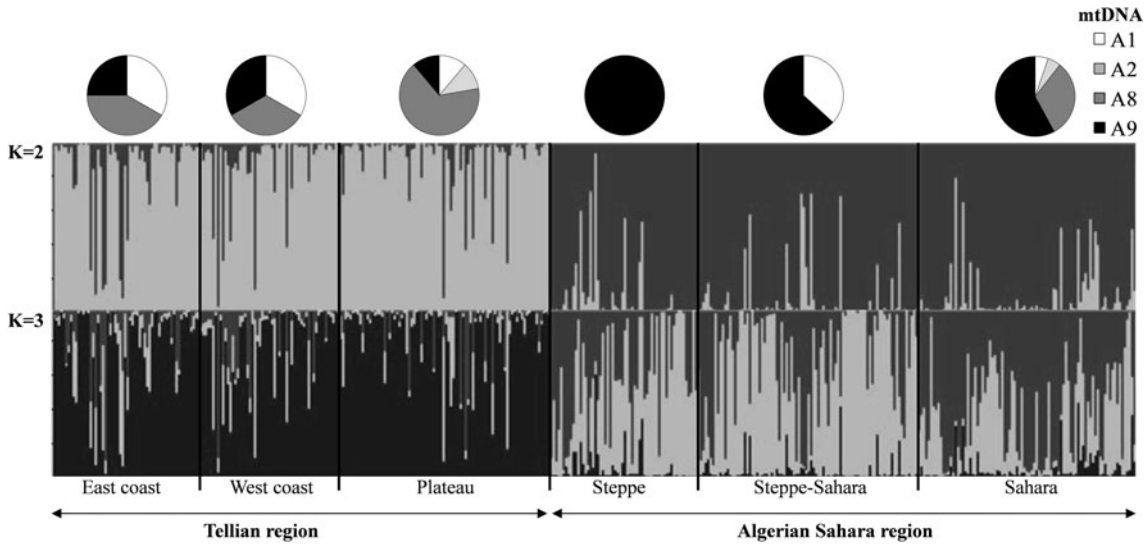

Fig. 2 Results of STRUCTURE analysis using admixture and correlated allele frequencies models. Individuals are represented by *vertical lines*, grouped by inferred populations (K = 2 but also K = 3). Division of individuals into coloured segment represents the assignment probability of that individual to each of the K groups. Haplotype frequencies in each population are shown at the *top*

other North African countries as Morocco (Franck et al. 2001), Tunisia (Lebdigrissa et al. 1991) and Libya (Shaibi et al. 2009), and also in Sudan (El-Niweiri and Moritz 2008). Repeated introduction of European honey bees as *A. m. carnica*, *A. m. ligustica* and *A. m. mellifera* (Moritz et al. 2005) into these countries have been detected through

mitochondrial analysis that revealed the presence of foreign haplotypes characterized by particular composition of the $tRNA^{leu}$-cox2 intergenic region.

Subspecies and population differentiation

The population structure analyses showed two well-defined clusters. Given the different geographic distribution pattern observed, these two groups could be assigned to the two subspecies naturally distributed in North Africa: the Tellian populations would correspond to *A. m. intermissa* whereas the populations located south to the Atlas Mountains would be assigned to *A. m. sahariensis*. This result needs further confirmation as the subspecies identification is based mainly on morphometric analysis (research in progress) following Ruttner (1988).

It is possible that the predominance of the A8 haplotype in *A. m. intermissa* and that of A9 in *A. m. sahariensis*, and the significant pairwise F_{ST} value between the two regions reflect a natural differentiation due to reduced gene flow between both subspecies, the Atlas Mountain acting as a barrier for the dispersion of the colonies. Hence, this isolation by distance could have not yet disappeared despite increased colony movements (migration, queen purchases, etc.) during the last decades.

At the population level, pairwise F_{ST} values revealed a weak differentiation among *A. m. intermissa* populations; the localities from East Coast and Plateau showed no significant difference despite the geographic distance between some of them. This observation may be due to the seasonal swarming behaviour exhibited by this subspecies over its distribution range (Ruttner 1988). It is also noteworthy that *A. m. sahariensis* populations were not significantly differentiated between them (low F_{ST} values), this suggests that the geographic distance is not an impediment to the gene flow among colonies south to the Atlas Mountains. Only the Desert population exhibits F_{ST} values higher than 0.01 in relation to the colonies from the Saharan steppe and the Steppe, this may indicate a slight level of differentiation, even if this result is not supported by a significant statistic analysis.

Gene diversity

Genetic variation in Algerian honey bees inferred from microsatellite markers is high, as expected for honey bee populations from the Middle East and African subspecies (Franck et al. 2001). This value is comparable to that found in Moroccan populations, with an estimated heterozygosity of 0.775 (*A. m. intermissa*) and 0.786 (*A. m. sahariensis*) (Franck et al. 2001). Similarly, De la Rúa et al. (2007) scored genetic diversity values of 0.79 ± 0.03 in *A. m. intermissa* and 0.82 ± 0.02 in *A. m. sahariensis*. In Libya, Shaibi and Moritz (2010) found higher values in the coastal ($He = 0.72 \pm 0.11$) than in oasis populations ($He = 0.63 \pm 0.11$), this may indicated long term isolation events in these last populations. This high genetic variability of African honey bee populations has been explained as a consequence of large effective population size through time, resulting from the pronounced migratory behaviour and tendency to swarm of these subspecies, and to their higher mating frequency (Franck et al. 1998, 2001). *A. m. intermissa* populations showed in this study a similar high genetic diversity that could also reflect the intensive migratory movements, whereas the values in *A. m. sahariensis* populations may alternatively be due to the introduction of honey bee queens from other origins.

Introgression and conservation of Saharan populations

The apiary established for the conservation of *A. m. sahariensis* at the Steppe area showed no indication of maternal introgression since all the colonies bear the A9 haplotype, which could be statistically considered as subspecies-specific marker. This population also showed the smallest gene diversity values within the Saharan region, in agreement with the suspected lack of queen introduction from other areas and isolation from neighbouring apiaries. The Hardy–Weinberg equilibrium observed in this population might be an indication that the apicultural practices in these colonies are appropriate for the conservation of *A. m. sahariensis.* Therefore, efforts to preserve and enlarge these "pure" populations must be supported if this subspecies is to be conserved.

On the other hand, colonies of *A. m. sahariensis* located in the Desert, south of the Steppe, showed a high percentage (32 %) of the A8 haplotype, which we have found to be closely associated to Algerian *A. m. intermissa* colonies. The most likely explanation for this finding is that colonies of this last subspecies were brought about from North Algeria in the 1960s, to recover decimated populations from pesticide campaigns for locust control. This maternal introgression only leaves as witness the mitochondrial haplotype if it is not repeated at a large scale. Notwithstanding, the Desert population showed a slight level of differentiation from the other two Saharan populations (as depicted from the F_{ST} and the STRUCTURE analyses), where no introductions took place. Colony translocation is also reflected in the gene diversity values, as the Desert population showed the highest value ($He = 0.752 \pm 0.029$), likely resulting from the introduction of novel genotypes. These introductions are also likely responsible of the Hardy–Weinberg disequilibrium observed in Table 2. This disequilibrium has been also found in populations from areas where honey bee introductions or migratory movements have been frequent

(Cánovas et al. 2011; De la Rúa et al. 2001, 2002, 2003; Muñoz et al. 2012). Therefore, not all Algerian populations located in the Saharan region resembling as *A. m. sahariensis* are adequate for managing conservation projects. We actively encourage the use of molecular tools to ensure the proper identification of the colonies to be conserved.

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References

Achou M (2007) Caractérisation morphométriques, biochimique et moléculaire des populations d'abeilles domestique de l'est Algerien. Effets physiopathologiques de son parasite majeur Varroa destructor. Thèse Doc. D'Etat, Univ.Baji Mokhtar, Annaba, Algérie

Baldensperger PJ (1924) North African bees. Bee World 5:175–176

Barry JP, Celles JC, Faurel L (1974) Carte Internationale du Tapis Végétal et des Conditions Ecologiques (1/1 000 000) et Notice explicative. Pub. Service d'écologie de l'Univ. d'Alger. édit. Par Soc. d'Histoire Naturelle de l'Afrique du Nord

Boore JL (2001) Mitochondrial gene arrangement source guide, version 6.1. Available at http://www.uni-ulm.de/fileadmin/website_uni_ulm/iui.inst.190/Forschung/Projekte/GENESIS/MGA_Guide.pdf. Accessed 18 July 2012

Buttel-Reepen H (1906) Apistica. Beiträge zur Systematik, Biologie, sowie zur geschichtlichen und geographischen Verbreitung der Honigbiene (*Apis mellifica* L), ihere Varietäten und der übrigen *Apis*-Arten. Veröff Zool Mus, Berlin, pp 118–120

Cánovas F, De la Rúa P, Serrano J, Galián J (2011) Microsatellite variability reveals beekeeping influences on Iberian honeybee populations. Apidologie 42:235–251

Cornuet JM, Garnery L (1991) Mitochondrial-DNA variability in honeybees and its phylogeographic implications. Apidologie 22:627–642

Cornuet JM, Daoudi A, Mohssine EH, Fresnaye J (1988) Étude biométrique de populations d'abeilles marocaines. Apidologie 19:355–366

De la Rúa P, Galián J, Serrano J (1998) Mitochondrial variability of honey bees populations from the Canary Islands. Mol Ecol 7:1543–1547

De la Rúa P, Galián J, Serrano J, Moritz RFA (2001) Genetic structure and distinctness of *Apis mellifera* L: populations from the Canary Islands. Mol Ecol 19:1733–1742

De la Rúa P, Galián J, Serrano J, Moritz RFA (2002) Microsatellite analysis of non-migratory colonies of *Apis mellifera iberica* from south-eastern Spain. J Zool Syst Evol Res 40:164–168

De la Rúa P, Galián J, Serrano J, Moritz RFA (2003) Genetic structure of Balearic honeybee populations based on microsatellite polymorphism. Gen Sel Evol 35:339–350

De la Rúa P, Radloff S, Hepburn R, Serrano J (2007) Do molecular markers support morphometric and pheromone analyses? A preliminary case study in *Apis mellifera* populations of Morocco. Arch Zootec 56:33–42

De la Rúa P, Jaffé R, Dall'Olio R, Muñoz I, Serrano J (2009) Biodiversity, conservation and current threats to European honey bees. Apidologie 40:263–284

El-Niweiri MAA, Moritz RFA (2008) Mitochondrial discrimination of honey bees (*Apis mellifera*) of Sudan. Apidologie 39:566–573

Estoup A, Garnery L, Solignac M, Cornuet JM (1995) Microsatellite variation in honey bee (*Apis mellifera*) populations: hierarchical genetic structure and test of the infinite and stepwise mutation models. Genetics 140:679–695

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Franck P, Garnery L, Solignac M, Cornuet JM (1998) The origin of West European subspecies of honey bees (*Apis mellifera*) new insights from microsatellite and mitochondrial data. Evolution 52:1119–1134

Franck P, Garnery L, Loiseau A, Oldroyd BP, Hepburn HR, Solignac M, Cornuet JM (2001) Genetic diversity of the honey bee in Africa: microsatellite and mitochondrial data. Heredity 86: 420–430

Garnery L, Solignac M, Celebrano G, Cornuet JM (1993) A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera* L. Experientia 49: 1016–1021

Garnery L, Mosshine EH, Oldroyd BP, Cornuet JM (1995) Mitochondrial DNA variation in Moroccan and Spanish honey bee populations. Mol Ecol 4:465–471

Garnery L, Franck P, Baudry E, Vautrin D, Cornuet JM, Solignac M (1998) Genetic biodiversity of the west European honey bee (*Apis mellifera* mellifera and *A. m. iberica*) I. Mitochondrial DNA. Genet Sel Evol 30:31–47

Grissa K, Cornuet JM, Msadda K, Fresnaye J (1990) Étude biométrique de populations d'abeilles tunisiennes. Apidologie 21:303–366

Haccour P (1960) Recherche sur la race d'abeille saharienne au Maroc. CR Soc Sci Nat Phys Extrait de La Belgique Apicole 25: 13–18

Hepburn HR, Radloff SE (1996) Morphometric and pheromonal analyses of *Apis mellifera* L. along a transect from the Sahara to the Pyrenees. Apidologie 27:35–45

Hepburn HR, Radloff SE (1998) Honey bees of Africa. Springer, Berlin

Lebdigrissa K, Msadda K, Cornuet JM, Fresnaye J (1991) The influence of European honeybees introduced in Tunisia on the Tunisian breed—*Apis mellifera intermissa*. Landbouwtijdschrift 44:631–636

Loucif-Ayad W (2009) Etude de la diversité génétique des abeilles domestiques Algériennes (*Apis mellifera* L.) et evaluation de l'effet de divers acaricides sur les abeilles et leur parasite *Varroa destructor*. Thèse doc., Univert. Baji Mokhtar, Annaba, p 171

Moritz RFA, Härtel S, Neumann P (2005) The western honeybee (*Apis mellifera* L.): an invasive species? Ecoscience 12:289–301

Muñoz I, Madrid-Jiménez MJ, De la Rúa P (2012) Temporal genetic analysis of an introgressed island honey bee population (Tenerife, Canary Islands, Spain). J Api Res 51:144–146

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–995

Quezel P, Santa S. (1962–1963) Nouvelle flore de l'Algérie et des régions désertiques méridionales, vol 1, CNRS, Paris

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact testsand ecumenicism. J Hered 86:248–249

Ruttner F (1975) African races of honey bees. In: Proc 25th Int Beekeep Congr. Bucharest, Apimondia

Ruttner F (1988) Biogeography and taxonomy of honey bees. Springer, Berlin

Shaibi T, Moritz RFA (2010) 10,000 years in isolation? Honeybees (*Apis mellifera*) in Saharan oases. Conserv Genet 5:2085–2089

Shaibi T, Muñoz I, Dall'Olio R, Lodesani M, De la Rúa P, Moritz RFA (2009) *Apis mellifera* evolutionary lineages in northern Africa: Libya, where orient meets occident. Insects Soc 56:293–300

Solignac M, Vautrin D, Loiseau A, Mougel F, Baudry E, Estoup A, Garnery L, Haberl M, Cornuet JM (2003) Five hundred and fifty microsatellite markers for the study of the honey bee (*Apis mellifera* L.) genome. Mol Ecol Notes 3:307–311

Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513