Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data

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A total of 738 colonies from 64 localities along the African continent have been analysed using the *Dra*I RFLP of the COI–COII mitochondrial region. Mitochondrial DNA of African honeybees appears to be composed of three highly divergent lineages. The African lineage previously reported (named A) is present in almost all the localities except those from north-eastern Africa. In this area, two newly described lineages (called O and Y), putatively originating from the Near East, are observed in high proportion. This suggests an important differentiation of Ethiopian and Egyptian honeybees from those of other African areas. The A lineage is also present in high proportion in populations from the Iberian Peninsula and Sicily. Furthermore, eight populations from Morocco, Guinea, Malawi and South Africa have been assayed with six microsatellite loci and compared to a set of eight additional populations from Europe and the Middle East. The African populations display higher genetic variability than European populations at all microsatellite loci studied thus far. This suggests that African populations have larger effective sizes than European ones. According to their microsatellite allele frequencies, the eight African populations cluster together, but are divided in two subgroups. These are the populations from Morocco and those from the other African countries. The populations from southern Europe show very low levels of ‘Africanization’ at nuclear microsatellite loci. Because nuclear and mitochondrial DNA often display discordant patterns of differentiation in the honeybee, the use of both kinds of markers is preferable when assessing the phylogeography of *Apis mellifera* and to determine the taxonomic status of the subspecies.

Keywords: Africa, *Apis mellifera*, microsatellite, mtDNA, phylogeography, population genetics.

Introduction

Biodiversity of the honeybee *Apis mellifera* was first assessed using morphometrics. Based on an extensive sample collection and multivariate analyses, Ruttner *et al.* (1978) proposed a classification which was long considered as definitive. These authors hypothesized that north-eastern Africa and the Near East would be the centre of origin of *Apis mellifera*. They proposed that the species invaded Africa and Europe in three distinct branches, a South and Central African branch (A), a North African and West European branch (M) and a North Mediterranean branch (C). This classification was further refined by the addition of a fourth evolutionary branch, called O, which included the Near and Middle Eastern subspecies (Ruttner, 1988). Data on mitoc-

hondrial DNA have confirmed the presence of three lineages A, M, C in Africa, western Europe and south-eastern Europe (Smith & Brown, 1988; Garnery *et al.*, 1992, 1993). The existence of a fourth mitochondrial lineage (O) in the Middle East has recently been confirmed (Arias & Sheppard, 1996; Franck *et al.*, 2000b; Palmer *et al.*, 2000). The same general structure of the species also emerged from microsatellite surveys (Estoup *et al.*, 1995; Franck *et al.*, 2000b). The main discrepancy provided by molecular markers was a clear genetic disruption between the branches M and A in the Iberian Peninsula (Smith *et al.*, 1991; Garnery *et al.*, 1995; Franck *et al.*, 1998). Consequently, honeybees from northern Africa and from western Europe are considered to have followed separate evolutionary histories.

The introduction of the African subspecies *A. m. scutellata* in Brazil (Kerr, 1957), its subsequent hybridization with previously imported subspecies (Michener,

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1975), and the rapid spread of this new hybrid strain (Africanized honeybee) throughout the Neotropics of America (Kerr, 1992) demonstrates how humans can greatly modify the genetic architecture of honeybee populations. Although most honeybee races present in Americas before the Africanization were native to Europe (Smith & Brown, 1988; Hall & Muralidharan, 1989; Hall & Smith, 1991; Sheppard *et al.*, 1991; McMichael & Hall, 1996), there are some clues that African honeybees from other subspecies than *scutellata* had been introduced into different American regions (Schiff & Sheppard, 1993; Sheppard *et al.*, 1999). It appears necessary to characterize the genetic diversity of honeybee races in their native range (Africa, Europe, and the Near-East). Only with this information will it be possible to accurately identify intrusion of unwanted genotypes and to develop coherent policies for conservation of local honeybee races. The present article focuses on African honeybees.

According to a recent review of the intraspecific nomenclature of *Apis mellifera* (Engel, 1999), 10 valid subspecies are recognized in Africa: *A. m. intermissa* (Maa, 1953) and *A. m. sahariensis* (Baldensperger, 1932) in Maghreb, *A. m. lamarckii* (Cockerell, 1906) and *A. m. jemenitica* (Ruttner, 1976) in north-eastern Africa, *A. m. monticola* and *A. m. litorea* (Smith, 1961) in south-eastern Africa, *A. m. unicolor* (Latreille, 1804) in Madagascar, *A. m. scutellata* (Lepeletier, 1836) and *A. m. capensis* (Eschscholtz, 1822) in southern Africa, and *A. m. adansonii* (Latreille, 1804) in western Africa. Hepburn & Radloff (1998) showed that these 10 subspecies have clearly separated morpho-clusters and they precisely delineated their geographical distribution. They also indicated geographical zones of high morphological variance within and between colonies, thus identifying hybrid zones among subspecies. Most African bees currently analysed belong to the A mitochondrial lineage (Smith, 1991; Garnery *et al.*, 1992, 1993; Arias & Sheppard, 1996; De la Rúa *et al.*, 1998). Only two colonies from Egypt have been recognized as belonging to lineage O (Arias & Sheppard, 1996; Franck *et al.*, 2000b). Microsatellite loci are extremely polymorphic in African populations compared to European honeybee populations and this has been interpreted as a consequence of larger effective population sizes in Africa (Estoup *et al.*, 1995; McMichael & Hall, 1996; Franck *et al.*, 1998). African populations would have been less influenced by quaternary ice episodes which are considered to be the main cause of honeybee subspecies differentiation in Europe (Ruttner, 1988). However, the question of a common evolutionary origin of the honeybees from Africa remains unanswered.

In this paper, several new African honeybee populations are investigated. In light of these new data, the

genetic variability of the honeybees from the African continent is reviewed by combining analyses of mtDNA and microsatellite polymorphisms.

Materials and methods

Sampling and DNA extraction

The origin of the samples is reported in Fig. 1. All African subspecies represented by a total of 738 honeybee colonies (254 colonies newly investigated) were sampled for use in this study. These colonies come from 64 locations in 21 African countries. In most cases the colonies have been morphometrically identified and several locations have been sampled within the endemic range of each subspecies (see Hepburn & Radloff [1998] for a review of original morphological data). A sample of 622 additional colonies from diverse locations in Europe, the Near East and America was used for comparison. Honeybee samples from each colony were brought back to the laboratory in vials containing 95% ethanol. The DNA was extracted from one leg with a chelex-based protocol (Estoup *et al.*, 1996).

Mitochondrial DNA

The mtDNA region including the tRNA^{leu} gene, the COI–COII intergenic region and the 5' end of the COII subunit gene was PCR-amplified according to a protocol detailed elsewhere (Garnery *et al.*, 1993). A fraction of the PCR product was run on a 1% agarose gel for total size determination and the remaining was restricted with *DraI* prior to electrophoresis on 7.5% polyacrylamide gel. The COI–COII mitotypes were determined for 404 new colonies. The newly found mitotypes were sequenced for further characterization as in Franck *et al.* (2000a). Additional data from De la Rúa *et al.* (1998), Garnery *et al.* (1993, 1995, 1998), Franck *et al.* (1998, 2000a,b) and Moritz *et al.* (1994) were included in the present study.

Microsatellite loci

Eight African populations for which a sufficient number of nonrelated honeybee individuals were available were scored at six microsatellite loci (*A113*, *A43*, *A28*, *A24*, *A88* and *B124*). These came from Morocco (Al-Hoceima and Kenitra, *A. m. intermissa*; Tiznit, *A. m. sahariensis*), Guinea (Nimba, *A. m. adansonii*), Malawi (Chelinda, *A. m. monticola*), and South Africa (Johannesburg and Pretoria, *A. m. scutellata*; Cape Town, *A. m. capensis*). They were compared to eight additional populations from France (Valenciennes, *A. m. mellifera*, branch M), Sweden (Umeo, *A. m. mellifera*, branch M), Spain

Fig. 1 Distributions of the COI–COII honeybee mitotypes in 21 African countries and nine other countries used as reference. Pie charts indicate the frequencies of the five mitochondrial lineages M, C, O, Y and A. In the A lineage, the three groups of mitotypes – A_I, A_{II} and A_{III} – are distinguished (see Fig. 4 for the details of mitotypes included within each lineage or group). Values under country names are the numbers of locations (right) and colonies (left) analysed per country (see <http://www.ensam.inra.fr/URLB> for a complete description of mitotypes observed at each location). The stars refer to the countries in which populations have been analysed using microsatellite markers. The right up corner map refers to the natural distribution of *Apis mellifera* subspecies studied.

(Sevilla, *A. m. iberiensis*, branch M), Portugal (Porto, *A. m. iberiensis*, branch M), Italy (Forlì, *A. m. ligustica*, branch C), Greece (Chalkidiki, *A. m. macedonica*, branch C), Sicily (Favara, *A. m. siciliana*, branch C), and Lebanon (El-Hermel, *A. m. syriaca*, branch O). The protocol followed and the original population data are reported in Estoup *et al.* (1995) and in Franck *et al.* (1998, 2000b).

Statistical and phylogenetic analysis

Unbiased estimates of gene diversity for microsatellite loci were calculated according to Nei (1978). Exact tests for genetic structure were computed using the GENEPOP package version 3.1 (Raymond & Rousset, 1995). The genetic differentiation between populations was computed using unbiased estimates of F_{ST} values provided by GENEPOP and the $(d\mu)^2$ microsatellite distance (Goldstein *et al.*, 1995).

A maximum parsimony tree of COI–COII mitotypes was constructed using the PHYLIP package version 3.5c (Felsenstein, 1993). Presence/absence of base pair substitutions and insertions/deletions along the COI–COII intergenic sequence were coded as 1/0. When a component was missing, the corresponding characters were coded as missing data (e.g. insertions/deletions and substitutions within the P sequence in lineage C). A neighbour-joining tree from population samples was calculated from microsatellite data using the chord distance of Cavalli-Sforza & Edwards (1967). Bootstrap values were computed over 2000 replications (Hedges, 1992) re-sampling individuals within population.

Results

Mitochondrial DNA

DraI RFLP of the COI–COII intergenic region provided a total of 42 different mitotypes among the 1359

colonies assayed (Fig. 2). Five mitotypes (A4', A25, A26, A27, Y1, Y2) were not reported in previous analyses (Garnery *et al.*, 1993, 1995, 1998; De la Rúa *et al.*, 1998; Franck *et al.*, 1998, 2000a,b; Palmer *et al.*, 2000). Their restriction maps, the length of their restriction fragments, and their P sequences are given in Figs 2 and 3.

A total of 50 characters provided by COI–COII restriction patterns were used to assess the phylogeny of COI–COII mitotypes (Fig. 4). The samples from Ethiopia were characterized by mitotypes which clearly belong to a fifth lineage that we have called Y. All other mitotypes are clearly assigned to their previously described lineages A, M, C, or O (Franck *et al.*, 2000b). The Y lineage has diverged from other lineages by around 2% (Table 1). Within lineage A, most of the mitotypes (group A_I) are not differentiated in the phylogenetic analysis (Fig. 4). Only two sublineages are identified. The first one is characterized by mitotypes displaying only restriction site 1 (group A_{II}). The second sublineage is characterized by mitotypes displaying the P₁ sequence (group A_{III}).

The distribution of COI–COII mitotypes per country is given in Fig. 1. African colonies display mainly mitotypes A1 and A4 (group A_I). Both mitotypes are also observed in high proportion in the Africanized bees from Mexico. In the populations from the Indian Ocean islands (*A. m. unicolor*), the proportion of mitotypes A1 is almost 100%. In continental African populations, the proportion of mitotypes A1 decreases progressively from Guinea toward south-eastern Africa and is replaced by mitotypes A4 in *A. monticola*, *A. m. scutellata* and *A. m. capensis*. From Guinea toward northern Africa, A1 is replaced by mitotypes A8, A9 and A10 (group A_{II}) in *A. m. sahariensis* and *A. m. intermissa*. Most of the mitotypes from northern Africa are also observed in *A. m. siciliana* and *A. m. iberiensis* populations from southern Europe. Nevertheless, these populations display a high proportion of mitotypes A which are extremely rare in continental Africa. Mitotypes A2 and A3 (group A_I) are principally observed in Spain and Sicily, and mitotypes of the A_{III} group are the most frequent within *A. m. iberiensis* populations from Portugal and the Canary Islands. Note that the populations from the Canary Islands also contain mitotypes C1 due to recent introduction of *A. m. ligustica* queens as in *A. m. mellifera* populations (Fig. 1). In north-eastern Africa, mitotypes belonging to three different lineages are identified (Fig. 1). Mitotypes A27 are observed only in *A. m. litorea* and they are highly divergent from other A mitotypes (Fig. 2). Mitotypes O and Y are observed only in *A. m. lamarckii* and *A. m. jemenitica*, respectively (Fig. 1).

Fig. 3 Sequences of the P region of COI–COII mitotypes indicating substitution sites (small characters), *DraI* restriction sites (bold characters) and insertion/deletion (dashes). Four diagnostic sequences are defined: the supposed ancestral P_0 sequence without deletion characterizing most of mitotypes of lineages A and O; the sequence P with the deletion *d* characterizing mitotypes of lineage M; the sequence P_1 with the deletion *d1* characterizing mitotypes of lineage A from the Atlantic coast; the sequence P_2 with the deletion *d2* characterizing mitotypes of lineage Y. Mitotypes C have no sequence P.

Microsatellite loci

Gene diversity estimates per population and microsatellite locus range from 0.671 (A24, Kenitra) to 0.922 (A88, Chelinda). Mean gene diversities per population are given in Table 2. The samples from Africa display gene diversity higher than those from Europe and Middle East (Mann–Whitney's *U*-test, $P < 10^{-6}$). Among the African samples, the Moroccan populations show the lowest values (Mann–Whitney's *U*-test, $P = 4 \times 10^{-5}$).

Fisher exact tests for genic differentiation indicate that all pairs of populations are significantly differentiated ($P_{\text{multilocus}} < 10^{-5}$). Among African populations F_{ST} values range from 0.0052 (Chelinda/Nimba) to 0.1239 (Kenitra/Cape Town) and $(d\mu)^2$ values range from 1.4535 (Pretoria/Nimba) to 12.1512 (Tiznit/Cape Town) (Table 3).

Fig. 2 Restriction maps (left) and restriction fragment sizes (right) of the 42 COI–COII mitotypes. The maps are deduced from *DraI* restriction pattern and sequences of the COI–COII intergenic mitochondrial region. Restriction sites are numbered from 1 to 10. Deletions and insertions are numbered preceded by characters *d* and *i*, respectively (see also Fig. 3). Exponent number refers to the number of equal size fragments.

In the neighbour-joining tree (Fig. 5) the populations are separated into four groups {Al-Hoceima, Kenitra, Tiznit, Cape Town, Johannesburg, Pretoria, Nimba and Chelinda}, {Seville, Porto, Valenciennes and Umea}, {Favara, Forli and Chalkidiki}, {El-Hermel} corresponding to the four branches A, M, C and O of Ruttner's classification (Ruttner, 1988). Within branch A, two sub-branches are identified. North-western African populations (Al-Hoceima, Kenitra and Tiznit) representative of the subspecies *A. m. intermissa* and *A. m. sahariensis* are clustered in the first sub-branch. The second sub-branch is composed of *A. m. adansonii* (Nimba) and *A. m. monticola* (Chelinda) populations in Equatorial Africa and *A. m. capensis* (Cape Town) and *A. m. scutellata* (Johannesburg, Pretoria) populations in southern Africa. Although *A. m. iberiensis* (Porto, Seville) and *A. m. siciliana* (Favara) populations display numerous A mitotypes, according to their morphological classification they cluster within branches M and C, respectively (Ruttner, 1988).

Discussion

The results can be summarized as follows. (1) Honeybees from north-eastern Africa contain three highly divergent mitochondrial lineages A, O, and Y, the third being newly characterized. (2) In the other parts of

Africa, honeybees carry only mitotypes of lineage A. Nevertheless, honeybees from north-western Africa and from tropical and southern Africa highly diverge when mitochondrial and microsatellite data are considered. (3) southern European and American populations also display several A mitotypes. In Spain, Portugal and

Fig. 4 Consensus tree (level 95%) of the COI-COII mitotypes over the equally parsimonious trees. The trees were established for 50 characters including restrictions sites, insertions and deletions (see Fig. 2). A, M, C, O and Y refer to the five major mitochondrial lineages. A_I, A_{II} and A_{III} refer to the three different groups of mitotypes within the lineage A.

Sicily they have several possible origins, presumably resulting from successive 'Africanizations'. In Mexico, the mitotypes A observed correspond to those displayed by *A. m. scutellata* populations from South Africa. (4) Microsatellite data indicate a high level of polymorphism in African populations compared to European ones.

The coexistence of three mitochondrial lineages in north-eastern Africa provides support for Ruttner *et al.*'s (1978) hypothesis that this area is the probable centre of origin for *Apis mellifera*. Note, however, that the Horn of Africa and the Rift Valley are the main channels of colonization from Asia to Africa. The presence of the mitochondrial lineage O in Egypt and Somalia and Y in Ethiopia may also result from successive honeybee invasions of Africa from the Middle East.

The quaternary climatic changes including desertification and vegetation shifts are considered quite important in explaining faunal distribution within the tropics (Potts & Behrensmeyer, 1992). They were probably responsible for honeybee subspecies diversification in Africa. The mitochondrial and microsatellite divergences between honeybee subspecies from northern and southern sides of the Sahara (mainly *A. m. intermissa* and *A. m. adansonii*) may have occurred during the late Pleistocene (around 15 000 years BP) when the Sahara extended largely southwards into the present Sahelian zone and the extreme north-west of Africa displayed favourable moist conditions (Mediterranean-like vegetation) for honeybees (Hooghiemstra *et al.*, 1992; Lioubimsteva, 1995) (Figs 1, 4 and 5). In the Middle Holocene (around 8000 years BP), African climates became moister and the Sahara desert almost completely disappeared (Gasse *et al.*, 1990; Hooghiemstra *et al.*, 1992). This change has probably facilitated gene flow between honeybees from the Maghreb and the Sahel. This hypothesis is further strengthened by contact zones along both sides of the Atlas range evidenced by mitochondrial DNA data (Garnery *et al.*, 1995). Another putative refuge for honeybees was the woodland and coastal forest along the borders of the Indian Ocean which persisted during the last arid period (around 15 000 years BP) when eastern Africa was largely characterized by open vegetation and the disappearance of forests at the top of eastern African mountains (Hamilton, 1982; Lovett,

	Lineage Y	Lineage O	Lineage A	Lineage M	Lineage C
Lineage Y	0.2–0.4	/	/	/	/
Lineage O	2.5–3.0	0.3–0.6	/	/	/
Lineage A	2.6–3.4	2.0–3.5	0.1–1.3	/	/
Lineage M	2.1–3.2	2.2–3.0	2.2–3.1	0.3–0.8	/
Lineage C	1.7–2.7	1.6–2.7	1.6–2.7	1.6–2.3	0.3–0.7

Table 1 Range of divergence percentage within and between lineages using 40 complete DNA sequences of the COI-COII intergenic region (sequence data are from Cornuet *et al.*, 1991; Garnery *et al.*, 1992; Franck *et al.*, 2000a,b; and this study)

1993a). This may explain the mitochondrial divergence observed between *A. m. scutellata* and *A. m. litorea* (Fig. 2) which was also recently reported by Meixner *et al.* (2000). The montane forest belt from the eastern African arc is rich in endemic species of plants (Lovett, 1993b). However the ecologically isolated subspecies *A. m. monticola* cannot be differentiated from *A. m.*

scutellata using mitochondrial DNA markers, although some differences can be detected using isoenzyme analyses (Meixner *et al.*, 1994, 2000). This may reflect recurrent introgression between both subspecies in the high tableland from northern Malawi studied here (N. Koeninger, personal communication). The honeybee subspecies from tropical and southern Africa (*A. m. adansonii*, *A. m. monticola*, *A. m. scutellata*, *A. m. capensis* and *A. m. unicolor*) are only slightly differentiated at the molecular level. The main difference among these five African subspecies arises from the variation in the proportion of mitotypes A1 and A4. It is noteworthy that both mitotypes only differ in the number of Q sequences (Fig. 2). Thus these differences

Table 2 Mean gene diversities and standard deviations for six honeybee microsatellite loci

Populations	$\bar{N}$	Gene diversities	SD
Al-Hoceima (<i>intermissa</i>)	54	0.775	0.129
Kenitra (<i>intermissa</i>)	50	0.756	0.058
Tiznit (<i>sahariensis</i>)	60	0.786	0.120
Nimba (<i>adansonii</i>)	56	0.894	0.023
Chelinda (<i>monticola</i>)	60	0.887	0.050
Pretoria (<i>scutellata</i>)	55	0.896	0.029
Johannesburg (<i>scutellata</i>)	36	0.867	0.046
Cape Town (<i>capensis</i>)	20	0.840	0.065
El-Hermel (<i>syriaca</i>)	86	0.636	0.192
Seville (<i>iberiensis</i>)	56	0.307	0.330
Porto (<i>iberiensis</i>)	56	0.259	0.352
Valenciennes (<i>mellifera</i>)	46	0.356	0.307
Umeo (<i>mellifera</i>)	44	0.314	0.322
Favara (<i>siciliana</i>)	100	0.663	0.114
Forli (<i>ligustica</i>)	60	0.406	0.226
Chalkidiki (<i>macedonica</i>)	58	0.468	0.255

$\bar{N}$, mean number of genes sampled per locus and per population.

Fig. 5 Neighbour-joining tree based on Cavalli-Sforza and Edwards' chord distance. Bootstrap values (2000 replications by resampling individuals) are indicated in percentages.

Table 3 Pairwise multilocus unbiased estimate of F_{ST} (below diagonal) and $(d\mu)^2$ genetic distance (above diagonal), in honeybee populations

	Hoc	Ken	Tiz	Nim	Che	Pre	Joh	Cap	Her	Sev	Por	Val	Ume	Fav	For	Cha
Hoc		1.86	1.77	4.88	2.97	3.45	5.75	10.58	20.84	27.70	33.88	17.16	17.83	13.32	40.83	23.28
Ken	0.02		2.18	1.92	3.32	1.86	3.59	8.73	13.58	20.34	24.96	11.52	13.72	7.45	27.09	15.16
Tiz	0.01	0.04		4.17	3.48	2.23	6.14	12.15	13.47	24.91	30.74	15.11	15.39	8.87	37.56	18.77
Nim	0.06	0.07	0.05		1.67	1.45	2.05	5.74	9.31	22.24	27.15	16.30	14.75	7.59	22.20	9.64
Che	0.06	0.07	0.05	0.01		2.05	2.52	4.64	14.18	31.34	37.97	23.19	20.32	11.75	32.10	14.09
Pre	0.07	0.08	0.06	0.01	0.01		5.18	6.96	12.46	21.13	26.65	12.97	13.00	9.59	28.57	13.65
Joh	0.07	0.09	0.06	0.02	0.01	0.01		4.85	9.32	34.09	39.28	25.83	25.37	6.06	21.09	8.39
Cap	0.10	0.12	0.09	0.04	0.02	0.02	0.03		17.18	45.97	52.66	33.40	35.76	13.87	22.00	8.94
Her	0.22	0.22	0.22	0.16	0.16	0.14	0.20	0.16		35.13	38.53	29.47	29.20	3.20	15.40	4.33
Sev	0.28	0.26	0.28	0.32	0.27	0.29	0.36	0.45	0.43		0.52	4.23	2.25	32.52	47.98	45.17
Por	0.30	0.28	0.31	0.35	0.30	0.31	0.39	0.49	0.46	0.01		5.91	4.82	35.69	48.65	49.11
Val	0.24	0.20	0.25	0.28	0.25	0.26	0.32	0.40	0.39	0.06	0.07		4.81	22.49	40.72	36.13
Ume	0.27	0.24	0.27	0.31	0.27	0.28	0.35	0.44	0.43	0.08	0.10	0.04		27.65	49.40	39.26
Fav	0.12	0.13	0.13	0.14	0.13	0.12	0.16	0.16	0.15	0.36	0.38	0.31	0.35		14.65	6.06
For	0.36	0.35	0.33	0.29	0.26	0.25	0.35	0.34	0.32	0.59	0.61	0.53	0.56	0.24		7.18
Cha	0.31	0.31	0.29	0.25	0.23	0.22	0.31	0.29	0.25	0.60	0.63	0.55	0.59	0.17	0.19	

Population names are abbreviated as follows: Hoc, Al-Hoceima; Ken, Kenitra; Tiz, Tiznit; Nim, Nimba; Che, Chelinda; Pre, Pretoria; Joh, Johannesburg; Cap, Cape Town; Her, El-Hermel; Sev, Seville; Por, Porto; Val, Valenciennes; Ume, Umea; Fav, Favara; For, Forli; Cha, Chalkidiki. See text and Fig. 5 for population locations.

are probably not evolutionarily significant across a Quaternary time scale. Consequently, the absence of endemic mitotypes and the absence of mitochondrial polymorphism in the *A. m. unicolor* populations could point to a recent colonization of the Indian Ocean islands by an extremely reduced number of colonies. Compared to *A. m. unicolor*, the 'Africanized honeybee' from Mexico displays both African mitotypes A1 and A4 whereas around 50 *A. m. scutellata* colonies have been introduced in Brazil in 1956.

The main explanation for the low molecular differentiation among African subspecies is probably a result of their highly migratory behaviour (absconding, swarming), specifically of *A. m. adansonii* and *A. m. scutellata* (Fletcher, 1978; Hepburn & Radloff, 1998). Direct genetic evidence of such behaviour is the high level of polymorphism at nuclear markers observed in all African populations assayed thus far (Table 2; Hall, 1992, 1998; Estoup *et al.*, 1995; McMichael & Hall, 1996; Franck *et al.*, 1998). This is also an explanation for the diffusion of mitotypes A outside the African continent into the Americas, the Iberian Peninsula and the Mediterranean Islands (Fig. 1; Hall & Muralidharan, 1989; Smith *et al.*, 1989, 1991; Hall & Smith, 1991; Garnery *et al.*, 1993, 1995; Sheppard *et al.*, 1997; Sinacori *et al.*, 1998). Interestingly, the diffusion of the African mitochondrial genome into southern Europe and the Americas does not necessarily correspond to the 'Africanization' of the nuclear genome (Lobo & Krieger, 1992, 2000a; Franck *et al.*, 1998). Conversely, whereas European subspecies such as *A. m. mellifera* and *A. m. ligustica* have been repeatedly introduced into northern Africa (Second, 1975), not a single M or C mitotype has been found in north-continental Africa (but see Fig. 1 and De la Rúa *et al.*, 1998 for the Canary Islands). Nevertheless, some evidence, including the sequences of microsatellite alleles (Franck, 1999), suggests nuclear introgression of Moroccan honeybees by some European alleles.

Consequently, mitochondrial data alone are probably insufficient to infer taxonomic and genetic status of honeybee colonies. Extending microsatellite analysis to honeybee subspecies from eastern Africa and the Middle East will be useful in the future for understanding the phylogeography of *Apis mellifera* and resolving relationships among all the African subspecies.

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