Original article

Genetic diversity of the endemic honeybee: *Apis mellifera unicolor* (Hymenoptera: Apidae) in Madagascar

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Abstract – *Apis mellifera unicolor* is a tropical honeybee endemic of Madagascar. Comprehensive knowledge about its mitochondrial and nuclear genetic diversity and structuration was our main purpose. Samples of worker bees were collected from 867 colonies in 76 sites in Madagascar and 1 reference population in South Africa. PCR-restriction fragment length polymorphism (RFLP) and sequencing were used to reveal variability in the COI–COII mtDNA region. Seventeen microsatellite *loci* were used for studying the nuclear diversity. Three PCR-RFLP profiles were observed, among which 99.4 % belonged to A1 haplotype, 0.2 % to a new A haplotype, and 0.4 % to A4 haplotype. In microsatellite analysis, moderate genetic diversity values were found for Madagascar, together with low mean number of alleles ranging from 2.47 to 3.88 compared to South Africa. Bayesian clustering assignment methods and principal component analysis (PCA) separated populations into two genetic clusters which matched with geographic areas. Several hypotheses are discussed regarding to the low genetic diversity of *A. m. unicolor* in its native range.

Apis mellifera unicolor / microsatellite / African lineage / genetic diversity / mtDNA

1. INTRODUCTION

Apis mellifera subspecies are the most economically valuable pollinators of crop monocultures worldwide (Klein et al. 2007). In addition, honeybee contribution to floral biodiversity and conservation through pollination is estimated to affect 80 % of wild flora (Batra 1995; De la Rua et al. 2009).

Remarkable morpho-geographical differentiations are found throughout honeybee distribution areas; 28 subspecies are endemic to Africa, Europe, and Middle East (Ruttner et al. 1978; Ruttner 1988; Sheppard et al. 1997; Sheppard and Meixner 2003). Four evolutionary lineages have been described based on phenotypes and molecular traits (Garnery et al. 1992, 1993; Estoup et al. 1995; Franck et al. 2000, 2001; Alburaki et al. 2013).

In Africa, 11 subspecies are found from all lineages except from the C lineage (Hepburn and Radloff 1998; Meixner et al. 2011). The African A lineage is native to Africa and subspecies include *Apis mellifera scutellata* (Lepeletier 1836), *Apis mellifera capensis* (Eschscholtz 1822), and *Apis mellifera unicolor* (Latreille 1804).

A. m. unicolor is the endemic subspecies of Madagascar, a drifted tropical island, located 400 km off the East coast of Africa. Madagascar is among the five richest biodiversity hotspots of the world in terms of endemic plants and vertebrate species (Myers et al. 2000). However, less than 10 % of its original habitat still remains (Myers et al. 2000). In such an area of endemism, with over 80 % of endemic phanerogam (Ganzhorn et al. 2001), the role of pollinating insects is vital for the reproduction of these plants. Although the pollinating role of the honeybee has never been investigated in detail for these floras, *A. m. unicolor* is thought to play a crucial role in the pollination of endemic phanerogam species (Ruttner 1975; Ralalaharisoa-Ramamonjisoa et al. 1996).

After molecular analysis, mitotype A1 was attributed to the honeybee of Madagascar, with a surprising absence of mitochondrial polymorphism within this endemic subspecies (Franck et al. 2001). The latest molecular study using SNP analyses suggested that *A. m. unicolor* had a specific SNP (Whitfield et al. 2006). However, those assumptions were based on a low number of samples (<50) taken from regions of the vast territory of Madagascar. In terms of morphological and behavioral criteria, two ecotypes were described, the first from the highlands (Hauts Plateaux) and the second from the coastal area (Ruttner 1988).

Since the recent arrival of the parasitic mite *Varroa destructor* documented in Madagascar in 2010, severe colony losses have been observed (Rasolofoarivao et al. 2013). This situation may dramatically impact the biodiversity of Madagascar. Therefore, considering the lack of genetic data and the threat to this subspecies, it appears necessary to improve our knowledge on *A. m. unicolor* genetic diversity in its native area.

2. MATERIAL AND METHODS

2.1. Area of study

The land area and relief of Madagascar gives rise to various climatic zones. The year is characterized by two distinct seasons: the austral winter (April to November) and the austral summer (December to March). The coastal zones enjoy a warm climate, and mean annual temperatures range between 22 and 25 °C. The upland areas of the

Figure 1. Madagascar map with ecological zonation ▶ and honeybee sampling sites (emplacements of each site are presented in *red polygons*). The 76 sites are spread in 6 geographic regions. *Each number* represents a site with correspondence in Table I.

island have a more temperate climate with a mean annual temperature of 20 °C. Tropical forests mainly consist of deciduous woodland in Western Madagascar and xerophytic thorn forests in the southern region (Figure 1).

2.2. Sampling

One adult worker honeybee per colony was sampled between August 2011 and March 2013 from 76 sites ($n =867$) in Madagascar (Figure 1, SD Table I). Sampling was performed on managed colonies and 33 wild colonies (collected in Djamajar (S8) $n =6$, Antsoha (S7) $n =9$, Rantolava (S24) $n =3$, and Tsararano (S21) $n =15$). As a reference population from African lineage, samples were collected from 22 managed colonies in South Africa (one apiary from Cape region) in 2013. Honeybees were preserved in ethanol (96 %) and kept at −20 °C until molecular analysis.

2.3. DNA extraction

The six worker legs were used for DNA isolation. DNA was extracted from individual honeybees as previously described (Delatte et al. 2010). All individuals were subjected to both microsatellite and mitochondrial analyses.

2.4. Microsatellite amplification and genotyping

Microsatellite population studies were carried out using 17 loci published in Solignac et al. (2003) and combined into four different mixes (mix 1: A024, A113, Ac306, Ap055, Ap081; mix 2: A (B)124, A028, A029, A088, Ap273, Ap289; mix 3: Ap033, A035, Ap036; mix 4: A014, Ap043, Ap066). PCR reactions were performed in a 10 μL final reaction volume with a primer mix (10 μM) using Type-it Multiplex PCR Master Mix (Qiagen) kits. PCR programs were run with an initial denaturation of 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s; annealing was 55 °C for 30 s for mix 1 and 52 °C for mixes 2–4, followed by elongation at 72 °C for 30 to 45 s. A final extension was done at 72 °C for 10 to 20 min.
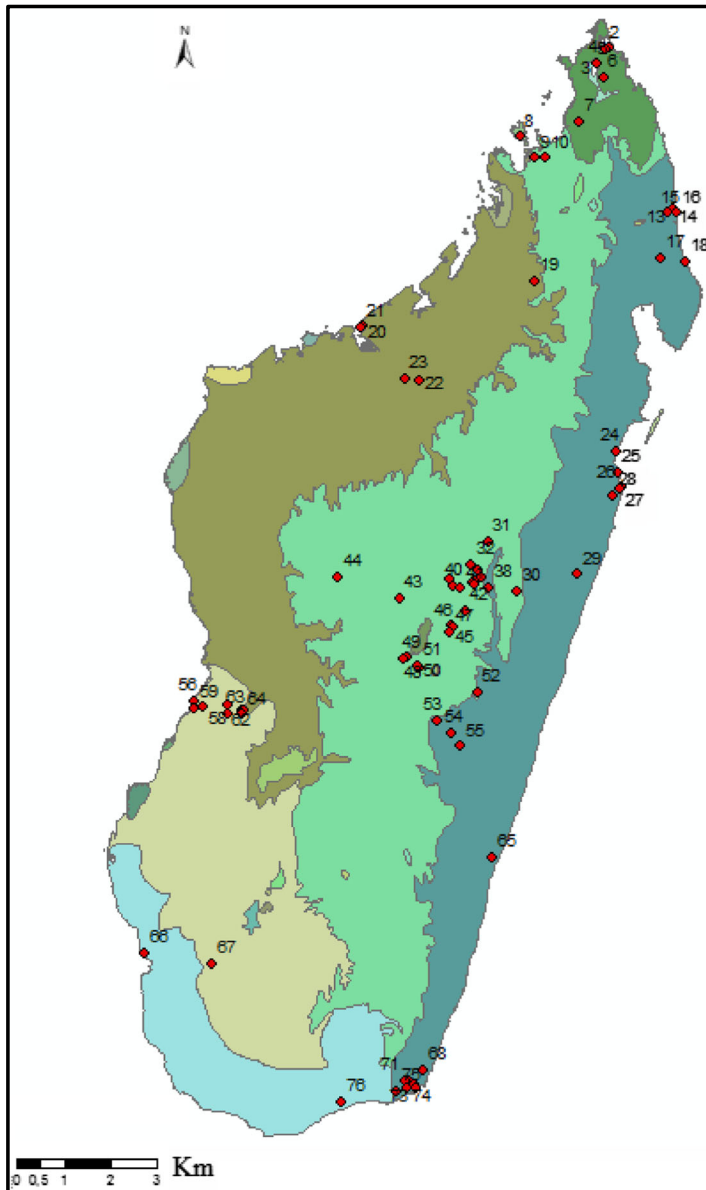
North: dry deciduous forest, dry tropical climate

West: dry deciduous forest, hot tropical climate during dry season

Center: high land, sub humid forest, altitude tropical climate

East: wetter area of island, lowland forest, sub equatorial climate

South: half desert, spiny thickets, dry tropical climate

South west: succulent woodlands, dry tropical climate

Samples were further genotyped using an automated DNA sequencer (Applied Biosystems 3130XL) with capillaries. Allele sizes were scored using GeneMapper 4.0 Software. Individuals with genotype data missing for more than 40 % of all loci were excluded from statistical analysis. Small sample sizes under 10 individuals per site were not included in the nuclear genetic analysis at population level.

2.5. Microsatellite analysis

Observed (H_o), expected (H_e), and Nei's 1987 unbiased expected ($H_{n.b}$) heterozygosity and fixation indices (F_{is}) (Weir and Cockerham 1984) were estimated using Genepop 4.2 (Rousset 2008) and Genetix 4.05 (Belkhir et al. 1996). All pairs of loci were tested for linkage disequilibrium using Genepop 4.2 (Rousset 2008). Deviations from Hardy–Weinberg equilibrium (HWE) were tested using a two-tailed Fisher exact test based on Markov chain (Rousset 2008). Permutation tests conducted by FSTAT (Goudet 2001) determined whether genetic diversity (H_e, $H_{n.b}$, H_o) and F_{is} differed significantly between geographical regions.

FreeNA (Chapuis and Estoup 2007) was used to estimate null allele frequencies. Population differentiation was quantified by calculating pairwise F_{ST} values (Weir and Cockerham 1984) and verifying their significance through the permutational test in Genetix 4.05 (Belkhir et al. 1996). Relationships between genetic and geographic distances at all sites were tested using the Mantel's test in Genepop 4.2 (Rousset 2008). The significance of the correlation between matrices of geographical and genetic distances among pairs of sites was tested using 1000 permutations of the data. As potential isolation by distance (ibd) patterns may not hold over the entire range (1470 km), because at some point the influence of gene flow is expected to be weak relative to the influence of genetic drift and homoplasy (Hutchison and Templeton 1999), ibd patterns were investigated at smaller spatial scales, i.e., in sites from the northern regions (500 km, n_{sites}=7), the central regions (370 km, n_{sites}=15), and southern regions of the island (380 km, n_{sites}=9). We further investigated the importance of scale on spatial genetic structuring by performing a hierarchical F analysis, which estimates the genetic variation found at each hierarchical level. A nested tree-level analysis of molecular variance (AMOVA, Excoffier and Lischer 2010) was performed by partitioning the total sum of squares into components representing variation between geographical regions, among sites within regions and among individuals within sites using Arlequin V3.5 (Excoffier and Lischer 2010).

Levels of population admixture were quantified using a number of Bayesian clustering procedures as implemented in Structure V2.3.4 (Pritchard et al. 2000). The number of population clusters was inferred according to Evanno et al. (2005) and the ad hoc statistic ΔK was calculated for K ranging from 1 to 10 for the full dataset comprising the reference population and 1 to 20 within the Madagascar dataset (1 million simulations and 100,000 burn-in with 10 iterations for each K). This ad hoc statistic was processed through the Structure Harvester website (http://taylor0.biology.ucla.edu/structureHarvester/). Clumpp v.1.1.2 (Jakobsson and Rosenberg 2007) was used to align the best of the five repetitions of the K. Distruct v.1.1 (Rosenberg 2004) was used to graphically display the results. A principal component analysis (PCA) was performed on the genetic data to visualize genetic differentiation among the population groups using adegenet 1.4 (Jombart 2008) in R software (Team 2005). Adegenet 1.4 was also used to check the dataset structure using an alternative clustering analysis with a discriminant analysis of principal component (DAPC).

2.6. Mitochondrial analysis

Mitochondrial DNA COI–COII region was amplified using two specific primers, E_2 and H_2 (Garnery et al. 1992). Amplification and PCR cycles were as described in Garnery et al. (1992). The size of the fragment amplified was visualized using 5 μL of the PCR products electrophoresed on 2 % agarose gel. PCR products were then used both for enzymatic restriction and sequencing.

Ten microliters of PCR products from Madagascar (n=867) and South Africa (n=22) were enzymatically digested by the *DraI* (Promega©) enzyme according to manufacturer recommendations. The resulting fragments were separated in 4 % agarose gel. Each enzymatic profile was scored and compared to expected sizes published (Garnery et al. 1992; Franck et al. 2001). Each profile with a different band pattern observed on gel in Madagascar was sent for sequencing (121 samples of the dominant profile, 2 per other profile). PCR products (n=173) were sent to Macrogen© for sequencing. Of these, some were taken from wild colonies (site Tsararano S21 n=10). DNA sequence

results were aligned using MEGA 5.04 (Kumar et al. 2008) then analyzed by BLAST search on GenBank. Genetic relationships between the different sequences obtained for the A1 haplotypes from Madagascar were investigated by constructing a minimum-spanning network of the haplotypes with TCS software (Clement et al. 2000).

3. RESULTS

3.1. Diversity indices

The amplification was successful for 710 out of 867 Malagasy and for all South African ($n=22$) individuals (with at least 10 loci amplified per sample). Of the 77 sampled sites, only 33 have more than 10 individuals (SD Table I). Analysis for linkage disequilibrium showed no significant deviation from equilibrium among the 17 microsatellite marker pairs (all $P>0.05$), except between loci A113 and A24 ($P<0.05$).

The average number of alleles per population and over 17 markers varied between 2.47 and 3.88 in Madagascar with an overall average of 7.76 alleles/*locus*, and for the single reference population of South Africa, the average number was 12.57 alleles/locus (Table I).

Unbiased estimated heterozygosity ($H_{n.b}$) ranged from 0.36 to 0.50 for Madagascar, and for the reference population $H_{n.b}=0.86$ (Table I). Across all loci, less than 10 % null allele was observed (Table I). Inbreeding coefficient F_{is} ranged from heterozygote excess (outbreeding, −0.07) to heterozygote deficiency (inbreeding, 0.15) compared with HWE expectations. No difference was detected among geographical regions within Madagascar (SD Table II, Comparisons among groups, all $P>0.4$).

3.2. Genetic structure

F_{ST} estimates among pairs of sites ranged from −0.02 to 0.17, with 318 out of 496 genetic differentiation estimates being significantly different from zero (SD Table III) within Madagascar dataset. F_{ST} estimates between the overall Malagasy populations and the reference population of South Africa were highly significant and equal to 0.34.

Within each of the northern, central, and southern parts of Madagascar (<500 km between sites), nuclear genetic differentiation between sites was positively correlated with the logarithm of geographical distance (Figure 2 a, b, c). However, at the island scale (north to south 1470 km, $n_{sites}=32$), the correlation between the two matrices was no longer significant (Mantel test, $P=0.87$; Figure 2d). The analysis of molecular variance comprising only Madagascar populations showed that the main contribution to the genetic variance was variation within sites (94 %). Differences among regions (2.3 %) and among sites within regions (3.34 %) were much lower but contributed significantly to the total genetic variation (SD Table IV). When all regions were taken into account (within Madagascar), all hierarchical levels accounted for a significant part of the genetic diversity.

Variation in allelic composition was highlighted by Structure. The optimal $K=2$ was the most strongly supported in likelihood using the reference population (Figure 3), with South African individuals being far apart from the Malagasy individuals. Then, looking at the substructure intra-Madagascar populations, the optimal and strongest K population is 2 (SD Fig. 1, SD Fig. 2). The DAPC analysis corroborates the same clustering analysis using both datasets. For the full dataset (South Africa and Madagascar), three genetic clusters were found (one for South African individuals, and two closer ones within Madagascar; Figure 4).

Under the $K=2$ population clustering assumption within Madagascar, a geographic structuring appears with populations from the north, northwest, and south together in cluster 1 as opposed to populations from the west and east (cluster 2). The highland populations were mostly a mix of two genetic clusters (Figures 1 and 4, SD Fig. 2).

3.3. PCR-restriction fragment length polymorphism patterns and sequences

After analysis of the Madagascar samples, three different PCR-restriction fragment length polymorphism (RFLP) profiles were detected. Two restriction profiles were congruent with ones already published. The dominant PCR-RFLP

Table I. Genetic diversity indices by sites.

Geographical regions	Map codes	Sites (S)	Number	H_e	$H_{n.b.}$	H_o	F_{is}	N_{br}	An
Madagascar									
North	2	Namakia	10	0.38	0.40	0.42	−0.07	2.47	0.05
Northeast	11	Analamandrorofo	16	0.42	0.44	0.38	0.11[a]	3.47	0.06
	13	Analapenja	11	0.35	0.37	0.34	0.08	2.76	0.02
	15	Antohomarina	22	0.38	0.39	0.34	0.14[a]	3.29	0.07
Northwest	20	Tsararano M.	10	0.38	0.40	0.39	0.01	3.00	0.02
	21	Tsararano M.	15	0.43	0.44	0.37	0.16[a]	3.35	0.07
	22	Tsaramandroso	10	0.42	0.45	0.35	0.21[a]	3.18	0.07
Hauts Plateaux	34	Ambotseheno	19	0.40	0.41	0.34	0.18[a]	3.24	0.08
	35	Betoho	16	0.38	0.39	0.35	0.10[a]	2.94	0.05
	36	Anjepy	12	0.42	0.44	0.42	0.04	3.24	0.04
	37	Maharidaza	10	0.38	0.41	0.40	0.02	2.76	0.03
	38	Mandraka	12	0.40	0.41	0.38	0.09	3.06	0.05
	40	Ivato	17	0.43	0.45	0.41	0.06	3.18	0.07
	42	Andramasina	10	0.43	0.46	0.40	0.14[a]	3.12	0.06
	43	Toronala	33	0.38	0.39	0.36	0.07[a]	3.53	0.05
	47	Ihazolava	12	0.38	0.40	0.36	0.11[a]	3.06	0.06
	49	Anativato	11	0.40	0.42	0.43	−0.02	3.00	0.05
	50	Mahazina	10	0.34	0.36	0.32	0.11	2.94	0.05
Hauts Plateaux south	52	Soanirina	11	0.37	0.40	0.40	−0.04	2.56	0.05
	53	Ambositra	10	0.36	0.39	0.32	0.17[a]	2.59	0.05
	54	Tsararano	43	0.38	0.39	0.36	0.07[a]	3.76	0.05
	55	Vohimasina	22	0.40	0.41	0.38	0.09[a]	3.29	0.07
West	63	Androvabe	11	0.47	0.50	0.43	0.15[a]	3.24	0.07
Southwest	66	Toliary	30	0.39	0.39	0.33	0.15[a]	3.88	0.06
	67	Bezaha	23	0.42	0.43	0.42	0.03	3.47	0.02
South	68	Mahatalaky	17	0.44	0.45	0.41	0.10[a]	3.47	0.06
	70	Ifarantsy	10	0.35	0.37	0.35	0.07	2.53	0.03
	71	Ifarantsy	16	0.39	0.40	0.38	0.04	3.24	0.04
	72	Ampasy N.	10	0.42	0.45	0.39	0.13[a]	3.12	0.05
	74	Soanierana	21	0.40	0.41	0.38	0.08[a]	3.47	0.06
	75	Ankaramena	13	0.35	0.36	0.36	0.03	2.71	0.05
	76	Ambovombe	15	0.42	0.44	0.39	0.09	3.35	0.04
South Africa	77	Cape	22	0.84	0.86	0.83	0.031	12.57	0.03

For each site, indicated are as follows: their geographical region, number of individuals per site, expected heterozygosity (H_e), expected unbiased heterozygosity ($H_{n.b}$), observed heterozygosity (H_o), estimate of Wright's (Weir and Cockerham 1984) fixation index (F_{is}),mean number of allele (N_{br}). Only sites with at least 10 individuals per apiary are considered

An mean null allele frequency

[a] Deviations from HWE

profile observed was the African A1 haplotype (99.4 %), which exhibited three lengths of fragments (~47, 108, and 483 bp) (Franck et al. 2001). Another PCR-RFLP profile referred to as
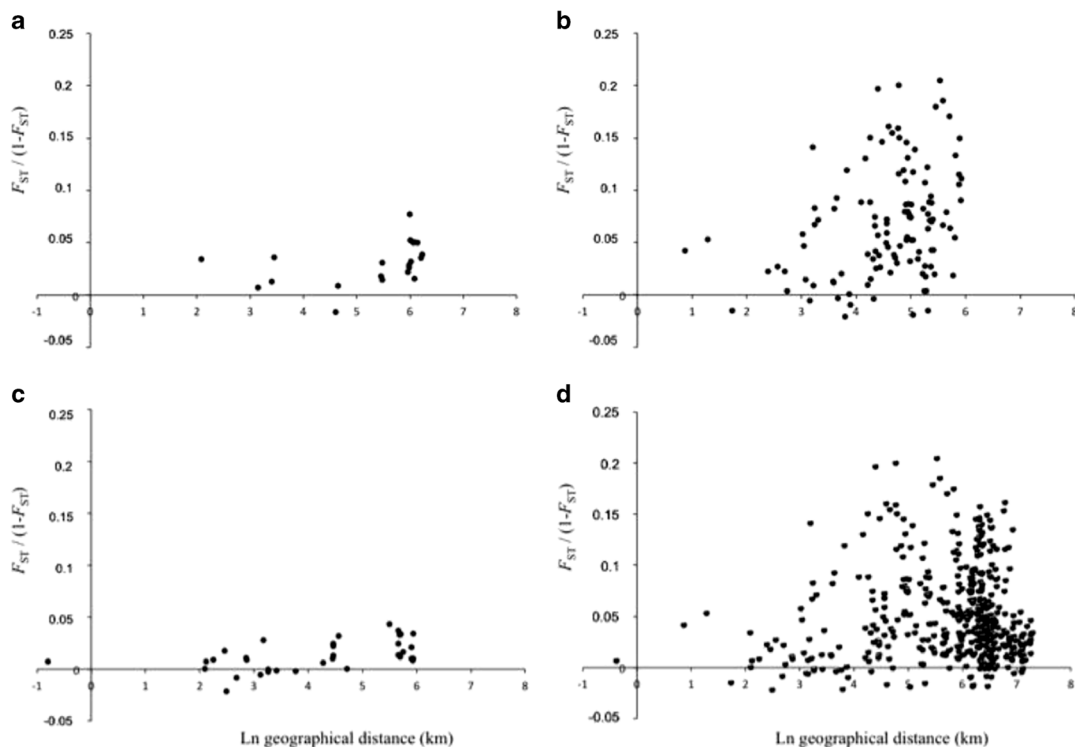
Figure 2. Relationship between logarithm of geographical distance and nuclear genetic differentiation as estimated as $F_{ST}/(1-F_{ST})$, between **a** sites from the northern region only ($n_{sites}=7$, $P=0.014$), **b** the southern region only ($n_{sites}=9$, $P=0.0027$), **c** sites from Hauts Plateaux only ($n_{sites}=16$, 120 combinations, Mantel test $P=0.014$), and **d** sites from all regions sampled in Madagascar ($n_{sites}=32$, 496 combinations, Mantel test $P=0.84$).

the A4 haplotype (~47, 108, 192, and 483 bp) was found in three individuals in our sample, two from S66 and one from S23. Another notable profile was found in two individuals from S22 and S29 (~47, 108, 150, 350 bp). The total sizes of each haplotype ranged from 638 bp for A1, 830 bp for A4, and 655 bp for the new haplotype. Within the South African samples, two different PCR-RFLP profiles were detected: A1 haplotype ($n=1$) and A4 haplotype ($n=21$).

A total of 173 samples collected in 49 sites were sequenced. A total of 18 different sequences were found with variable sequence sizes belonging to the A lineage (SD Table V). The 16 sequences obtained for the A1 restriction profile have never been reported and were named A1_Mad1 to A1_Mad16 (accession numbers KF976992 to KF977009). These 16 sequences were characterized by one unit P_0 and one unit Q. The most frequent sequence, A1_Mad3, was

present in all sites ($n=121$, 70 %). The sequences of the two individuals exhibiting the new PCR-RFLP haplotype (in S29, S22) cluster within the *A. m. unicolor* group (it was subsequently named A1_Mad13) and we propose to classify it as a subtype of A1. The two other sequences (A4_Mad1 and A4_Mad2) were genetically close to the A4 haplotypes of *A. m. scutellata* (accession number FJ 477987 (Franck et al. 2001), similarity=98 %) and found in two different regions of the West coast of Madagascar (R4 and R16) (SD Table V, SD Fig. 2). The A4 haplotypes were characterized by one unit P_0 and two units Q sequences.

4. DISCUSSION

Previous PCR-RFLP analyses performed on 48 individuals from Madagascar by several authors (Garnery et al. 1992; Franck et al. 2001) detected
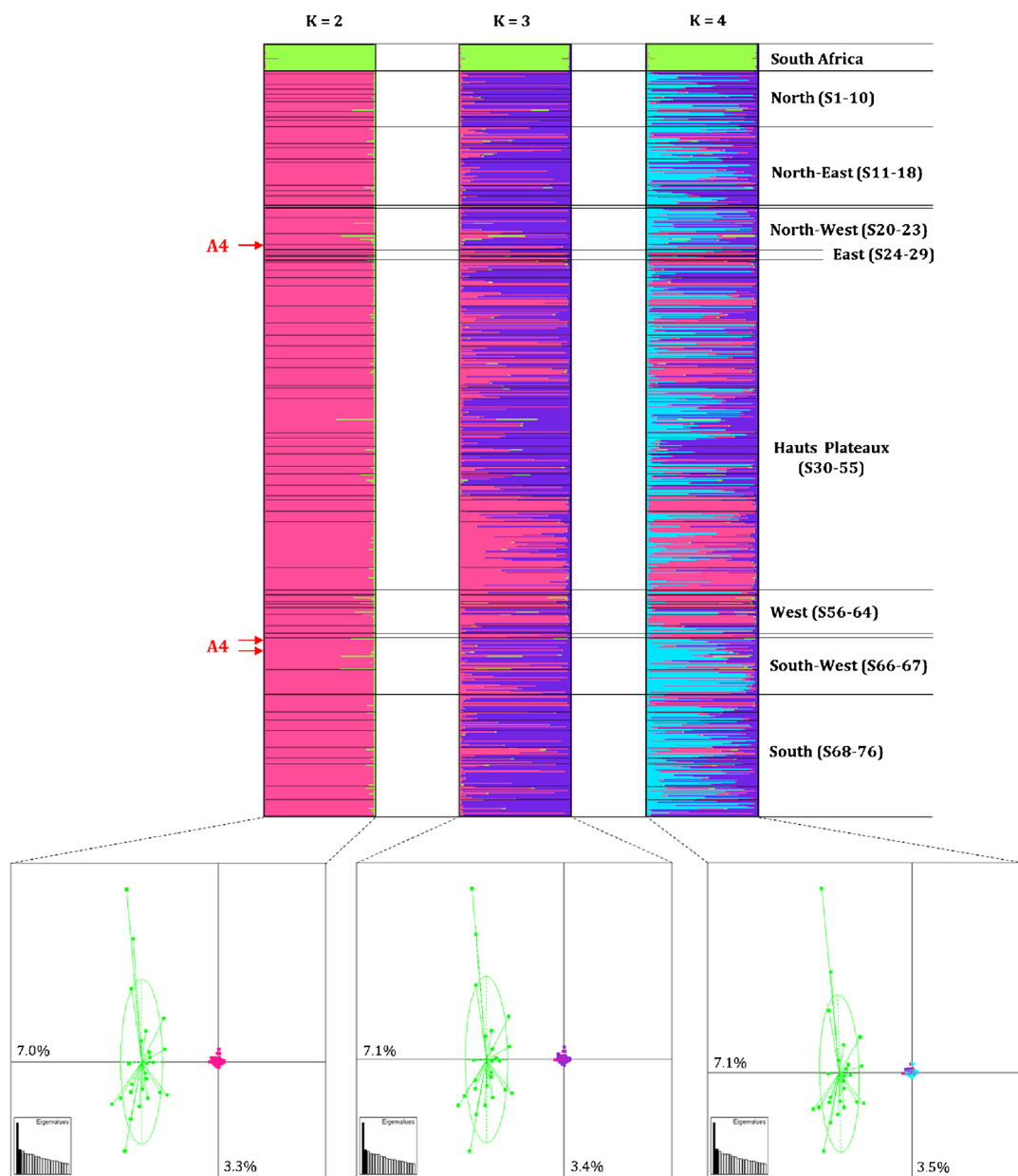
Figure 3. Population structure and Euclidean distances among genetic clusters based on 17 microsatellites loci. *Top* : Structure bar plots (*K* =2, 3, and 4) with 22 reference samples from South Africa and 710 individuals of Madagascar organized by sampling sites (S1–76). *Each horizontal bar* represents one individual, and sites are delimited by *black lines* . The *height of each bar* represents the probability of assignment to a genetic cluster (*one color*). *Bottom* : PCAs among individual genotypes assigned to the different clusters (*K* =2, 3, and 4). Inertia percentage of each axis is indicated (using 232 variables).

a single restriction profile (A1). The larger sampling scale of this study in Madagascar detected a new A1 restriction profile and the occurrence of an A4 restriction profile. The sequencing approach used in our study revealed mtDNA variability with 16 new sequences. Haplotype
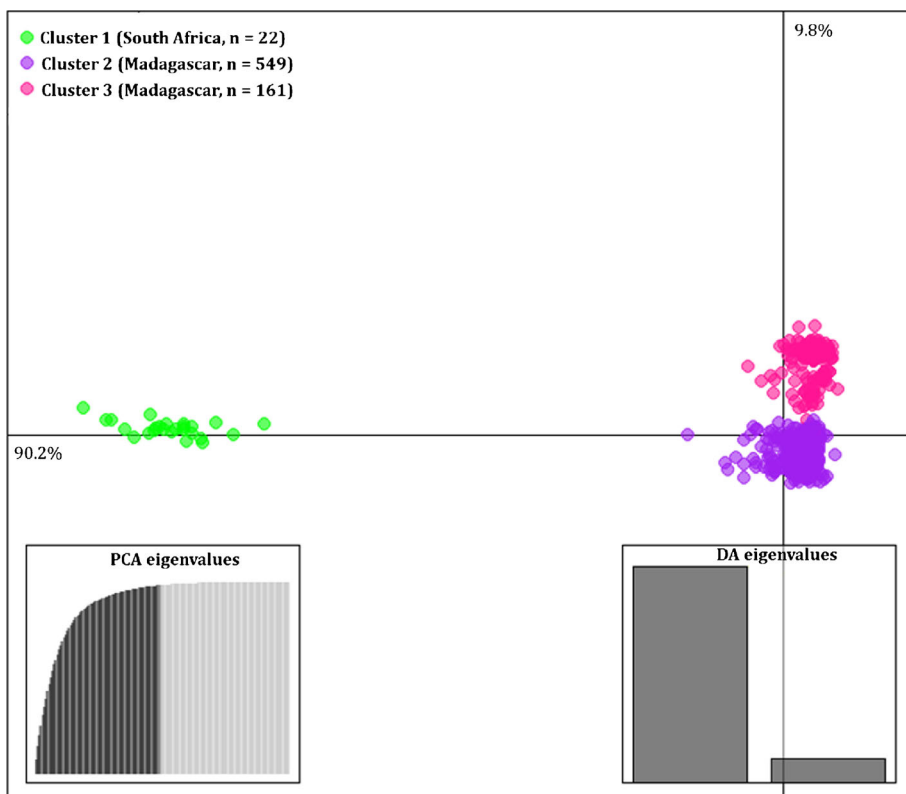
Figure 4. DAPC based on individual genotypes of Madagascar (n =710) and reference population of South Africa (n =22). *Dots of different colors* indicate honeybee samples from different genetic clusters (K =3). Inertia percentage of each axis, PCs eigenvalues, and discriminant factors retained are indicated.

frequencies and network analyses suggested that divergences are quite recent (SD Fig. 3), with all A1 sequences in Madagascar diverging from the predominant one through one single mutation (except for A1Mad_12). The A1 haplotype is widespread. It was found in this study in South Africa, and it has also been reported in Morocco (De la Rua et al. 2006), Algeria (Chahbar et al. 2013), Sudan (El-Niweiri and Moritz 2008), and in the Middle East (Alburaki et al. 2011). Three samples from A4 were found in the Western region of Madagascar. Two of these samples show high genetic diversity compared to A4 published sequences. A4 haplotypes might probably result from ancestral introductions to the island together with ancestral A1, as both haplotypes (belonging to A$_\mathrm{I}$ sublineage) are commonly distributed within the different African subspecies populations (Franck et al. 2001).

The absence of haplotypes belonging to other lineages in our study implies that introductions of foreign queens are rare. This can be explained by the fact that other subspecies i) if imported/ introduced were too few to be seen in our sampling, ii) are not well adapted to this environment (climate and specific endemic vegetation), or iii) are not selected by traditional Malagasy beekeepers, *A. m. unicolor* being easy to handle compared to other much more aggressive subspecies like the Africanized honeybee (Ruttner 1988; Winston 1992).

Madagascar populations were highly different (F_ST=0.34) to the reference population (comprising A1 and A4 haplotypes). Furthermore, *A. m. unicolor* has a poor allelic diversity in terms of number of alleles for each population marker (N_br=2.47 to 3.88), in comparison to our reference population (N_br=12.57) or even with other studies performed on African honeybee populations with

allelic diversity ranging from 7.9 (*A. m. capensis*) to 9 (*Apis mellifera intermissa*) and even 11 (*A. m. scutellata*) (for 7 to 12 microsatellite loci, with most loci being the same in both studies) (Estoup et al. 1995).

The levels of heterozygosity observed in Malagasy populations were also much lower than the reference population used in this study and those reported from African populations. Across Madagascar, levels varied from 0.34 to 0.47 (H_e), while the reference population $H_e=0.84$, and the average H_e ranged between 0.78 and 0.90 (*A. m. intermissa* to *A. m. scutellata)* in African populations (Franck et al. 2001). In parallel, the lowest levels ranged from 0.26 to 0.66 in west Mediterranean subspecies (*Apis mellifera iberiensis* and *Apis mellifera siciliana*, respectively) (Garnery et al. 1998).

High levels of nuclear polymorphism in African populations have been explained by i) quaternary climate changes that could be responsible for honeybee subspecies diversification and expansion in Africa (Franck et al. 2001), ii) larger effective population size (Estoup et al. 1995), allowing more alleles to be maintained, and iii) the high migratory behavior of colonies which is typical for African honeybees south of the Sahara (Hepburn and Radloff 1998; Jaffe et al. 2009). Allelic richness within populations can also be increased by introgression of foreign genes into zones with other subspecies. Due to the lack of data on the biology of the Malagasy subspecies, it is difficult to compare effective population size of *A. m. unicolor* to other subspecies. However, its insular situation prevents frequent natural introductions and may in part explain the low nuclear polymorphism. Both the significant pairwise F_{ST} values observed between neighboring sites (i.e., S34–S35 only 10 km apart, SD Table III) and the significant isolation by distance patterns observed among sites 500 km apart (Figures 1 and 2) suggest that gene flow is restricted. The larger variance of F_{ST} at longer distances (>500 km, Figure 2d) indicates that at the island scale, the influence of genetic drift is strong relative to gene flow (Hutchison and Templeton 1999) and that problems of homoplasy could be more important (Jarne and Lagoda 1996). As observed in *A. m. capensis* from South Africa, *A. m. unicolor* populations may be less mobile than other African subspecies (Estoup et al. 1995)

because of the topography of the island (coastal vs. Hauts Plateaux areas) and the climatic variations between regions. Indeed this was underlined with the results of Structure indicating genetic subclustering of the observed populations into at least two major clusters. The observed genetic subclustering did not match the distribution of the two honeybee ecotypes described by Ruttner (1988). Furthermore, we found such an admixture of genetic clusters between populations from different regions and the region surrounding the capital (on the Hauts Plateaux) that those ecotypes might have been mixed in the recent past. Indeed, in Madagascar, most goods pass through the capital, central market, and free commercial exchanges, which facilitate honeybee movement. Transport routes around the island are limited but all of them lead to the capital.

Wild populations, uninfluenced by beekeeping, exist in many regions of Africa, and honeybees from natural habitats have been shown to have a higher genetic diversity than managed populations (Allsopp 2004), so more intensive studies of wild colonies in protected and wild zones of Madagascar would be interesting to confirm or not our findings on the genetic diversity of *A. m. unicolor*.

Several clues and hypotheses point out the fact that *A. m. unicolor* might be derived from a recent (in geological time) colonization event of this continental island: i) relatively low mitochondrial and nuclear genetic diversity were found on *A. m. unicolor* in Madagascar, compared to other subspecies of the A lineage (Estoup et al. 1995; Franck et al. 1998, 2001). ii) The hypothesis on molecular data showing A lineage split from other lineages 6 million years ago with *A. m. unicolor* divergence from other subspecies more recently (1 million years ago) (Han et al. 2012). iii) The prehistoric breakup of the supercontinent Gondwana which separated Madagascar from mainland Africa is dated much earlier (around 135 million years ago; Rabinowitz et al. 1983) than the first honeybee species.

Nevertheless, its morphological (two ecotypes) and behavioral differences (one of the most gentle honeybees in the world (Ruttner 1988)) from other African honeybees suggest that, such as the flora of the island, *A. m. unicolor* seems to have evolved in relative isolation. Furthermore, the low

genetic diversity observed, if confirmed in wild and conserved areas, might also be the result of over 1400 years of high deforestation rates and habitat fragmentation on the island (Campbell 1993; Gade 1996) which has been increasing over the last 50 years (Harper et al. 2007).

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Diversité génétique de l'abeille endémique de Madagascar: *Apis mellifera unicolor* **(Hymenoptera: Apidae)**

Microsatellite / lignée africaine / ADNmt / aire de répartition / haplotype

Genetische Diversität der endemischen Honigbiene von Madagaskar, *Apis mellifera unicolor* **(Hymenoptera: Apidae)**

Apis mellifera unicolor **/ Mikrosatelliten / Afrikanische Lineage / genetische Diversität / mtDNA**

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