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Original article

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Abstract – The genetic diversity and structure of 414 honeybee workers from eight different populations in Algeria were analyzed using 14 polymorphic DNA microsatellite loci. The results showed that the honeybee populations were characterized by substantial genetic variation in terms of the average number of alleles and the degree of heterozygosity. Most populations were at Hardy–Weinberg equilibrium. Phylogenetic and population structure analyses confirmed the African origin of the studied Algerian populations and clustered them in a group distinct from evolutionary lineages West Mediterranean (M), North Mediterranean (C), and Oriental (O). Structure analyses revealed weak allelic introgression from both lineages M and C. High genetic variability was found within the Algerian populations. Two honeybee subspecies, *Apis mellifera intermissa* and *Apis mellifera sahariensis*, were present. However, to delimit the natural spread area of *A. mellifera sahariensis*, more samples from southern Algerian are needed.

African honeybee / DNA microsatellite markers / *Apis mellifera intermissa* / *Apis mellifera sahariensis* / genetic diversity

1. INTRODUCTION

Honeybees (Hymenoptera, Apidae) were naturally distributed in Africa, Europe, and Western Asia before they were spread around the world by human beekeeping activities. The intraspecific taxonomy of *Apis mellifera* L. has been based mainly on morphometric approaches, which subdivide this honeybee species into four different evolutionary lineages: West Mediterranean (M), which includes the Western European subspecies *Apis mellifera mellifera*; North Mediterranean (C); African (A), comprising the African honeybee subspecies; and Oriental (O), located geographically in the Middle East and represented mainly by Caucasian (*Apis mellifera caucasica*) and Turkish (*Apis mellifera anatoliaca*) honeybees (Ruttner 1988). Genetic studies have confirmed the morphometric classification (Garnery et al. 1993; Han et al. 2012).

Honeybee genetic diversity has been extensively studied using mitochondrial DNA (mtDNA) (Garnery et al. 1991, 1993, 1995, 1998a, b; Hall and Smith 1991; Arias and Sheppard 1996; De la Rua et al. 1998; Sheppard et al. 1999; Alburaki et al. 2011; Rortais et al. 2011) as well as different DNA microsatellite markers (Estoup et al. 1993,

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1995; Garnery et al. 1998a, b; Solignac et al. 2003, 2004; Alburaki et al. 2013). The intensive molecular studies during the last two decades have undoubtedly contributed to a better understanding of *A. mellifera* genetic diversity. However, some of the 26 known honeybee subspecies around the world (Ruttner et al. 1978; Ruttner 1988; Kauhausen-Keller et al. 1997; Sheppard et al. 1997; Sheppard and Meixner 2003) have been less well studied than others, especially those located in the African continent.

The North African honeybee populations are currently regarded as being members of two African honeybee subspecies: *Apis mellifera intermissa* (Buttel-Reepen 1906) and *Apis mellifera sahariensis* (Baldensperger 1924). Based on morphometric and geographical traits, these two subspecies were originally classified by Ruttner (1988) as intermediate between African and West Mediterranean groups. However, later molecular studies of some northern African honeybee populations, including samples of *A. mellifera intermissa* and *A. mellifera sahariensis*, confirmed that these subspecies are more closely related to the African lineage (Garnery et al. 1992, 1995; Franck et al. 1998, 2001).

A. mellifera intermissa is found in Tunisia, Algeria, and Morocco between the Atlas Mountains and the Mediterranean and Atlantic coasts (Ruttner 1988). In contrast, *A. mellifera sahariensis* ranges from Ain Sefra in Algeria through the oases of the Sahara south of the Atlas Mountains to Figuig in the west of Morocco (Baldensperger 1924, 1932; Haccour 1960; Adam 1983; Ruttner 1988). This subspecies has been also recorded in several oases in Southern Morocco (Ruttner 1988). Several morphometric studies were carried out on *A. mellifera sahariensis* to delimit the geographic areas in which this subspecies occurs (Cornuet et al. 1988; Grissa et al. 1990; Loucif and Tahar 2001; Barour et al. 2005).

Algerian honeybee diversity has not previously been studied on a large scale, especially at the molecular level. mtDNA is a suitable tool for characterizing honeybee colonies within populations and consequently detecting foreign queen importations. Microsatellite DNA is also a valuable molecular marker that has been widely used on honeybees and has proved to be highly efficient in differentiating populations and groups of populations and in detecting recent bottleneck events as well (Cornuet and Luikart 1996). Microsatellites have been intensively used on the European honeybee and much less so on the North African populations (Franck et al. 1998, 2000, 2001; Garnery et al. 1998a, b; De la Rua et al. 2001a, b, 2003; Miguel et al. 2007; Alburaki et al. 2013).

Very little is known about Algerian honeybee diversity. Modern beekeeping practices, particularly the importation of foreign queens and transhumance practices, are factors that can affect the genetic structure of a local honeybee population through genetic introgression (Garnery et al. 1998a, b; Jensen et al. 2005). To preserve the genetic diversity of Algerian honeybee populations, the genetic structure of the populations must first be understood. In this work, we studied the genetic structure of Algerian honeybee populations, their phylogenetic relationships with other honeybee subspecies, and probable genetic introgression and/or gene flow that may have resulted from beekeeping transhumance and commercial breeding.

2. MATERIALS AND METHODS

2.1. Sampling and DNA extraction

A total of 414 colonies from eight Algerian populations of *A. mellifera* were sampled in 2004–2008. The location of each sampled region as well as the number of sampled colonies for each population are detailed in Figure 1 and Table I. One worker bee per colony was sampled and individually preserved in absolute ethanol and subsequently analyzed using microsatellite markers. DNA was extracted from the bee head using a phenol–chloroform extraction method (Garnery et al. 1998a, b) and stored at −20 °C.

Figure 1. Geographical locations of the eight sampled honeybee populations in Algeria. *N* is the number of sampled colonies per population.

2.2. Microsatellite analysis

DNA samples were amplified using multiplex PCR with 14 microsatellite loci. Ten of these microsatellite loci were already known (B124, A43, A88, B24, A113, Ap43, A28, A7, Ap55, and Ap81) (Estoup et al. 1995; Garnery et al. 1998a, b; Franck et al. 2001; Perrier et al. 2003; Munoz et al. 2009), while four others (Ap33, A8, Ap36, Ap66) were newly identified from the *Apis* genome (Consortium 2006) and tested for diversity against a representative panel of individuals (Alburaki et al. 2013). PCR amplifications were carried out in a total volume of 10 μL, containing 1 μL of Taq 5× buffer (Promega, Fitchburg, WI, USA), 1.2 mM $MgCl_2$, 25 pmol of each primer, 1 μL bovine serum albumin, 25 nmol of each dNTP, 0.6 units of Promega Taq polymerase, and 1.0 μL of DNA extract. Annealing temperature was 54 °C for each plex. Reference populations previously analyzed were included in the analyses; they were from Georgia and Armenia (lineage O), Italy and Greece (lineage C), Morocco and Guinea (lineage A), and France and Belgium (lineage M) (Estoup et al. 1995; Garnery et al. 1998a, b; Franck et al. 2000; Alburaki et al. 2013).

2.3. Statistical analyses

Population parameters (expected and observed heterozygosity and number of alleles) were calculated according to Nei (1987). The exact test for Hardy–Weinberg equilibrium, genotypic linkage disequilibrium, was computed with the GENEPOP package (Raymond and Rousset 1995). To evaluate the genetic relationships among populations, a neighbor-joining (NJ) tree was constructed from microsatellite data using the chord distance of Cavalli-Sforza and Edwards (1967). Bootstrap values were computed over 2000 replications (Hedges 1992), resampling individuals within populations. To visualize the

Table I. Numbers of honeybee colonies sampled in each population and region in Algeria.

Population	Abbreviation	Region	GPS coordinates	No. of worker bees sampled
1-Annaba	Ana	Annaba	36° 53′ N 7° 45′ E	31
2-Chlef	Chl	Chlef	36° 10′ N 1° 20′ E	8
		Ain Defla	36° 04′ N 1° 59′ E	12
3-Mostaganem	Mos	Mostaganem	35° 55′ N 0° 05′ E	22
		Tlemcen	34° 52′ N 1° 18′ W	16
		Mascara	35° 23′ N 0° 08′ E	16
		Arzew	35° 51′ N 0° 18′ W	8
4-Boufarik	Bou	Boufarik	36° 34′ N 2° 54′ E	16
		Blida	36° 31′ N 2° 58′ E	40
		Kolea	36° 38′ N 2° 45′ E	16
		Tipaza	36° 36′ N 2° 23′ E	24
		Miliana	36° 18′ N 2° 14′ E	12
		Medea	36° 16′ N 2° 45′ E	38
		Dar-El Beida	36° 42′ N 3° 12′ E	8
		Tizi-Ouzou	36° 43′ N 4° 30′ E	5
		Algiers	36° 45′ N 3° 31′ E	6
5-Ain Sefra	Ain	Ain Sefra	32° 45′ N 0° 34′ W	44
6- Constantine	Con	Constantine	36° 21′ N 6° 36′ E	36
7-Tebessa	Teb	Tebessa	35° 23′ N 8° 65′ E	22
8-Biskra	Bis	Biskra	34° 51′ N 5° 44′ E	34
Total	–	–	–	414

groups in multivariate space, NUEES software (Langella 2001) was used to perform principal component analyses (PCA) using the distance matrix between populations. Finally, pairwise F_{st} values based on microsatellite variability were calculated as a short-term genetic distance between pairs of Algerian populations and pairs of Algerian and reference populations using GENEPOP (Raymond and Rousset 1995).

2.4. Population structure analysis

STRUCTURE software v2.3.3 (Pritchard et al. 2000) was used to identify genetically similar groups of individuals in our data set. The most likely number of clusters, K, was mathematically calculated according to Evanno et al. (2005) by using the ΔK value. The results were generated using an admixture model based on simulations of 100,000 burn-in steps and 500,000 Markov Chain Monte Carlo iterations after burn-in.

3. RESULTS

3.1. Genetic diversity

The numbers of detected alleles and observed and expected heterozygosities (H_o and H_e) per microsatellite locus were calculated. The overall population parameters are shown in Table II. For the 14 loci scored, the numbers of alleles in the sampled populations ranged from 2 for locus (B24) to 22 for locus (Ap43) (Table S1). Per-locus expected heterozygosity as a measure of the gene diversity ranged from 0.678±0.043 (Biskra) to 0.756±0.031 (Boufarik), while the average of the observed heterozygosity ranged from 0.661±0.051 (Annaba) to 0.717±0.041 (Mostaganem) (Table II). All loci were in Hardy–Weinberg equilibrium with respect to the studied populations except for two populations, Tebessa and Biskra. Fisher's probability value of

Table II. Multilocus microsatellite variability in honeybee populations from Algeria.

Population	N	n	H_o	H_e
Annaba	62	9.786±0.933	0.661±0.051	0.730±0.039
Chlef	40	8.50±0.803	0.682±0.039	0.748±0.033
Mostaganem	124	12.29±1.04	0.717±0.041	0.745±0.347
Boufarik	324.43±1.30	13.79±1.17	0.713±0.034	0.756±0.031
Ain Sefra	88	8.0±0.902	0.716±0.037	0.737±0.034
Constantine	71.571±0.228	8.714±0.801	0.706±0.048	0.691±0.044
Tebessa	43.857±0.143	7.286±0.73	0.681±0.038	0.697±0.039
Biskra	68	7.643±0.731	0.679±0.047	0.678±0.043

Values are averages and standard errors for each population

N mean sample size per locus, n mean observed number of alleles per locus, H_o observed heterozygosity, H_e expected heterozygosity

overall loci was highly significant for all populations expected for Biskra (0.0013) and Tebessa (0.048) (data not shown).

3.2. Phylogenetic analysis

Phylogenetic relationships among the Algerian honeybee and reference populations are presented in Figure 2. The microsatellite data clustered the honeybee populations into five groups depending on their origin: lineages M (France, Belgium), O (Armenia, Georgia), C (Greece, Italy), and A (Morocco, Guinea), and, finally, the Algerian group with 92 % bootstrap support (Figure 2). Clearly, none of the analyzed populations were closely related to the reference populations from lineages M, C, to O. In the NJ tree, the Algerian populations clustered near the populations from Morocco and Guinea, confirming their common African origin.

The projections on the first three PCA axes based on inter-population distances showed the position of each sampled population with the reference populations of the four evolutionary

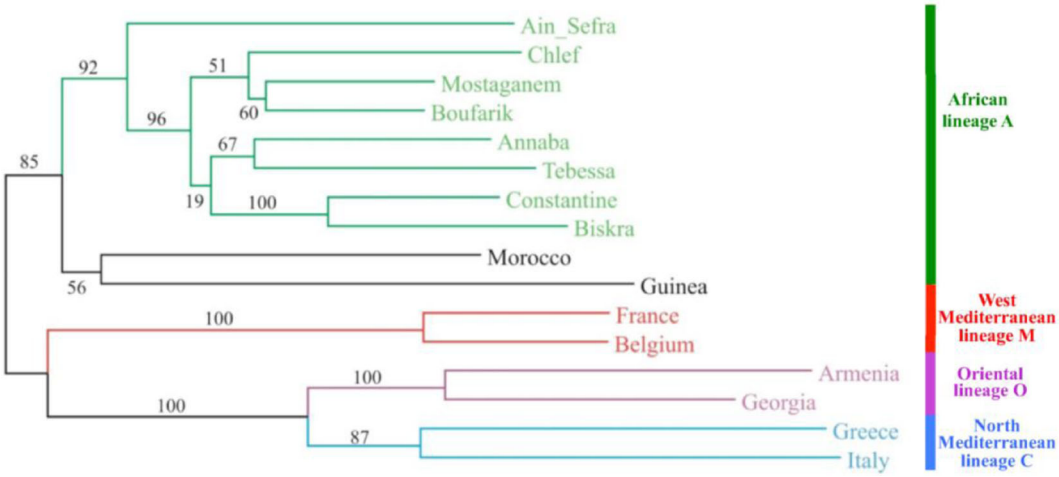

Figure 2. A neighbor-joining tree calculated based on 14 microsatellite loci using the chord distance of Cavalli-Sforza and Edwards (1967). Reference populations for each of the four evolutionary lineages of honeybees were included.

lineages. The first axis, which represented 37.86 % of the total variability, separated the reference populations of lineages M, C, and O from those of lineage A and from the studied populations (Figure 3a). On the third axis, which accounted for 10.14 % of the variability, the population of Ain Sefra differed slightly from the other Algerian populations toward the Moroccan populations (Figure 3b).

3.3. Population relationships

To assess the genetically differentiated populations in our data set, microsatellite pairwise F_{st} values were calculated among the eight Algerian populations and the reference populations of the four lineages (Table S2). Within the Algerian populations, multilocus F_{st} values varied between 0.002 (Annaba and Tebessa) and 0.089 (Ain Sefra and Biskra); the highest values were between Ain Sefra and Biskra (0.089) and between Ain Sefra and Tebessa (0.075). The F_{st} estimates of genetic distance revealed weak differentiation among the Algerian populations and reference populations of both Morocco and Guinea. The distance values between the Algerian populations were 0.002–0.089 (Morocco) and 0.060–0.118 (Guinea) for the African lineage. However, the distances were greater with other populations of lineages M (0.201–0.308), O (0.196–0.276), and C (0.264–0.333).

3.4. Population structure and introgression

The genetic structure of the studied populations was defined using the STRUCTURE software. The program was run for values $K=1$–10 with five repetitions. The most likely number of clusters, K, was mathematically calculated according to Evanno et al. (2005). The highest ΔK was detected with a model assuming four populations. The three most significant values of K, including the optimal one ($K=4$) are all summarized in Figure 4.

When $K=3$, three lineages were distinguished: African lineage A, including our studied samples; lineage M, which grouped the populations of France and Belgium; and lineages C and O together (Figure 4). At the optimal K value ($K=4$), a new cluster appeared grouping three African populations (Ain Sefra, Morocco, and Guinea). This classification remained almost the same when assuming five clusters, and no separation occurred between lineages C and O in this analysis. The optimal clustering ($K=4$) clearly indicated that the Algerian populations contain weak allelic introgression from both the M and C and/or O lineages. In addition, a remarkable genetic diversity within the African populations could be easily noticed from both $K=4$ and $K=5$.

4. DISCUSSION

In this study, an analysis of 14 microsatellite markers from eight local honeybee populations showed that Algerian *A. mellifera* populations are characterized by high variability in terms of number of alleles per microsatellite locus and heterozygosity (Tables II and S1). Similar observations in Algerian (Chahbar et al. 2012) and Moroccan bees have been previously obtained (Franck et al. 2001; De la Rua et al. 2007).

A comparison of the genetic variability between the Algerian populations and Moroccan (De la Rua et al. 2007), Canarian (De la Rua et al. 2001a, b), or Balearic (De la Rua et al. 2003) honeybee populations revealed that the microsatellite allele number per locus (A113, A28, and A7) is higher in the Algerian than in the Morocco and Spanish populations. Also, the heterozygosity values were higher than those in the Canarian and Balearic populations for the loci B124, A88, B24, A8, A113, A28, and A7 (De la Rua et al. 2001a, b, 2003). This variability in allele number could be also due to differences in bee sample sizes, whether within the Algerian populations or among the Algerian, Moroccan, and Spanish populations. In contrast, compared with our populations, the Canarian honeybee populations showed low levels of genetic variability in terms of average number of alleles and degree of heterozygosity (De la Rua et al. 2001a, b).

African honeybee populations are often characterized by high levels of microsatellite polymorphism (Estoup et al. 1995; Franck et al. 1998), which has been explained as a result of their pronounced migratory behavior and tendency to swarm (Franck et al. 1998). These two behaviors

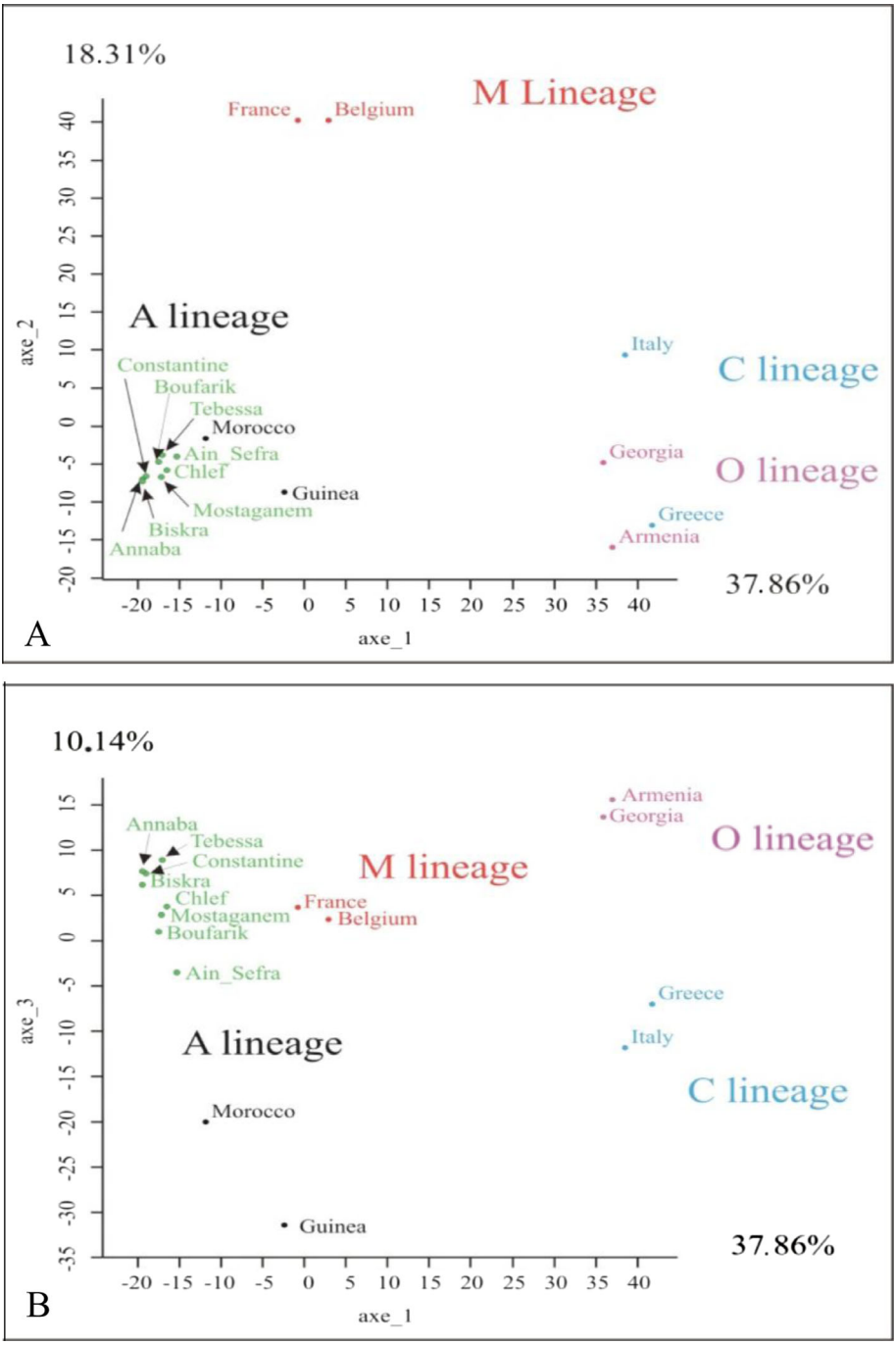

Figure 3. Principal component analysis of 14 microsatellite loci showing patterns of genetic variation in the studied populations. The four evolutionary lineages M, A, C, and O are differentiated along axes 1 and 2 (**a**) and axes 1 and 3 (**b**).

are well documented in Algerian honeybee hives. Our results showed that almost all of the studied Algerian populations were panmictic, except for those from Tebessa and Biskra, probably due to

the Wahlund effect (De la Rua et al. 2003; Perrier et al. 2003).

Both PCA and STRUCTURE results, as well as the NJ analysis, confirmed that Algerian honeybees belong to the African (A) lineage and are completely separate from other evolutionary lineages (M, C, and O). Locally, the population of Ain Sefra seemed to be most distant (Figure 2). In fact, the shorter the geographic distance between populations, the greater their genetic similarity. In this sense, the locations of the populations in the NJ tree coincided largely with their geographic distributions. The Ain Sefra population is particularly interesting, as it clustered independently with both Moroccan and Guinean populations, quite distinct from other Algerian honeybees (Figure 4). This clustering was confirmed by the higher F_{st} between Ain Sefra (0.045–0.089) and the other Algerian populations (0.002–0.042) (Table S2), as well as the clear separation of this population on axis 3 of the PCA (Figures 3 and 4). Honeybees of this population, which are very near the Moroccan border, could well probably be the subspecies *A. mellifera sahariensis*, which is known to spread along this area (Ruttner 1988). The ability to clearly discriminate Algerian honeybees from other honeybee populations has great economic importance for beekeepers in Algeria. It can help Algerian beekeepers to maintain local honeybee colonies, which are better adapted to the local climate and flowering periods.

The different methods used to analyze our data (PCA, NJ, and STRUCTURE) all showed similar results, confirming the robustness of our

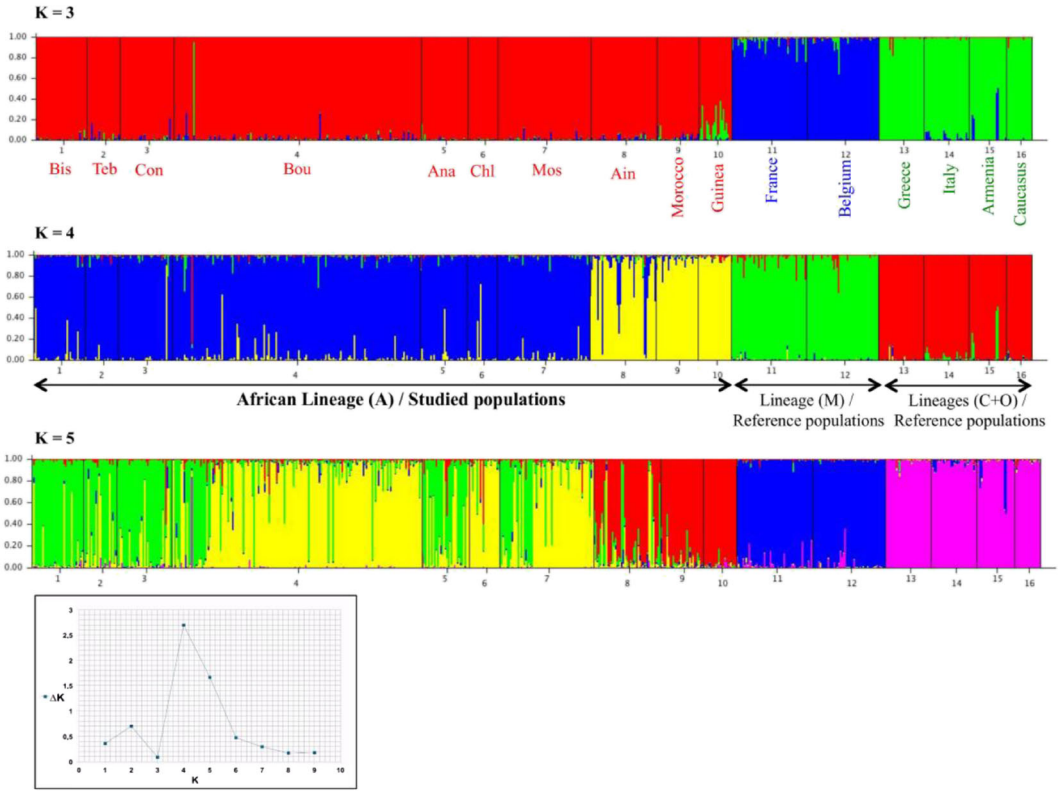

Figure 4. Structure analysis of 14 microsatellite loci from eight Algerian populations as well as reference populations from the four evolutionary lineages (M, A, C, and O) of honeybees. The most likely number of clusters (K =4) was mathematically calculated according to Evanno et al. (2005). The Algerian populations clustered with the reference African populations when K =3. When K =4 and K =5, the Ain Sefra population differed from the other Algerian populations and clustered with the populations from Morocco and Guinea.

sampling. However, the distinction of the Ain Sefra population was much more apparent in the STRUCTURE analysis than in the PCA on axis 3 (Figures 3b and 4). In contrast, while both lineages C and O were clearly separated in the PCA, STRUCTURE cluster them together because of the higher diversity expressed within the African lineage than in the C and O lineages (Estoup et al. 1995; Franck et al. 1998). The STRUCTURE results also clearly indicated that very weak allelic introgression from other lineages occurred in the African populations (Figure 4). However, these recent introgression events have not changed the gene pool of the local Algerian populations. Therefore, beekeepers must be aware of the impact that imported queens can have on the native *A. mellifera intermissa* populations. Previous studies have demonstrated similar results in Moroccan, Tunisian, and Algerian honeybee populations, in which African haplotypes were strongly dominant despite past importations of European honeybees (Lebdi-Grissa et al. 1991; Garnery et al. 1995; De la Rua et al. 2007; Chahbar et al. 2012). However, in other populations from Morocco, such as Tiznit, European microsatellite alleles were found, suggesting that introgression of foreign nuclear genes occurred via the drones in these populations (Estoup et al. 1995).

A. mellifera intermissa has, with no doubt, a superior ability to develop strong colonies in its native environment compared to that with European subspecies, which are generally much slower and less adapted in a North African environment (Garnery et al. 1995). Consequently, colonies headed by European queens in Algeria may not swarm properly and cannot survive for more than a few generations (Costa et al. 2012).

5. CONCLUSION

In conclusion, the microsatellite data of our study clearly indicated that Algerian honeybee populations belong to the African (A) lineage. Low levels of allelic introgression from both the M and C lineages were identified in some populations. The African honeybee samples analyzed in this study have remarkable genetic diversity within the African lineage. Expanding the sampling to the southern Algerian honeybee populations is of major importance, as it will allow a better understanding of the diversity of *A. mellifera sahariensis* subspecies and delimiting its territories. From a conservation point of view, limiting foreign queen importations to preserve the local genetic diversity of the Algerian honeybee populations is recommended.

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Conflict of interest The authors declare that they have no conflict of interest.

Evaluation génétique des populations d'abeilles algériennes par l'utilisation de marqueurs microsatellites

abeille africaine / *Apis mellifera intermissa* / *Apis mellifera sahariensis* / microsatellite / diversité génétique

Genetische Beurteilung der Honigbienenpopulation in Algerien anhand von Mikrosatelliten

Afrikanische Honigbienen / DNA Mikrosatelliten / *Apis mellifera intermissa* / *Apis mellifera sahariensis* / genetische Diversität

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