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# Temporal changes in mitochondrial diversity highlights contrasting population events in Macaronesian honey bees

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**Abstract** – Temporal studies of the genetic diversity are important as they enable detection of genetic changes that can constitute threats to the honey bee populations. In this study, we analyzed the present mitochondrial diversity in honey bee populations inhabiting the Macaronesian archipelagos (Azores, Madeira and Canary), and compared with previous data over a 10-year period. Present populations showed mainly haplotypes characteristic of the evolutionary African sub-lineage ( $A_{III}$ ), but foreign mitochondrial haplotypes (M7, C1 and C2) were also observed suggesting honey bee queens introductions with different origins. Differential patterns of change in mitochondrial diversity and introgression were detected: whereas a low decrease was detected on Madeira and São Miguel, major changes were observed on El Hierro, La Palma and La Gomera. Despite loss of  $A_{III}$  haplotypes relative to previous data, extant frequency and distribution of the African sub-lineage in the Macaronesian archipelagos appears sufficiently large to propose potential conservation policies for protecting local ecotypes.

**Macaronesian honey bees / mtDNA / genetic diversity / subspecies introduction / temporal variation**

## 1. INTRODUCTION

Pollination is an important ecosystem service for many wild plants and commercial crops provided mainly by bees (Klein et al. 2007). The recent evidence of bees decline is of an overall great concern (Murray et al. 2009). This decline is mainly anthropogenically driven by factors as fragmentation and degradation of habitats, pesticides and introduction of invasive species and diseases, exploitation and climate change (Brown and Paxton 2009). In relation to the western honey bee (*Apis mellifera*), human

activity, especially in modern apiculture, has a considerable impact on its natural distribution which has led to the spread, fragmentation and replacement of several subspecies (Jensen et al. 2005; Soland-Reckeweg et al. 2009). In such situation, efforts have to be made to preserve local ecotypes and wild honey bees as they are important reservoirs of local adaptations that could determine the survival of honey bees in the future (Jaffé et al. 2009).

Islands offer an interesting scenario to study the consequences of anthropogenic influences on honey bee populations and to implement management plans for conservation. Archipelagos located in the northeast Atlantic Ocean, next to Europe and North Africa (Azores, Madeira, Savage, Canary and Cape Verde), collectively known as the

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Macaronesia, are such a case study. As a result of isolation and adaptation to the particular environmental island conditions, many organisms, including honey bees, have evolved giving rise to locally adapted populations (Gillespie and Roderick 2002). In fact, genetic analysis of honey bee populations from the Canary (De la Rúa et al. 1998, 2001, 2002), Azores and Madeira (De la Rúa et al. 2006) archipelagos revealed that they are, to some extent, differentiated from European continental populations. These Macaronesian populations are included in a subset of the African evolutionary lineage of subspecies with Atlantic distribution (known as sub-lineage A<sub>III</sub>). They are characterized by mitochondrial haplotypes rarely found in populations from eastern Iberia and Africa (Franck et al. 2001; Miguel et al. 2007; De la Rúa et al. 2007; Cánovas et al. 2008) and more common in the Atlantic side of the Iberian Peninsula (Pinto et al. 2012).

Genetic structure of Macaronesian honey bee populations has been modified due mainly to the introduction of other subspecies which has led to introgression (defined as “introduction of genetic material from another subspecies into a population” Frankham et al. 2002) with an impact on the regional genetic composition (De la Rúa et al. 2001, 2006; Muñoz et al. 2012). Eastern European honey bees (belonging to the evolutionary lineage C) are such introduced subspecies through queen trade, more specifically *Apis mellifera ligustica* and *Apis mellifera carnica* from the Italian and Balkan peninsulas, respectively (vanEngelsdorp and Meixner 2010). These subspecies are imported globally by beekeepers (De la Rúa et al. 2009), jeopardizing native honey bee subspecies, populations or ecotypes (Moritz et al. 2005). Genetic introgression due to queens introduction has also been noted in other honey bee populations with African origin from Mediterranean islands as the Balearic Islands (De la Rúa et al. 2003) and Sicily (Dall’Olio et al. 2008). Introduction of other subspecies has been detected through the analysis of the mitochondrial DNA that allows to infer the maternal origin of the honey bee colonies (Bouga et al.

2011). This molecule is specially suitable to detect introduction events as it shows sequence variation specific to the different *A. mellifera* evolutionary lineages and subspecies (Garnery et al. 1998).

The honey bees of the Macaronesian islands deserve conservation interest, especially on the Canary Islands where a program to select and preserve the local black honey bee was initiated on La Palma in 2001. The regional laws included the prohibition of the introduction of other honey bee subspecies (B.O.C. 49 of 20/4/2001) and the establishment of a natural mating area for breeding local black honey bee queens. This activity has had an impact on the mitochondrial diversity of the colonies located in the mating area, as indicated by a significant increase of queens with haplotypes characteristic of the Atlantic African A<sub>III</sub> sub-lineage (Muñoz and De la Rúa 2012).

The scope of this study is to examine the possible changes in maternal variation occurring over the past decade among Macaronesian honey bees. Specifically, we want to determine: (1) whether the genetic variation has changed, (2) whether there have been changes in the queens’ introduction pattern, and (3) whether there is a temporal variation in the genetic structure influenced by beekeeping activities. Previously, temporal analyses have been reported in honey bee samples from one of the Canary Islands (La Palma, Muñoz and De la Rúa 2012) where a conservation program was established, and from Tenerife, a highly introgressed honey bee population (Muñoz et al. 2012). However, no long-term genetic study has so far been conducted at a large geographical scale in the Macaronesia.

## 2. MATERIAL AND METHODS

### 2.1. Sampling

The Canary archipelago is composed of seven main islands (La Palma, El Hierro, La Gomera, Tenerife, Gran Canaria, Lanzarote and Fuerteventura). The Azores archipelago includes nine main islands (Corvo, Flores, Faial, Pico, Graciosa, São Jorge, Terceira, São

Miguel and Santa Maria), while Madeira comprises two inhabited islands, Madeira and Porto Santo, three islets of only 15 km<sup>2</sup> known as the Desertas Islands and about ten offshore rocks. In this work, we have analyzed honey bee populations from the larger islands (São Miguel and Madeira) of the Portuguese archipelagos and from five of the seven islands of the Canary archipelago (Spain) where beekeeping is more intensively performed (Table I).

Worker honey bees from the inner frames of 443 colonies were sampled during 2006–2011. Different islands were sampled in different years but each island was sampled once (Table I). These honey bees were kept in absolute ethanol at -20 °C until laboratory processing. For temporal analysis, we included the results of previous studies (313 honey bee colonies, De la Rúa et al. 2001, 2006). A summary of these data has been included in Figure 1 and Table I.

## 2.2. Mitochondrial DNA analysis

The method to identify the maternal origin of the honey bee colonies and to include them in the different evolutionary lineages and sub-lineages is based on the variation of the mitochondrial intergenic region located between the tRNA<sup>leu</sup> and cox2 genes (Garnery et al. 1993). Two types of sequences are present in this region: P and Q. The sequence P has three forms: P (with 54 bp typical of subspecies from the Western Europe lineage as *A. m. mellifera* and northern *A. m. iberiensis* populations), P<sub>o</sub> (with 67 bp, typical of African subspecies and southern *A. m. iberiensis* populations), or P<sub>1</sub> (with 50 bp, characteristic of Atlantic honey bee populations). Up to four copies of the sequence Q can be found, except in the honey bees from the C lineage which intergenic region is composed by only one copy (reviewed by De la Rúa et al. 2009). The combined length and sequence variation (detected with a restriction fragment length polymorphism or RFLP approach) determine the haplotype of each worker directly inherited from the queen.

One worker per colony was used for mtDNA identification. DNA was extracted from a pair of legs using the Chelex<sup>®</sup> method (Walsh et al. 1991). The mitochondrial analysis was carried out following Garnery et al. (1993). The intergenic tRNA<sup>leu</sup>-cox2 region was PCR-amplified in a total volume of 25 µL using the primers E2 (5'-GGCAGAATAAGTGCATTG-3'), located at the 5' end of the gene tRNA<sup>leu</sup>, and H2 (5'-CAATATCATTGATGACC-3') located close

to the 5' end of the gene cox2. The annealing temperature was set at 48 °C following the conditions described by Garnery et al. (1993). The size of the PCR-amplified products was determined after the electrophoretic separation in a 1.5 % agarose gel. Twenty microliters of the amplified products were then digested with the *Dra*I enzyme at 37 °C overnight. Restricted fragments were visualized in a 4 % NuSieve<sup>®</sup> agarose gel stained with ethidium bromide and photographed under UV light. Amplicon of a novel restriction pattern was purified with isopropanol and 5 M ammonium acetate, and sequenced (Secugen S.L., Madrid, Spain) with both E2 and H2 primers. This sequence was manually checked for base calling and a multiple sequence alignment was performed with other sequences retrieved from Genbank using the MEGA program version 4 (Tamura et al. 2007).

## 2.3. Data analyses

Frequency of haplotypes, evolutionary sub-lineages and lineages were calculated and compared with data previously reported by De la Rúa et al. (2001, 2006). Mitochondrial introgression was calculated from the percentage of foreign haplotypes (M7, C1 and C2) present on each island. Fischer's exact test was used to test the significance of the association (contingence) between the haplotype frequency and temporal survey. Genetic diversity was calculated with GENALEX version 6.1 (Peakall and Smouse 2006). *F* statistics values and analysis of molecular variance (AMOVA) were performed to evaluate the present genetic differentiation and the temporal genetic variation using ARLEQUIN version 3.1 (Excoffier et al. 2005).

## 3. RESULTS

### 3.1. Extant mitochondrial DNA diversity and introgression in Macaronesian honey bee populations

The combination of length and *Dra*I restriction patterns yielded 18 different haplotypes in the 443 colonies assayed, mostly (15) of African ancestry (Table I). A novel African haplotype that belongs to the Atlantic sub-lineage (A<sub>III</sub>) has been described for the first time. Following the nomenclature established earlier (Garnery et al. 1998) and recently

**Table 1.** Haplotype frequencies, mitochondrial introgression (*I*) and genetic diversity in honey bee populations from Macaronesian islands of the previous (1998-2001) (data from De la Rúa et al., 2001, 2006) and present (2006-2011) surveys

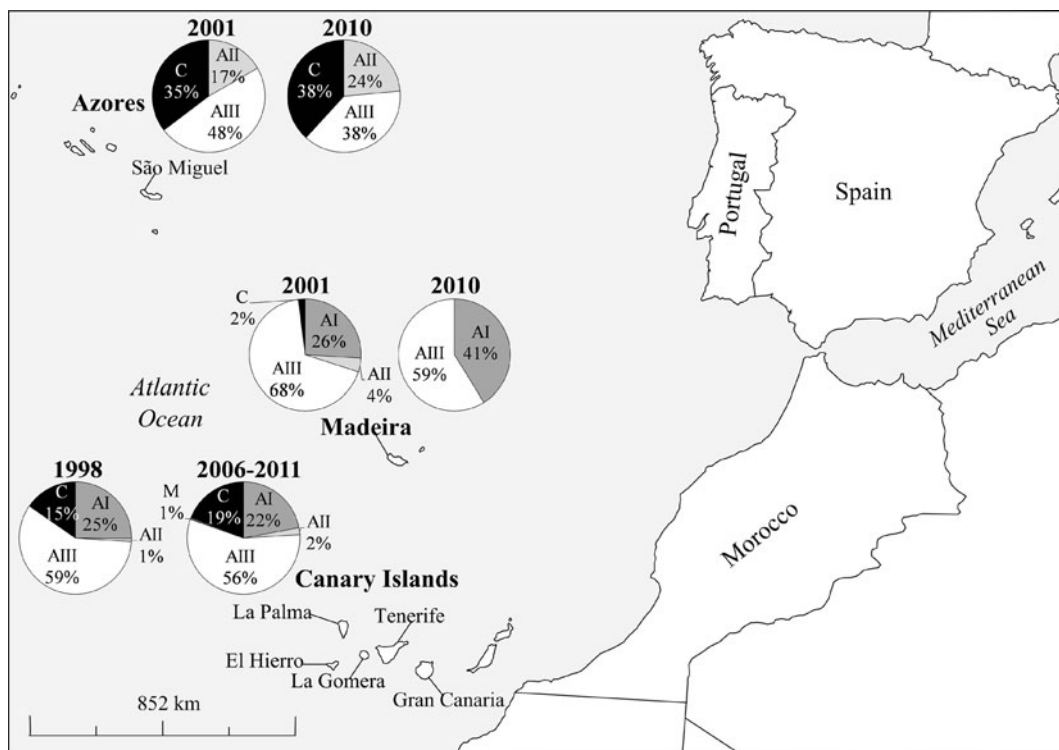
Survey	1998-2001							2006-2011									
	Island Year	SM 2001	MD 2001	HI 1998	LP 1998	GO 1998	TE 1998	GC 1998	Total	SM 2011	MD 2011	HI 2011	LP 2006	GO 2010	TE 2008	GC 2010	Total
n		48	50	17	41	57	76	24	313	34	51	47	119	49	53	90	443
A1		0.200	0.200		0.707	0.123	0.158	0.250	0.204	0.333			0.361	0.184	0.151	0.200	0.214
A2		0.040							0.006	0.039							0.004
A3																0.011	0.002
A4'			0.020						0.003	0.039					0.038		0.004
A8										0.029							0.007
A9		0.042						0.083	0.013	0.059						0.056	0.016
A10		0.125	0.040						0.026	0.147							0.011
A11			0.060					0.125	0.019	0.029	0.021	0.176	0.184	0.113	0.211	0.128	0.128
A14		0.417	0.020		0.024	0.175	0.118	0.042	0.134	0.294		0.101	0.061	0.038	0.311	0.124	0.124
A15			0.480	0.824	0.268	0.684	0.368	0.417	0.402		0.412	0.489	0.294	0.143	0.358	0.178	0.273
A16		0.063	0.100						0.026	0.020							0.002
A20			0.020						0.003								
A34										0.059							0.005
A40'										0.078							0.009
A42'										0.078							0.009
A47''														0.020			0.002
M7																0.022	0.004
C1		0.354	0.020	0.176		0.018	0.355	0.083	0.163	0.382	0.149						0.045
C2											0.340	0.067	0.408	0.302	0.011	0.138	0.138
<i>I</i>		0.354	0.020	0.176		0.018	0.355	0.083	0.163	0.382	0.489	0.067	0.408	0.302	0.033	0.187	0.187
<i>D</i>		0.694	0.726	0.309	0.438	0.494	0.708	0.764	0.591	0.759	0.718	0.636	0.743	0.757	0.756	0.792	0.737
<i>D</i> <sup>w</sup> M-C		0.551	0.714		0.438	0.476	0.592	0.723	0.499	0.729	0.718	0.083	0.709	0.764	0.676	0.778	0.637

n number of sampled colonies; SM São Miguel; MD Madeira; HI El Hierro; LP La Palma; GO La Gomera; TE Tenerife; GC Gran Canaria; *D* genetic diversity calculated from all detected haplotypes; *D*<sup>w</sup> M-C genetic diversity calculated without foreign haplotypes

reviewed for lineages M (Rortais et al. 2011) and A (Pinto et al. 2012), this new haplotype was named as A47'' (accession number JX898863). This haplotype was found in one colony from La Gomera (Canary Islands) and its sequence confirmed the presence of P<sub>1</sub> and four repeats of the Q element.

The distribution of haplotypes per island and lineages and sub-lineages per archipelago are given in Table 1 and Figure 1, respectively. Fifteen haplotypes belonged to the African lineage, mainly to the A<sub>III</sub> sub-lineage (haplotypes A11, A14, A15, A16, A34, A40', A42' and A47''). This sub-lineage corresponded to 38, 59 and 56 % of the sampled colonies from Azores, Madeira and Canary archipelagos, respectively (Figure 1). The most common A<sub>III</sub> haplotypes were A11, A14 and A15. Haplotype A11 was particularly frequent on Gran Canaria (0.211), haplotype A14 was present at high frequency on

São Miguel (0.294) and Gran Canaria (0.311), and haplotype A15 was the most frequent on Madeira (0.412), El Hierro (0.489) and Tenerife (0.358). Interestingly, haplotype A15 was common on every surveyed island but absent on São Miguel. The sub-lineage A<sub>I</sub> (haplotypes A1, A2, A3 and A4') was absent on São Miguel and El Hierro but present at considerable frequency on the other islands. Indeed, it was more frequently observed on Madeira (0.410) and La Palma than on the other islands, although A1 was amongst the most frequent haplotype ranging from 0.151 on Tenerife to 0.361 on La Palma (Table 1). Haplotypes of sub-lineage A<sub>II</sub> ancestry (A8, A9 and A10) were detected only on three islands; they exhibited a high frequency on São Miguel (0.240) and were rare on Tenerife (0.038) and Gran Canaria (0.056). Several private African haplotypes were detected at a low frequency: two on São Miguel (A10 and A34) and on the



**Figure 1.** Map of the study area showing the location of the sampled Macaronesian archipelagos and the distribution of the evolutionary lineages (M and C) and African sub-lineages (A<sub>I</sub>, A<sub>II</sub> and A<sub>III</sub>) of the previous (1998–2001) and present surveys (2006–2011).

Canaries (A3 on Gran Canaria and A47'' on La Gomera) and five on Madeira (A2, A4', A16, A40' and A42').

Of the three non-African haplotypes, one was of M lineage ancestry (M7) and two belonged to the C lineage (C1 and C2). Haplotype M7 has been reported in northern *A. m. iberiensis* and *A. m. mellifera* (Garnery et al. 1993, 1998; Miguel et al. 2007; Cánovas et al. 2008) but also in *A. m. ligustica* (Franck et al. 2000). In this study, M7 was exclusively found on Gran Canaria with a frequency of 0.022 (Table I). Haplotypes C1 and C2 are characteristic of the commonly worldwide imported subspecies *A. m. ligustica* and *A. m. carnica*, respectively (Franck et al. 2000). While haplotype C1 was found on both São Miguel (0.382) and El Hierro (0.149), C2 was detected only on the Canary Islands showing a high frequency on La Gomera (0.408), El Hierro (0.340) and Tenerife (0.302) (Table I). Therefore, mitochondrial introgression (*I*) has been detected in every honey bee population surveyed in the Macaronesia, except on Madeira. Introgression values ranged from 0.489 (El Hierro) to 0.033 (Gran Canaria, Table I).

Extant genetic diversity (*D*) (Table I) in the Macaronesian islands ranged from 0.792 (Gran Canaria) to 0.636 (El Hierro), when all the observed haplotypes were taken into account. When foreign haplotypes were removed from the calculations ( $D^w_{M-C}$ ), a decrease of genetic diversity

was observed on every island population except on La Gomera (from 0.757 to 0.764), being significantly reduced on El Hierro (from 0.636 to 0.083).

Pairwise  $F_{ST}$  values obtained for the current survey showed a highly significant differentiation in most population comparisons, except for Tenerife and La Gomera ( $F_{ST}=0.024$ ,  $P>0.05$ ; Table II). The greatest differentiation was observed between the population of São Miguel (Azores) and the remaining islands (0.136–0.263, Gran Canaria and El Hierro, respectively).  $F_{ST}$  values of Madeira with respect to the other islands, ranged from 0.037 (La Palma) to 0.263 (São Miguel). Lower  $F_{ST}$  values were observed among Canarian populations (0.024–0.205).

### 3.2. Temporal mtDNA variation in Macaronesian honey bee populations

Temporal mtDNA variation analyses were performed comparing the data from the two surveys (1998–2001 and 2006–2011) on each Macaronesian island as shown in Table III. Fischer's exact test yielded significant differences in the genetic diversity variation for every pairwise comparison between time periods. Overall, the temporal analysis of genetic diversity showed no differences for São Miguel ( $\Delta D=0.065$ ), a significant decrease for Madeira ( $\Delta D=-0.008$ ;  $P<0.05$ ) and a significant increase for every Canary island

**Table II.** Present genetic differentiation between honey bee populations from Macaronesian islands by pairwise  $F_{ST}$  values.

	São Miguel	Madeira	El Hierro	La Palma	La Gomera	Tenerife	Gran Canaria
São Miguel		0.26277	0.26320	0.22270	0.22381	0.23037	0.13577
Madeira	***		0.15228	0.03642	0.16229	0.08119	0.12064
El Hierro	***	***		0.16342	0.11544	0.03236	0.20473
La Palma	***	*	***		0.09187	0.05829	0.04791
La Gomera	***	***	***	***		0.02356	0.11462
Tenerife	***	***	*	***	ns		0.10653
Gran Canaria	***	***	***	***	***	***	

ns non-significant; \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$

**Table III.** Temporal variation of genetic diversity with all detected haplotypes ( $\Delta D$ ) and without foreign haplotypes ( $\Delta D^{w\ M-C}$ ), variation of introgression rate ( $\Delta I$ ) and  $F_{ST}$  values between previous and present surveys.

	$\Delta D$	$\Delta D^{w\ M-C}$	$\Delta I$	$F_{ST}$ (present–previous)
São Miguel	0.065 <sup>ns</sup>	0.178 <sup>ns</sup>	0.028	-0.007 <sup>ns</sup>
Madeira	-0.008*	0.004**	-0.020	0.013 <sup>ns</sup>
El Hierro	0.327**	0.083 <sup>ns</sup>	0.313	0.143**
La Palma	0.306***	0.271***	0.067	0.095***
La Gomera	0.262***	0.288***	0.390	0.284***
Tenerife	0.048***	0.084**	-0.053	0.129***
Gran Canaria	0.028***	0.055*	-0.050	0.063**

ns nonsignificant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

( $P < 0.01$ ; Table III). Specifically, a significant increase of genetic diversity was detected on El Hierro ( $\Delta D = 0.327$ ;  $P < 0.01$ ), La Palma ( $\Delta D = 0.306$ ;  $P < 0.001$ ) and La Gomera ( $\Delta D = 0.262$ ;  $P < 0.001$ ). When the comparisons were performed excluding colonies with foreign haplotypes (M7, C1 and C2), the major change in significance or magnitude of genetic diversity variation was found on El Hierro, which showed in this case non-significant differences ( $\Delta D^{w\ M-C} = 0.083$ ; Table III).

The temporal variation of the mitochondrial introgression ( $\Delta I$ ) showed a high increase on El Hierro ( $\Delta I = 0.313$ ) and La Gomera ( $\Delta I = 0.390$ ), whereas a low decrease was observed on Madeira ( $\Delta I = -0.020$ ), Tenerife ( $\Delta I = -0.053$ ) and Gran Canaria ( $\Delta I = -0.050$ ; Table III). Regarding temporal changes in population structure (Table III),  $F_{ST}$  estimates for populations of São Miguel and Madeira were negligible and non-significantly different from zero ( $F_{ST} = -0.007$  and  $0.013$ , respectively). In contrast,  $F_{ST}$  values showed significant temporal differentiation among the Canarian honey bee populations (Table III). The lowest  $F_{ST}$  value was observed in the population from Gran Canaria (0.063) and the highest on La Gomera (0.284; Table III).

To quantify the degree of temporal genetic divergence in the Macaronesian honey bee populations, an AMOVA was performed comparing

the two time periods (pooled across island populations). The results revealed that most of the variation was within islands (83.75 %) rather than among islands (15.25 %) with only a 1 % due to the temporal genetic variation between surveys (1998–2001 vs. 2006–2011). Overall, the  $F_{ST}$  value ( $F_{ST} = 0.162$ ) was significant ( $P < 0.001$ ), mainly due to the significant temporal structure observed on the Canary Islands populations ( $F_{ST} = 0.159$ ;  $P < 0.001$ ) rather than to the differentiation on São Miguel ( $F_{ST} = 0.024$ ;  $P = 0.200$ ) and Madeira ( $F_{ST} = -0.023$ ;  $P = 0.830$ ).

#### 4. DISCUSSION

The results of this work have been obtained with the *DraI* test of the mitochondrial intergenic region, which allows (1) depiction of the maternal origin of honey bee colonies, (2) spatial representation of haplotypes, which is important for establishing protected areas, (3) identification of honey bee colonies harbouring haplotypes that need proper management and (4) assessment of temporal changes in the haplotypic diversity (Bouga et al. 2011; Rortais et al. 2011). In this sense, our study demonstrates the utility of the mitochondrial DNA for detecting changes on recent timescales of the level of subspecies introduction and subsequent changes in the population structure for the Macaronesian honey bees. We have provided strong support for the



view that most honey bee colonies from the Macaronesian islands belong to the African Atlantic sub-lineage A<sub>III</sub> (38, 59 and 56 % in Azores, Madeira and Canary archipelagos, respectively). We found a novel haplotype (A47'') belonging to the A<sub>III</sub> sub-lineage, which increases the genetic diversity within this sub-lineage. The number of haplotypes reported for sub-lineage A<sub>III</sub> has been recently increased 188 % after the study of honey bee colonies from the Atlantic side of the Iberian Peninsula (Pinto et al. 2012). Therefore this sub-lineage comprehends the highest number of haplotypes (24) within the African evolutionary lineage.

Preserving the genetic diversity is crucial for maintaining the overall genetic health of the honey bee populations (Delaney et al. 2009). Therefore, long-term studies are important for detecting changes that can constitute threats to this biological resource. In this study, we analyzed the genetic variation of honey bee populations inhabiting the main islands of the three Macaronesian archipelagos (Azores, Madeira and Canary) over a 10-year period. We could detect different patterns of change in mitochondrial genetic diversity: whereas a low decrease was detected on Madeira, major changes were observed on El Hierro, La Palma and La Gomera, mainly due to the introduction of honey bee queens of European origin. Interestingly, this increase was non-significant on El Hierro when the colonies with these foreign haplotypes were discarded from the analysis. This result indicates that introduction of foreign honey bee queens may modify the genetic diversity of local honey bee populations.

On other islands such as Tenerife and Gran Canaria the observed increase of the genetic diversity, although significant, was smaller, and on São Miguel, this increment was non-significant. Despite the fact that the introduction of honey bee queens from different origins may increase the maternal genetic diversity, it should be also inferred from the results that native honey bees (i.e. those belonging to the sub-lineage A<sub>III</sub>) are not seriously displaced may be due to the large adaptive capacity of the honey bee and its uncontrolled reproductive system

(Moritz et al. 2005). Although losses of 3 % (São Miguel and Canary Islands) and 9 % (Madeira) of A<sub>III</sub> haplotypes have been observed, four A<sub>III</sub> haplotypes have been detected in the present survey: A34, A40, A42' and A47''. The haplotype A42' is frequent in continental Portugal (Pinto et al. 2012), so colonies with this haplotype may have been imported from mainland Portuguese beekeepers.

We have also found evidence of mtDNA introgression as foreign haplotypes belonging to European M and C lineages were detected, specially at high frequency on El Hierro and La Gomera. Previous studies detected haplotypes typical of European honey bee subspecies as *A. m. ligustica* (belonging to C lineage) in the Macaronesian islands (De la Rúa et al. 2001, 2006). In the present study, we observed again high frequencies of C haplotypes (C1 from Italian and C2 from carniolan honey bees) and a low frequency of one M haplotype (M7) typical of the Western European honey bee populations as northern *A. m. iberiensis* and *A. m. mellifera* but also present in *A. m. ligustica* (Franck et al. 2000). These data suggest that there have been changes in the origin of imported queens on the Canary Islands but not on São Miguel. The absence of C1 haplotypes on the Canaries in 2006–2011 (except on El Hierro where both C1 and C2 haplotypes were observed in the new sampling) imply that importation of *A. m. ligustica* did not generate long-term maintained hybridization with consequences of the gene pool of local honey bee populations, at least at the mitochondrial level, because in few years it is not detected, maybe reflecting adaptation problems.

A low mitochondrial variation was observed on São Miguel and Madeira suggesting that the beekeepers on these islands have not changed the level of purchase honey bee queens with European origin: while this practice is widely performed on São Miguel, it is practically inexistent on Madeira. In the Canarian honey bee populations, we detected differential introgression levels which can be also explained by beekeeping activity. While in the islands with intense beekeeping

(Tenerife and Gran Canaria) there was a decrease of mitochondrial introgression ( $-5\%$ ), in the islands of El Hierro and La Gomera, where beekeeping is practiced mostly by amateurs, there was an increase of introgression in the last 10 years (31.3 and 39.0 %, respectively). Beekeepers on these islands have suffered colony losses due to a drought period, fire and other threats, so they are introducing colonies from different origins (mainly from Eastern Europe but probably also from America) to recover the colonies density. This practice is probably giving rise to introgressive hybridization with consequences not only in the gene pool of local honey bee populations, but in the behaviour as hybrid colonies behave more aggressively.

Present population structure revealed by pairwise significant  $F_{ST}$  values among islands, suggests spatial differentiation in the Macaronesian honey bee populations. This demographic independence allows different conservation strategies, since colonies settled on each island may be considered as one population, except for Tenerife and La Gomera, possibly due to their geographical proximity and to a strong collaborative history among beekeepers of both islands. The most differentiated population is that of São Miguel, probably due to the presence of colonies with foreign origin and to the demonstrated close connection to mainland Portugal (De la Rúa et al. 2006). As stated in that previous work, Madeira and the Canary Islands showed a closer connection due to the presence in both archipelagos of a particular mtDNA haplotype (A15) in high frequency that suggests a similar ancient colonization event with a moderate degree of local differentiation in each archipelago. The population structure has changed differently between the two analyzed periods for the Macaronesian honey bee populations. The non-significant  $F_{ST}$  values between surveys indicate temporal stability of mitochondrial genetic structure of honey bee populations from São Miguel and Madeira. Assuming that in the short-term, human-assisted processes involving selection and gene flow are the most influent drivers of

change in the genetic composition; these results suggest that beekeeping practices have been maintained in the last decade in both Portuguese islands. The high proportion of C lineage colonies in the population of São Miguel has been maintained by colony reproduction and/or by continued queen importations. The disappearance of C lineage on Madeira suggests that queen importation is not regularly practiced by beekeepers or that either genetic drift or selection may have extirpated these foreign haplotypes from the population. The Canary Islands have the highest beekeeping activity in the Macaronesian region and conservation policies on the black Canarian honey bee have been implemented since 2001 (B.O.C. 49 of 20/4/2001). The temporal patterns of maternal introgression varied dramatically among Canarian islands. While a slight temporal variation in the introgression levels was observed on La Palma (increase), Tenerife and Gran Canaria (decrease), on El Hierro and La Gomera the frequency of foreign haplotypes increased considerably. It is possible that the undergoing conservation program on La Palma and the greater awareness of Tenerife and Gran Canaria beekeepers for preserving native honey bee populations are refraining importation of foreign queens (Muñoz and De la Rúa 2012; Muñoz et al. 2012).

In summary, analysis of temporal samples spanning the last decade proved informative for detecting and confirming spatio-temporal patterns of honey bee populations of the Macaronesia region. Despite strong anthropogenic influences, the high frequency of Atlantic (A<sub>III</sub>) haplotypes is still evident in the Macaronesian honey bee populations. However, our results also suggest an important mitochondrial introgression from European honey bee subspecies. This disturbance can be minimized by strengthening conservation policies oriented for protecting local ecotypes. This study is a contribution to the preservation of genetic diversity within the native Macaronesian honey bees as it provides baseline data for designing conservation strategies.

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**Des modifications temporelles de la diversité de l'ADN mitochondrial soulignent des événements de population contrastés chez les abeilles de Macaronésie**

**Abeille / Macaronésie / ADNm / diversité génétique / introduction de sous-espèce / variation temporelle**

**Zeitliche Änderungen in der mitochondrialen DNA der Honigbienen Macaronesiens zeigen unterschiedliche Populationsereignisse an**

**Honigbienen Macaronesiens / mtDNA / genetische Diversität / Einfuhr von Unterarten / zeitliche Änderung**

## REFERENCES

- Bouga, M., Alaux, C., Bienkowska, M., Büchler, R., Carreck, N.L., et al. (2011) A review of methods for discrimination of honey bee populations as applied to European beekeeping. *J. Apic. Res.* **50**, 51–84
- Brown, M.J.F., Paxton, R.J. (2009) The conservation of bees: a global perspective. *Apidologie*. **40**, 410–416
- Cánovas, F., De la Rúa, P., Serrano, J., Galián, J. (2008) Geographical patterns of mitochondrial DNA variation in *Apis mellifera iberiensis* (Hymenoptera: Apidae). *J. Zool. Syst. Evol. Res.* **46**, 24–30
- Dall'Olio, R., Muñoz, I., De la Rúa, P., Lodesani, M. (2008) Estimating introgression in *Apis mellifera sicula* populations: are the conservation islands really effective? In Proceeding of the Third European Conference of Apidologie, Belfast UK, 8–11 September 2008. **3**, 23–24.
- De la Rúa, P., Serrano, J., Galián, J. (1998) Mitochondrial DNA variability in the Canary Island honey bees (*Apis mellifera* L.). *Mol. Ecol.* **7**, 1543–1548
- De la Rúa, P., Galián, J., Serrano, J., Moritz, R.F.A. (2001) Genetic structure and distinctness of *Apis mellifera* L. populations from the Canary Islands. *Mol. Ecol.* **10**, 1733–1742
- De la Rúa, P., Galián, J., Serrano, J. (2002) Biodiversity of *Apis mellifera* populations from Tenerife (Canary Islands) and hybridisation with East European races. *Biodivers. Conserv.* **11**, 59–67
- De la Rúa, P., Galián, J., Serrano, J., Moritz, R.F.A. (2003) Genetic structure of Balearic honey bee populations based on microsatellite polymorphism. *Genet. Sel. Evol.* **35**, 339–350
- De la Rúa, P., Galián, J., Pedersen, B.V., Serrano, J. (2006) Molecular characterization and population structure of *Apis mellifera* from Madeira and the Azores. *Apidologie* **37**, 699–708
- 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 honeybees. *Apidologie*. **40**, 263–284
- Delaney, D.A., Meixner, M.D., Schiff, N.M., Sheppard, W.S. (2009) Genetic characterization of commercial honey bee (Hymenoptera: Apidae) populations in the United States by using mitochondrial and microsatellite markers. *Ann. Entomol. Soc. Am.* **102**, 666–673
- Excoffier, L., Laval, G., Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinformatic Online* **1**, 47–50
- Franck, P., Garnery, L., Celebrano, G., Solignac, M., Cornuet, J.M. (2000) Hybrid origins of honey bees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). *Mol. Ecol.* **9**, 907–921
- Franck, P., Garnery, L., Loiseau, A., Oldroyd, B.P., Hepburn, H.R., Solignac, M., Cornuet, J.M. (2001) Genetic diversity of the honey bee in Africa: microsatellite and mitochondrial data. *Heredity* **86**, 420–430
- Frankham, R., Ballou, J.D., Briscoe, D.A. (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge
- Garnery, L., Solignac, M., Celebrano, G., Cornuet, J.M. (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., Franck, P., Baudry, E., Vautrin, D., Cornuet, J.M., Solignac, M. (1998) Genetic diversity of the

- west European honey bee (*Apis mellifera mellifera* and *A. m. iberica*). II. Mitochondrial DNA. *Genet. Sel. Evol.* **30**, 31–47
- Gillespie, R.G., Roderick, G.K. (2002) Arthropods on islands: colonization, speciation, and conservation. *Annu. Rev. Entomol.* **47**, 595–632
- Jaffé, R., Dietemann, V., Allsopp, M.H., Costa, C., Crewe, R.M., Dall’Olio, R., De La Rúa, P., El-Niweiri, M.A.A., Fries, I., Kezic, N., Meusel, M.S., Paxton, R.J., Shaibi, T., Stolle, E., Moritz, R.F.A. (2009) Estimating the density of honey bee colonies across their natural range to fill the gap in pollinator decline censuses. *Conserv. Biol.* **24**, 583–593
- Jensen, A.B., Palmer, K.A., Boomsma, J.J., Pedersen, B.V. (2005) Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honey bee, *Apis mellifera mellifera*, in northwest Europe. *Mol. Ecol.* **14**, 93–106
- Klein, A.M., Vaissière, B., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremer, C., Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *P. Roy. Soc. Lond. B Biol.* **274**, 303–313
- Miguel, I., Iriondo, M., Garnery, L., Sheppard, W.S., Estonba, A. (2007) Gene flow within the M evolutionary lineage of *Apis mellifera*: role of the Pyrenees, isolation by distance and post-glacial recolonization routes in the Western Europe. *Apidologie* **38**, 141–155
- Moritz, R.F.A., Härtel, S., Neumann, P. (2005) Global invasions of the western honey bee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience* **12**, 289–301
- Muñoz, I., De la Rúa, P. (2012) Temporal analysis of the genetic diversity in a honey bee mating area of an island population (La Palma, Canary Islands, Spain). *J. Apic. Sci.* **55**, 141–148
- Muñoz, I., Madrid-Jiménez, M.J., De la Rúa, P. (2012) Temporal genetic analysis of an introgressed island honey bee population (Tenerife, Canary Islands, Spain). *J. Apic. Res.* **51**, 144–146
- Murray, T.E., Kuhlmann, M., Potts, S.G. (2009) Conservation ecology of bees: populations, species and communities. *Apidologie* **40**, 211–236
- Peakall, R., Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295
- Pinto, M.A., Muñoz, I., Chávez-Galarza, J., De la Rúa, P. (2012) The Atlantic side of the Iberian Peninsula: a hot-spot of novel African honey bee maternal diversity. *Apidologie*. doi:10.1007/s13592-012-0141-1
- Rortais, A., Arnold, G., Alburaki, M., Legout, H., Garnery, L. (2011) Review of the Dral COI-COII test for the conservation of the black honeybee (*Apis mellifera mellifera*). *Conserv. Genet. Res.* **3**, 383–391
- Soland-Reckeweg, G., Heckel, G., Neumann, P., Fluri, P., Excoffier, L. (2009) Gene flow in admixed populations and implications for the conservation of the western honey bee, *Apis mellifera*. *J. Insect Conserv.* **13**, 317–328
- Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596–1599
- vanEngelsdorp, D., Meixner, M.D. (2010) A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J. Invertebr. Pathol.* **103**, 80–95
- Walsh, P.S., Metzger, D.A., Higuchi, R. (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506–513