

Stock composition of northern neotropical honey bees: mitotype and morphotype diversity in Mexico (Hymenoptera: Apidae)

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Abstract – The stock of honey bees in the northern neotropics is likely a composite of European and African lineages, but the genetic makeup of most populations in this region has remained unstudied. We analyzed the genetic composition of honey bees across temperate and tropical regions of Mexico using mitochondrial and morphometric analyses. The results showed that honey bees from Mexico are descendent almost in similar proportion from matrilineal African and European origins. However, morphometrics indicate that most colonies are the result of extensive introgressive hybridization with Africanized bees. While large-scale displacement of European honey bees seems to have occurred in the tropical regions, higher frequencies of colonies with a mixed range of African-European markers were identified in the temperate areas. Our results suggest that the outcome of the hybridization between Africanized and European honey bees in Mexico has been significantly associated with climate.

Apis mellifera / Africanization / introgressive hybridization / mtDNA / morphometrics

1. INTRODUCTION

In the northern neotropics, Mexico has some of the highest concentrations of managed *Apis mellifera* colonies worldwide (Rinderer et al. 1991; Quezada-Euán 2007). With an estimated number of 1.5 million managed colonies, beekeeping is widely practiced across the country making it one of the top five honey producers in the world (Winston 1979; Gu et al. 2002; Quezada-Euán et al. 2008). Since the sixteenth century, several introductions of honey bees to Mexico have occurred, and three main evolutionary branches have

been reported to date: the M west-European lineage, the C east-European lineage, and the A African lineage (Labougle-Rentería and Zozaya-Rubio 1986; Clarke et al. 2001). However, by the mid 1980s, most commercial beekeeping in Mexico had been based on descendants of the east-European lineage, mainly *Apis mellifera ligustica* and *Apis mellifera carnica* (Labougle-Rentería and Zozaya-Rubio 1986; Labougle 1991). In 1985, swarms of Africanized honey bees (descendants of *Apis mellifera scutellata*) entered the country near the border with Guatemala (Fierro et al. 1987). In their northern migratory expansion, Africanized honey bees established feral populations and interbred with resident European stocks (Quezada-Euán and Hinsull 1995; Clarke et al. 2001). The expansion of the Africanized population northwards seems to have stabilized (Mortensen and Ellis 2015). After almost 30 years of interaction, the

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present populations of honey bees in the northern neotropics are a composite of European- and African-derived genes (Quezada-Euán 2000; Clarke et al. 2002; Pinto et al. 2004; Kraus et al. 2007; Zamora et al. 2008). Nevertheless, apart from a few populations where the introgression of African genes has been investigated in detail, most honey bee populations in North America have remained unstudied. This type of study is important because the large European-derived resident population of honey bees in Mexico could numerically favor a symmetric introgression between European- and African-derived subspecies compared to other areas of the neotropics (Del Lama et al. 1990; Sheppard et al. 1991).

Climate is probably the main abiotic factor determining the distribution of Africanized bees in the Americas (Sheppard et al. 1991; Hall and McMichael 1992; Quezada-Euán et al. 2003). Mexico is a climate-diverse country, and accordingly, there are five recognized beekeeping regions, namely the following: North, Central highlands, Pacific coast, Gulf coast, and the Yucatan Peninsula (Labougle 1991). The North and Central highlands encompass the temperate areas of Mexico with the highest altitudes (between 1500 and 2800 m), while the other three regions include the tropical areas (Labougle-Rentería and Zozaya-Rubio 1986). It is important to note that colonies in apiaries in most of Mexico are subject to little management, especially queen replacement. Additionally, queen honey bee producers can only supply ca. 5 % of the potential demand (Guzmán-Novoa and Page 1994; Guzmán-Novoa 2004); thus, the vast majority of beekeepers continue replacing queens from their own stock (Winston 1979). In addition, the density of managed European colonies varies across the country with the tropical areas having 12–25 colonies per km² and the temperate zones 5–10 per km² (Quezada-Euán et al. 2008). Given the natural adaptation of European stocks to temperate areas and African to tropical ones (Winston 1987) and assuming similar densities of managed colonies across Mexico, selection should favor a higher introgression of African-derived genes in the tropical regions compared to the temperate Northern and Central areas.

To determine the maternal origin of honey bees, a diagnostic mtDNA marker exists that can

distinguish the different mitotypes related to four recognized lineages of *A. mellifera*, namely the following: African (A), west-European (M), east-European (C), and middle-East (O) (Ruttner 1988; Smith 1991; Arias and Sheppard 1996; Garnery et al. 1993). This marker has a size polymorphism owing to a combination of two sequence elements P and Q, of which the P element has three forms, P, P0, and P1 (Cornuet et al. 1991; De la Rúa et al. 1998). The various associations of the P and Q elements coupled with a polymorphism for the restriction enzyme *Dra*I are indicative of a particular lineage (Garnery et al. 1993; Franck et al. 1998; 2001). Mitochondrial DNA can trace the maternal origin of colonies, and for paternal genetic contribution, morphometric traits are good indicators of nuclear introgression (Rinderer et al. 1991; Mortensen and Ellis 2015). Morphometric traits can also be combined to determine a probability of Africanization using various methods (Sylvester and Rinderer 1987).

In this study, we analyzed for the first time the genetic composition of honey bees from the five beekeeping regions of Mexico using mitochondrial and morphometric markers in order to assess (1) the degree of European and African ancestry of stocks in northern neotropical regions and (2) the relationship between climate and the distribution of European and African mitotypes and morphotypes.

2. MATERIALS AND METHODS

2.1. Sampling

A total of 500 samples of worker bees were collected from the same number of managed colonies in the different beekeeping regions of Mexico in 2008. The number of samples per state and region is presented in Table I. With the exception of Campeche (Yucatan Peninsula) and Veracruz (Gulf coast), the most important beekeeping states in Mexico are represented in the sample (Labougle-Rentería and Zozaya-Rubio 1986).

The samples were collected from small independent beekeepers that owned colonies in the range of 10 to 100. We avoided sampling apiaries in which the owner reportedly replaced queens introducing stock purchased elsewhere. The number of apiaries sampled per state

Table 1. Summary of sample data including beekeeping region, states, apiary, and colony numbers. The frequencies of mitotypes for each of the main *Apis mellifera* lineages are presented in percentage per state, region, and total. The frequency of Africanized morphotype is presented in accordance to the classification of the FABIS system (Sylvester and Rinderer 1987) per state, region, and total. The values of the forewing and hind femur lengths are presented with the results of Duncan's multiple comparison tests per region

Region	State	N colonies (apiaries)	Mitotype (%)			African morphotype (%)	Forewing length (mm)	Hind femur length (mm)
			A	C	M			
North	1) Chihuahua	36 (7)	0.25	0.75	0	0.55	9.112±0.07	2.526±0.03
	2) Coahuila	35 (7)	0.4	0.6	0	0.914	8.968±0.15	2.492±0.04
	3) Sonora	35 (7)	0.142	0.857	0	0.942	8.936±0.07	2.520±0.04
	4) Zacatecas	16 (3)	0.125	0.875	0	0.812	9.045±0.13	2.505±0.04
	5) Baja California sur	12 (3)	0.33	0.5	0.16	0.75	9.015±0.15	2.499±0.03
	<i>Mean</i>	<i>134 (27)^a</i>	<i>0.249</i>	<i>0.716</i>	<i>0.032</i>	<i>0.793</i>	<i>9.015 ± 0.11a</i>	<i>2.508 ± 0.04a</i>
Central highlands	6) Estado de México	14 (3)	0.285	0.714	0	0.285	9.158±0.12	2.563±0.04
	7) Morelos	30 (6)	0.4	0.6	0	0.9	8.975±0.10	2.519±0.03
	8) Puebla	47 (9)	0.404	0.531	0.065	0.446	9.106±0.18	2.543±0.04
	<i>Mean</i>	<i>91 (18)^a</i>	<i>0.363</i>	<i>0.615</i>	<i>0.021</i>	<i>0.543</i>	<i>9.079 ± 0.13b</i>	<i>2.541 ± 0.04b</i>
Pacific coast	9) Jalisco	40 (8)	0.175	0.8	0.025	0.65	9.054±0.17	2.506±0.05
	10) Michoacán	26 (5)	1.00	0	0	1.00	8.721±0.10	2.466±0.05
	11) Oaxaca	23 (5)	0.869	0.130	0	0.956	8.817±0.21	2.459±0.04
	12) Chiapas	50 (5)	0.48	0.52	0	0.9	8.879±0.15	2.485±0.04
	<i>Mean</i>	<i>139 (23)^a</i>	<i>0.631</i>	<i>0.362</i>	<i>0.0062</i>	<i>0.876</i>	<i>8.867 ± 0.16c</i>	<i>2.479 ± 0.05c</i>
Gulf coast	13) Tamaulipas	31 (6)	0.580	0.419	0	0.87	8.904±0.15	2.482±0.06
	14) Tabasco	49 (10)	0.816	0.163	0.021	1.00	8.779±0.15	2.435±0.03
	<i>Mean</i>	<i>80 (16)^a</i>	<i>0.698</i>	<i>0.291</i>	<i>0.01</i>	<i>0.935</i>	<i>8.841 ± 0.15d</i>	<i>2.458 ± 0.04d</i>
Yucatan Peninsula	15) Quintana Roo	31 (6)	0.677	0.322	0	1.00	8.764±0.10	2.429±0.03
	16) Yucatán	25 (5)	0.6	0.24	0.16	1.00	8.873±0.10	2.492±0.03
	<i>Mean^a</i>	<i>56 (11)</i>	<i>0.638</i>	<i>0.281</i>	<i>0.08</i>	<i>1.00</i>	<i>8.818 ± 0.10d</i>	<i>2.461 ± 0.03d</i>
	<i>Overall mean</i>	<i>500 (95)^a</i>	<i>0.515</i>	<i>0.453</i>	<i>0.029</i>	<i>0.829</i>	<i>8.924 ± 0.14</i>	<i>2.489 ± 0.04</i>

Different literals in the same column indicate significant differences at $P < 0.01$

^aAll values in italics per region represent the mean, except for N colonies and apiaries which represent the subtotal per region

ranged between 5 and 10, except for Baja California, Zacatecas, and Estado de Mexico where only 3 apiaries were sampled (Table I).

2.2. Mitotype analysis

Total DNA was extracted from the thoracic muscles of one worker honey bee per colony using a high salt protocol (Paxton et al. 1997). To assign bees to lineages, primers E2 and H2 were utilized for the DNA amplification as described by Garnery et al. (1993) but with some modifications. The 25- μ L reactions contained 5 μ L of high salt-extracted DNA as the template, $\times 1$ reaction buffer, 20 mM of each dNTP, 1 mM MgCl₂, 1 mM of each primer, and 0.6 units of *Taq* DNA polymerase (Sigma). Polymerase chain reaction (PCR) temperature profiles were those described by Clarke et al. (2001). An aliquot of 10 μ L of PCR product was analyzed on a 1 % agarose gel to calculate the size of the PCR fragment. The remainder of the PCR product was digested with the restriction enzyme *Dra*I (Boehringer Mannheim). Restriction fragments were electrophoretically separated on 10 % denaturing polyacrylamide gels [27 mL of $\times 0.5$ TBE, 3 mL of 40 % acrylamide/bis (Astral Scientific Gymea)] and visualized under UV light after staining with ethidium bromide.

*Dra*I allowed the identification of the mitotypes and respective assignment to one of the mitotypes in the maternal lineages: African (A), east European (C), and west European (M) (Franck et al. 1998).

We calculated the frequencies of the different mitotypes in the A, C, and M lineages per region and Nei's unbiased D_{ST} (1978) across states and regions. The genetic distance matrix of unbiased D_{ST} was used to construct an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree using Poptree (Takezaki et al. 2010). The association between the overall percentage of African and European mitotypes with beekeeping region was assessed using Fisher's exact test with a Monte Carlo estimate.

2.3. Morphometric analysis

Ten bees from each colony sample were dissected, and the right forewing and hind leg from each were mounted on slides. Two morphometric characters, the length of the forewing and the hind femur, were measured on each bee with the help of an inverted

microscope and a digitizer tablet (Summasketch Professional Plus) that converted the distances to millimeters using the software AFUSDA[®] 7 (Rubink unpublished). The lengths of the forewing and femur were compared between beekeeping regions using the mean values obtained for each trait per colony by means of ANOVA followed by Duncan's multiple comparison test using SPSS, Inc. (2006) statistical software. Additionally, the colonies were assigned a probability of Africanization calculated by multiplying the average value of each morphometric trait by the coefficients provided in the Fast Africanized Bee Identification System (FABIS) (Sylvester and Rinderer 1987). Colonies with a FABIS value below -0.56 were considered with an Africanized morphotype (A), colonies with values between -0.212 and 0.56 were considered with an intermediate morphotype (I), and colonies with values above 0.56 were classified as a European morphotype (E). The percentage of Africanization of each state and region was calculated as the percentage of the Africanized morphotype present in that population sample. The association between the percentage of Africanized morphotypes with the beekeeping region was evaluated using Fisher's exact test with a Monte Carlo estimate using SPSS, Inc. (2006) statistical software.

2.4. Association between mitotype and morphotype

Finally, the overall and within-region association between morphotype and mitotype were also evaluated by means of Fisher's exact statistic followed by a Monte Carlo estimate for each exact test performed. When the result was statistically significant, an analysis of adjusted residuals was performed with SPSS, Inc. (2006) in order to identify the cell (mitotype-morphotype association) causing the deviation from expected (Everitt 1992).

3. RESULTS

3.1. Mitotype analysis

Six different mitotypes were identified in Mexican apiaries: three for the African lineage (A_1 , A_4 , and A_{30}), one for the east-European lineage (C_1), and two for the west-European lineage (M_3 and M_8). Our results showed that over half of the whole colonies under study had mitotypes of

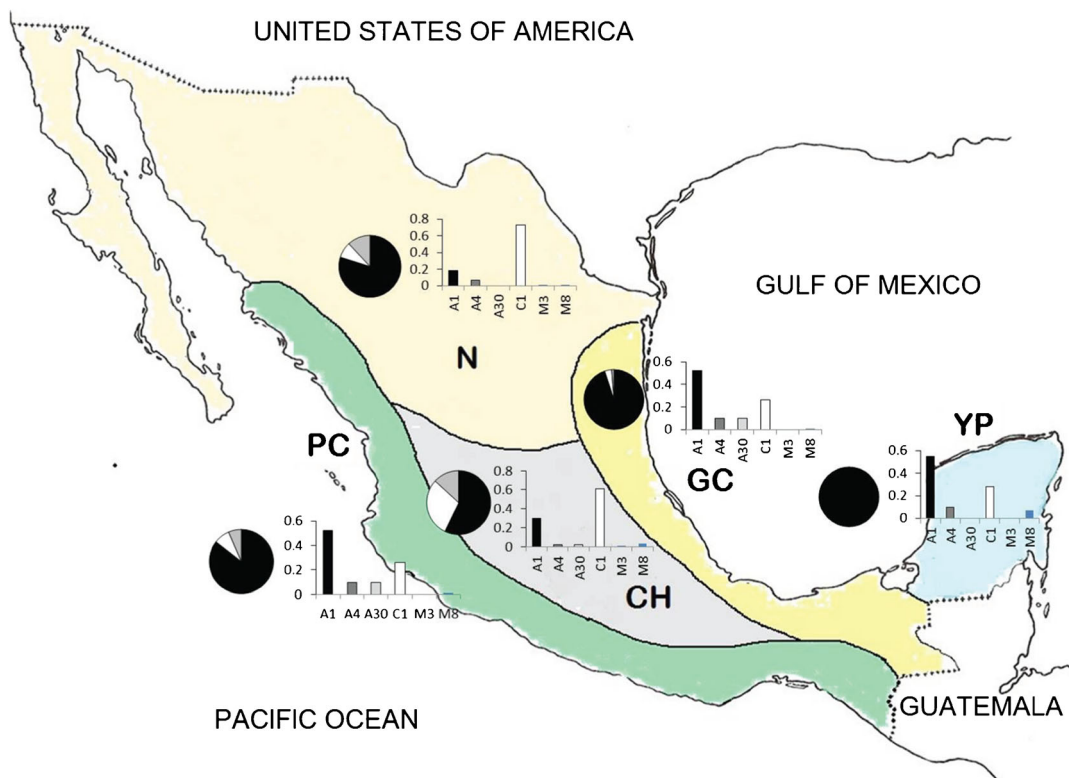


Figure 1. Map of Mexico showing the five main beekeeping regions: North (*N*), Central highlands (*CH*), Pacific coast (*PC*), Gulf coast (*GC*), and Yucatan Peninsula (*YP*). The relative frequencies of the different alleles per mitotype lineage on each region are presented in *bar graphs*. The *pies* represent the relative frequencies of morphotypes per region: Africanized in *black*, European in *white*, and intermediate in *gray*

African origin (51.5 %, Table I), the most frequent being mitotype A_1 (Figure 1). Regarding the other two African mitotypes, mitotype A_4 was present in all regions but was highest on the Pacific and Gulf coasts. The mitotype A_{30} was also highest in these latter two regions but was absent in the samples from the Yucatan Peninsula and in the North (Figure 1).

The overall distribution of African mitotypes was heterogeneous across the country (Table I, Figure 1). The highest frequency of African mitotypes was obtained in the Gulf coast (69.8 %) and the lowest in the Northern region (24.9 %) where European mitotypes were predominant (Table I, Figure 1). The state with the lowest proportion of African mitotypes was Zacatecas (12.5 %) in the Northern zone, and the highest was Michoacan (100 %) in the Pacific coast (Table I). Amongst the European mitotypes,

the most frequent in all regions was C_1 ; its highest frequency was found in the North and Central regions whereas its lowest was found in the tropical areas of the Pacific, the Gulf, and the Yucatan Peninsula. The M mitotypes were detected in much lower proportions; the M_8 mitotype was highest in the Yucatan Peninsula (5 %), and the M_3 mitotype was only detected in the temperate regions (1 % in both the North and Central highlands) (Figure 1).

An UPGMA tree based on mitotype differences across regions showed that the temperate North and Central regions were more similar to each other and that the three tropical regions (Pacific, Gulf, and Yucatan Peninsula) clustered in a separate group in which the first two regions were more similar to each other (Figure 2). The Yucatan Peninsula was different from those of the Pacific and the Gulf due to the presence of a higher

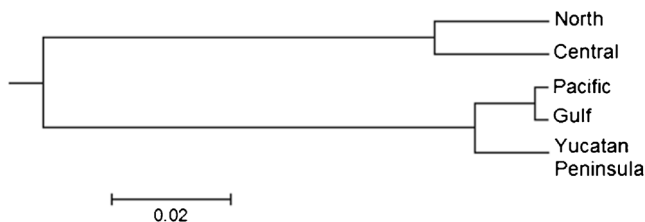


Figure 2. UPGMA tree produced from a distance matrix based on Nei's (1978) unbiased distance across the five beekeeping regions from Mexico

frequency of mitotype M_8 and the absence of mitotype A_{30} . A significant overall association between the frequency of mitotypes and region ($P=0.001$) confirmed the distribution on the UPGMA tree. This result indicates that a climatic gradient of African mitotype distribution exists in Mexico, with high frequencies in the tropical regions and decreasing in the temperate Central and North regions (Figures 2 and 3).

3.2. Morphometric analysis

The results of the morphometric analysis are presented in Table I. There were significant differences in the length of both the forewing ($F_{4,495}=41.7$; $P<0.001$) and femur ($F_{4,495}=47.17$; $P<0.001$) across regions. The values for both traits were highest in the Central region (Table I) and lowest in the Gulf coast and Yucatan Peninsula, which were not significantly different from each other. The values of the forewing and femur length for the Pacific coast and the North regions were intermediate between the Central highlands and the Gulf coast plus the Yucatan Peninsula regions but were significantly different from each other and amongst the other regions ($P<0.001$; Table I).

The values of the forewing and femur length were submitted to the FABIS system to calculate the percentage of Africanization in accordance to morphotype across the five beekeeping regions. The percentage of Africanization calculated using the FABIS classification for the whole country was higher (83 %) compared with the proportion of colonies carrying mitotypes of African origin (51.5 %). The lowest percentage of morphometrically Africanized colonies was obtained in the Central and North regions (54.3 and 79.3 %, respectively) and the highest in the Yucatan

Peninsula (100 %) (Table I; Figure 3). The Gulf and Pacific regions also had high proportions of morphometrically Africanized colonies (93.5 and 87.6 %, respectively). The largest proportion of European (8 and 30 %) and intermediate morphotypes (12 and 13 %) were found in the North and Central regions, respectively (Figure 1).

Similar to the results of mitotype distribution, there were differences in the percentage of morphometrically Africanized colonies across regions (Table I; Figure 1). Similarly, there was a significant association between Africanized morphotype with beekeeping region ($P=0.001$). This result also suggests that a climatic gradient of Africanization exists in Mexico, with the highest frequency of Africanized honey bees observed in the Yucatan Peninsula, slightly decreasing in the Gulf and Pacific, and decreasing in the temperate Central and North regions (Figure 3).

3.3. Association between mitotype and morphotype

The overall association between mitotypes and morphotypes showed that a large proportion of colonies (46 %) carrying African mitotypes had Africanized morphology (Table II). In contrast, only 8 % of the colonies in the country showed no evidence of introgression of African genes, i.e., European mitotypes (C_1) coupled with European morphology. However, over a third of all colonies showed European mitotypes associated with Africanized morphology (34.2 %). Fisher's exact statistic to evaluate the association between mitotype and morphotype corroborated an overall significant association of both markers for the whole country ($P=0.001$). The analysis of adjusted residuals revealed that three associations

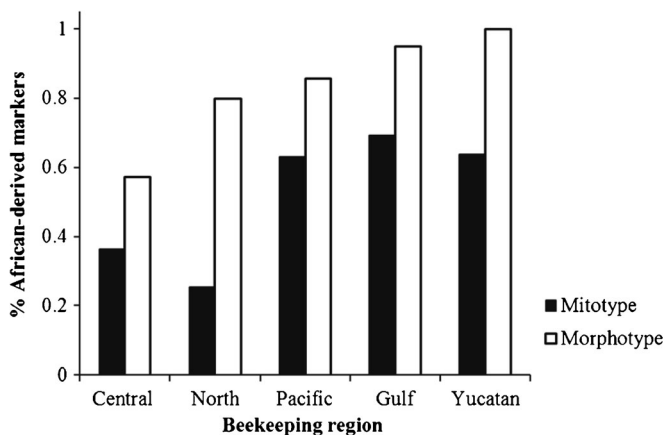


Figure 3. Percentage of African-derived markers; mitotypes in *black bars* and morphotypes in *white bars* found in the five beekeeping regions from Mexico

contributed to the observed significance: colonies with African mitotypes and Africanized morphotypes, colonies carrying the European C mitotypes with Africanized morphotype, and those carrying European mitotype C with European morphotype (Table II). However, within regions, the analysis of mitotype-morphotype association showed that both markers were not homogeneously linked. In the North ($P=0.13$) and Central regions ($P=0.08$), morphotype and mitotype were not significantly linked, meaning that the association between mitotypes and morphotypes within these two regions was randomly determined in contrast with the tropical ones ($P=0.001$).

4. DISCUSSION

We used morphometric and mtDNA markers to assess the relative contribution of African- and

European-derived genes to stocks in the northern neotropics and the possible effect that climate may have on their distribution. The mitochondrial results showed that after two decades of Africanization, honey bees in Mexico are descendent almost in similar proportion from matriline of African and European ancestries. Morphometrics indicate that extensive introgressive hybridization with Africanized bees while large-scale displacement of pure European honey bees seems to have occurred in the tropical regions of Mexico. However, in the temperate regions, we found a higher frequency of colonies with mixed mitotypes and morphotypes with a lower introgression of African-derived genes.

The mitochondrial analysis revealed that the displacement of European matriline in managed colonies from Mexico has been negligible. Since 1986, African matriline have probably entered apiaries via the capture of feral swarms by the

Table II. Contingency table presenting the overall number of honey bee colonies per morphotype (rows) and mitotype (columns) found in Mexico. The percentages per cell are presented within brackets

Morphotype	Mitotype			Total
	A	C	M	
Africanized	230 (0.46)	171 (0.342)	9 (0.018)	410
European	10 (0.02)	40 (0.08)	0 (0)	50
Intermediate	18 (0.036)	16 (0.032)	6 (0.012)	40
				500

beekeepers or by takeover of weak or queenless European colonies by African swarms, as reported elsewhere (Vergara et al. 1993; Quezada-Euán et al. 1996; Clarke et al. 2001; Collet et al. 2006). Notoriously, European matriline are still found in high frequencies even in the most Africanized areas (up to 30 %) of the tropical Gulf coast and Yucatan Peninsula. In particular, the east-European mitotype Cs are high in managed Mexican bees in contrast with those found in feral neotropical populations (Hall and Muralidharan 1989; Hall 1992) and managed populations from South America, except in the Yucatan Peninsula (Clarke et al. 2001; Collet et al. 2006). Interestingly, the frequency of C matriline in the three tropical areas in our study is similar to that found in the Yucatan Peninsula in 1998 (Clarke et al. 2002). The large density of European colonies before Africanization may be evoked to explain the frequency of European mitotypes in Mexican managed populations, but additionally, the persistence of C matriline in apiaries may be the result of differences in the sessility of Africanized and European honey bees (Winston 1987). Absconding and migration are highly frequent in African bees and their hybrids (Schneider et al. 2004). However, European colonies are less inclined to abscond, and this may determine a seasonal reduction of their Africanized counterparts in apiaries. During the annual colony multiplication practiced by beekeepers, colonies with European mitotypes should be more frequent in apiaries and, thus, more likely involved in colony multiplication. Such a cyclical colony reproduction from colonies having European matriline may have determined the persistence of high frequencies of the C lineage in managed Mexican stocks.

The morphometric results suggest that a large-scale introgression of nuclear African genes has occurred into tropical Mexican populations with the concurring displacement of nuclear European genes. This finding is in agreement with results in tropical and subtropical regions elsewhere (Clarke et al. 2002; Pinto et al. 2005; Whitfield et al. 2006). A numerical asymmetry between Africanized and European honey bees can explain these results (Rinderer et al. 1991). The rapid buildup of large feral Africanized populations together with

preferential mating between queens produced in apiaries with Africanized drones is considered the main force displacing European genes (Clarke et al. 2002; Schneider et al. 2004). The numerical superiority of African drones in congregation areas where queens mate mainly arises from proportionately greater male production by Africanized colonies (Rinderer et al. 1987) and Africanized drones' parasitism of European colonies, which also suppress the production of European drones (Rinderer et al. 1985). In combination, these factors favor the introgression of African paternal alleles into European colonies while resulting in the eventual displacement of European nuclear markers (Rinderer et al. 1991; Pinto et al. 2005; Whitfield et al. 2006). However, in temperate zones, feral populations with African ancestry may not reach the high densities observed in tropical areas, giving their European counterparts better opportunities to compete reproductively (Sheppard et al. 1991; Quezada-Euán 2007) as our findings suggest. Factors such as partial seasonal isolation could also favor the preservation of European genes in temperate zones (Quezada-Euán and May-Itzá 2001).

When both types of markers were combined, it was noticed that the majority of colonies in Mexico seems to be the product of African matriline mating with African patriline. However, the association of African-derived mitotype and morphotype was only significant in the tropical regions compared with the North and Central highlands where colonies exhibit a mixed range of both markers. Altogether, these results suggest that the outcome of the hybridization between Africanized and European honey bees in Mexico has been significantly associated with the climatic region. A lack of association between morphology and mitotypes in the North and Central highlands indicates extensive gene flow between European and Africanized bees as predicted from the selective disadvantage of the latter in temperate regions (Sheppard et al. 1991). Selection rather than competitive displacement seems a more plausible hypothesis for the genetic composition of Mexican bees. Selection would favor genes advantageous to survival in tropical or temperate climate (Moritz and Meusel 1992; Quezada-Euán et al. 2003) which is in agreement to our findings of

Africanized-derived markers more frequent in tropical regions and European-derived markers more frequent in temperate regions. Concomitantly, a gradient of Africanization seems to exist with higher frequencies of Africanized colonies in the Mexican tropical regions and lower in the temperate ones, similar to those found in the southern neotropics (Sheppard et al. 1991; Quezada-Euán et al. 2003).

Factors other than climate may also account for the outcome of Africanized-European honey bee hybridization, notably the higher parasite disease resistance of Africanized stocks (Medina-Medina and Martin 1999; Pinto et al. 2004; Martínez-Puc et al. 2011). For instance, the expansion of Africanized honey bees in the temperate region of the southern United States seems to have been greatly enhanced by the decimation of resident feral European honey bee colonies following the arrival of the *Varroa* mite (Pinto et al. 2005). Similarly, in Puerto Rico, a genetically stable honey bee population has formed, with European- and African-like traits associated with *Varroa* resistance (Galindo-Cardona et al. 2013). The reported spread of Africanized colonies in neotropical highlands (Quezada-Euán et al. 2003; Kraus et al. 2007) may have been helped by similar parasite-related events.

Future studies of Mexican honey bees involving high-density molecular markers should be useful in revealing the population genetic structure and the level of selection that has occurred for African- and European-derived genes. Such studies should be of special interest to also interpret the apparent absence in Mexico of the colony collapse affecting European stocks in North America (Ellis et al. 2010; Vandame and Palacio 2010). A corollary is that breeding programs in Mexico may benefit from using honey bees that are already present and probably better adapted to their respective tropical and temperate zones. These populations have been largely confronted to various environmental conditions and in the process have probably better adapted to their regions compared with foreign stock. Strong considerations should be distinctly made on the risk of disease introduction (Vandame and Palacio 2010). Africanized honey bee colonies are amongst the most genetically diverse *A. mellifera*

yet recorded (Tarpay et al. 2010; Harpur et al. 2012). When colonies have been assessed on an individual basis, they clearly display a wide range of behavioral characteristics (Spivak 1992; Quezada-Euán et al. 2008). Such amount of variation could serve as the basis of successful breeding programs.

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Composition génétique du stock d'abeilles du nord de la région néotropicale: diversité du mitotype et du morphotype au Mexique (Hymenoptera: Apidae)

Apis mellifera / africanisation / hybridation introgressive / ADNmt / morphométrie

Genetische Zusammensetzung des Honigbienenbestands in den nördlichen Neotropen: Mitotypen und morphometrische Diversität in Mexico (Hymenoptera: Apidae)

Apis mellifera / Afrikanisierung / introgressive Hybridisierung / mtDNA / Morphometrie

REFERENCES

- Arias, M.C., Sheppard, W.S. (1996) Molecular phylogenetics of honey bee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequences. *Mol. Phyl. Evol.* **5**, 557–566
- Clarke, K.E., Oldroyd, B.P., Quezada-Euán, J.J.G., Rinderer, T.E. (2001) Origin of honeybees (*Apis mellifera* L.) from the Yucatan peninsula inferred from mitochondrial DNA analysis. *Mol. Ecol.* **10**, 1347–1355
- Clarke, K.E., Rinderer, T.E., Quezada-Euán, J.J.G., Oldroyd, B.P. (2002) The Africanization of the honey bees (*Apis mellifera* L.) of the Yucatan: a study of a

- massive hybridization event across time. *Evolution* **56**, 1462–1474
- Collet, T., Ferreira, K.M., Arias, M.C., Soares, A.E.E., Del Lama, M.A. (2006) Genetic structure of Africanized honeybee populations (*Apis mellifera* L.) from Brazil and Uruguay viewed through mitochondrial DNA COI-COII patterns. *Heredity* **97**, 329–335
- Cornuet, J.M., Garnery, L., Solignac, M. (1991) Putative origin and function of the intergenic region between COI and COII of *Apis mellifera* L. mitochondrial DNA. *Genetics* **1128**, 393–403
- De la Rúa, P., Galián, J., Serrano, J. (1998) Mitochondrial variability of honeybees populations from the Canary Islands. *Mol. Ecol.* **7**, 1543–1547
- Del Lama, M.A., Lobo, J.A., Soares, A.E.E., Del Lama, S.N. (1990) Genetic differentiation estimated by isozymic analysis of Africanized honeybee populations from Brazil and from Central America. *Apidologie* **21**, 271–280
- Ellis, J.D., Evans, J.D., Pettis, J. (2010) Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. *J. Apic. Res.* **49**, 134–136
- Everitt, B.S. (1992) The analysis of contingency tables. 2a Ed. Chapman and Hall, London
- Fierro, M., Barraza, A., Maki, D.L., Moffet, J.O. (1987) The effects of the first year of Africanization on honey bee populations in Chiapas, México. *Am. Bee J.* **127**, 845
- Franck, P., Garnery, L., Solignac, M., Cornuet, J.M. (1998) The origin of west European subspecies of honeybees (*Apis mellifera*): New insights from microsatellite and mitochondrial data. *Evolution* **52**, 1119–1134
- Franck, P., Garnery, L., Loiseau, A., Oldroyd, B.P., Hepburn, H., Solignac, M., Cornuet, J.M. (2001) Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. *Heredity* **86**, 420–430
- Galindo-Cardona, A., Acevedo-González, J.P., Rivera-Marchand, B., Giray, T. (2013) Genetic structure of the gente Africanized honey bee population (gAHB) in Puerto Rico. *BMC Genetics* **14**, 65
- 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**, 1016–1020
- Gu, G., Zhang, C., Hu, F. (2002) Analysis on the structure of honey production and trade in the world. *Apiacta* **2** <http://www.apimondiafoundation.org/foundation/files/2002/GU%20G.%20ZHANG%20CH.pdf>
- Guzmán-Novoa, E. (2004) Impacto de la africanización de las abejas en México. *Imagen Veterinaria* **4**, 22–25
- Guzmán-Novoa, E., Page Jr., R.E. (1994) The impact of Africanized bees in Mexican beekeeping. *Am. Bee J.* **134**, 101–106
- Hall, H.G. (1992) DNA studies reveal processes involved in the spread of New World African honeybees. *Fla. Entomol.* **75**, 51–59
- Hall, H.G., McMichael, M.A. (1992) European honey bee (*Apis mellifera* L.) (Hymenoptera: Apidae) colonies kept at high elevations in Costa Rica tested for African DNA markers. *Bee Science* **2**, 25–32
- Hall, H.G., Muralidharan, K. (1989) Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. *Nature* **339**, 211–213
- Harpur, B.A., Minae, S., Kent, C.F., Zayed, A. (2012) Management increases genetic diversity of honey bees via admixture. *Mol. Ecol.* **21**, 4414–4421
- Kraus, F.B., Franck, P., Vandame, R. (2007) Asymmetric introgression of African genes in honeybee populations (*Apis mellifera* L.) in Central Mexico. *Heredity* **99**, 233–240
- Labougle, R.J.M. (1991) Beekeeping in Mexico: past, present and future. *Am. Bee J.* **131**, 132–135
- Labougle-Rentería, J.M., Zozaya-Rubio, J.A. (1986) La apicultura en México. *Ciencia y desarrollo* **12**, 17–36
- Martínez-Puc, J.F., Medina, M.L.A., Catzín-Ventura, G.A. (2011) Frecuencia de *Varroa destructor*; *Nosema apis* y *Acarapis woodi* en colonias manejadas y enjambres silvestres de abejas (*Apis mellifera*) en Mérida, Yucatán, México. *Revista Mexicana de Ciencias Pecuarias* **2**, 25–38
- Medina-Medina, L., Martin, S.J. (1999) A comparative study of *Varroa jacobsoni* reproduction in worker cells of honey bees (*Apis mellifera*) in England and Africanized bees in Yucatan, Mexico. *Exp. App. Acar.* **23**, 659–667
- Moritz, R.F.A., Meusel, M. (1992) Mitochondrial gene frequencies in Africanized honeybees (*Apis mellifera* L.): theoretical model and empirical evidence. *J. Evol. Biol.* **5**, 71–81
- Mortensen, A.N., Ellis, J.D. (2015) The frequency of African (*Apis mellifera scutellata* Lepeletier) matrilineal usurpation of managed European-derived honey bee (*A. mellifera* L.) colonies in the southeastern United States. *Insectes Soc* **62**, 151–155
- Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590
- Paxton, R.J., Weibschuch, N., Quezada-Euán, J.J.G. (1997) Characterization of dinucleotide microsatellite loci for stingless bees. *Mol. Ecol.* **8**, 690–691
- Pinto, A.M., Rubink, W.L., Coulson, R.N., Patton, J.C., Johnston, J.S. (2004) Temporal pattern of Africanization in a feral honeybee population from Texas inferred from mitochondrial DNA. *Evolution* **58**, 1047–1055
- Pinto, A.M., Rubink, W.L., Patton, J.C., Coulson, R.N., Johnston, J.S. (2005) Africanization in the United States: replacement of feral European honeybees (*Apis mellifera* L.) by an African hybrid swarm. *Genetics* **170**, 1653–1665
- Quezada-Euán, J.J.G., De Araujo-Freitas, C., Marrufo Olivares, J.C., Velásquez Madrazo, A., Medina Medina, L.A. (2008) Selección en colonias de abejas africanizadas: una alternativa viable para el trópico mexicano. In *Memorias 2º. Apimondia Simposio mundial de criadores de abejas reina e inseminación artificial*, Nayarit México 15–17 Octubre 2008
- Quezada-Euán, J.J.G. (2000) Hybridization between European and Africanized honeybees in tropical Yucatan; Mexico. II. Morphometric, allozymic and

- mitochondrial DNA variability in feral colonies. *Apidologie* **31**, 443–453
- Quezada-Euán, J.J.G. (2007) A retrospective history of the expansion of Africanized honey bees in Mexico. *J. Apic. Res. and Bee World* **46**, 295–300
- Quezada-Euán, J.J.G., Hinsull, S.M. (1995) Evidence of continued European morphometrics and mtDNA in feral colonies of honey bees (*Apis mellifera*) from the Yucatan peninsula, Mexico. *J. Apic. Res.* **34**, 161–166
- Quezada-Euán, J.J.G., May-Itzá, W.d.J. (2001) Partial seasonal isolation of African and European-derived *Apis mellifera* (Hymenoptera: Apidae) drones at congregation areas from subtropical México. *Ann. Entomol. Soc. Am* **94**, 540–544
- Quezada-Euán, J.J.G., Echazarreta, C.M., Paxton, R.J. (1996) The distribution and range expansion of Africanized honey bees (*Apis mellifera*) in the state of Yucatan. Mexico. *J. Apic. Res.* **35**, 85–95
- Quezada-Euán, J.J.G., Perez-Castro, E., May-Itza, W.d.J. (2003) Hybridization between European and African-derived honey bee populations (*Apis mellifera*) at different altitudes in Perú. *Apidologie* **34**, 217–225
- Rinderer, T.E., Hellmich, R.L., Danka, R.G., Collins, A.M. (1985) Male reproductive parasitism: a factor in the Africanization of European honey bee populations. *Science* **228**, 1119–1121
- Rinderer, T.E., Collins, A.M., Hellmich, R.L., Danka, R.G. (1987) Differential drone production by Africanized and European honey bee colonies. *Apidologie* **18**, 61–68
- Rinderer, T.E., Stelzer, J.T., Oldroyd, B.P., Bucu, S.M., Rubink, W.L. (1991) Hybridization between European and Africanized honeybees in the Neotropical Yucatan peninsula. *Science* **253**, 309–311
- Ruttner, F. (1988) *Biogeography and taxonomy of honey bees*. Springer, Berlin
- Schneider, S.S., Degrandi-Hoffman, G., Smith, D.R. (2004) The African honey bee: Factors contributing to a successful biological invasion. *Annu. Rev. Entom.* **49**, 351–376
- Sheppard, W.S., Soares, A.E., De Jong, D., Shimanuki, H. (1991) Hybrid status of honey bee populations near the historic origin of Africanization in Brazil. *Apidologie* **22**, 643–652
- Smith, D.R. (1991) Mitochondrial DNA and honeybee biogeography. In: Smith, D.R. (ed.) *Diversity in the genus Apis*, pp. 131–176. Westview Press and IBH Publications, Oxford
- Spivak, M. (1992) The relative success of Africanized and European honey-bees over a range of life-zones in Costa Rica. *J. Appl. Ecol.* **29**, 150–162
- SPSS, Inc. (2006) *SPSS for Windows version 15*. Chicago, USA
- Sylvester, H.A., Rinderer, T.E. (1987) *Fast Africanized bee identification system (FABIS) manual*. *Am. Bee J.* **127**, 511–516
- Takezaki, N., Nei, M., Tamura, K. (2010) POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with windows interface. *Mol. Biol. Evol.* **27**, 747–752
- Tarpy, D.R., Caren, J.R., Delaney, D.A., Sammataro, D., Finley, J., Loper, G.M., Degrandi-Hoffman, G. (2010) Mating frequencies of Africanized honey bees in the south western USA. *J. Apic. Res. and Bee World* **49**, 302–310
- Vandame, R., Palacio, M.A. (2010) Preserved honey bee health in Latin America: a fragile equilibrium due to low-intensity agriculture and beekeeping? *Apidologie* **41**, 243–255
- Vergara, C., Dietz, A., Perez De Leon, A. (1993) Female parasitism of European honey bees by Africanized honey bee swarms in Mexico. *J. Apic. Res.* **32**, 34–40
- Whitfield, C.W., Behura, S.K., Berlocher, S.H., Clark, A.G., Johnston, J.S., Sheppard, W.S., Smith, D.R., Suarez, A.V., Weaver, D., Tsutsui, N.D. (2006) Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science* **314**, 642–645
- Winston, M.L. (1979) The potential impact of the Africanized honey bee on apiculture in Mexico and Central America. *Am. Bee J.* **119**, 584–586
- Winston, M.L. (1987) *The biology of the honey bee*. Harvard University Press, Massachusetts
- Zamora, O., Domínguez, R.J., Alaniz-Guiterrez, L., Quezada-Euán, J.J.G. (2008) Frequency of European and African-derived morphotypes and haplotypes in colonies of honey bees (*Apis mellifera*) in NW Mexico. *Apidologie* **39**, 388–396