

## Africanization of honey bees (*Apis mellifera*) in three climatic regions of northern Mexico

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### Abstract

This study was conducted to analyze the process of Africanization of managed honey bee (*Apis mellifera*) colonies by determining the frequency of African and European morphotypes and mitotypes ( $m_{mt}$ DNA) in three different ecological environments of northern Mexico. Colonies ( $n=151$ ) were sampled in 1) temperate semi-dry; 2) semi-warm semi-dry; and 3) temperate sub-humid regions in the state of Zacatecas. The  $m_{mt}$ DNA type was determined by PCR-RFLP and the morphotype by the Fast Africanized Bee Identification System (FABIS). Out of all the colonies sampled in all areas, the  $m_{mt}$ DNA analysis showed a significantly higher frequency of European maternal lineage (77.5%) than of African maternal lineage (22.5%;  $P < 0.0001$ ). The morphometric analysis classified 47% of the colonies as European and 42.4% of them as Africanized. The frequency of colonies with African or European mitotypes and morphotypes varied significantly between regions ( $P < 0.05$ ) with results indicating a higher degree of Africanization in the semi-warm semi-dry region. Conversely, the highest frequency of colonies with the European morphotype and mitotype occurred in the temperate semi-dry region. These results suggest that the environment affects the degree of Africanization of honey bee colonies in northern Mexico. Colonies established at higher altitudes and in more temperate climates have more European genotypes than colonies established in tropical regions. Several hypotheses are discussed to explain these results.

**Keywords:** *Apis mellifera*; Africanization; Morphotype; Mitotype; Climate; Zacatecas; Mexico.

### Introduction

The so-called Africanized honey bees are a hybrid population derived from the interbreeding of the European honey bee subspecies (e.g., *Apis mellifera mellifera*) and the African subspecies *Apis mellifera scutellata* imported into Brazil in 1956 (Kerr, 1967; Whitfield *et al.*, 2006). Africanized bees are characterized by their highly defensive and migratory behaviors (Nogueira-Neto, 1964). The phenomenon of Africanization began following the release of the African bee (*A. m. scutellata*) colonies in 1956 (Kerr, 1967). The African genotype was favored by the

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tropical conditions of Brazil and the absence of natural predators (Guzmán-Novoa *et al.*, 2011); Africanized bee colonies dispersed at a rate of over 300 km per year, displacing European honey bees in the tropics (Taylor, 1977; Hall, 1992). At the present time, Africanized bees are well established in most of South America, virtually all of Central America (Smith, 1991), Mexico and the Southern USA, occupying a range of approximately 20 million km<sup>2</sup> (Rinderer *et al.*, 1993). The high colonizing ability of these bees is one of the fastest and most spectacular biological invasions known to date (Guzmán-Novoa *et al.*, 2011).

The frequency of the European genotypes has decreased in the tropics due to the Africanization of honey bee colonies as a result of factors such as numerical superiority and the behavior of Africanized drones (which gives them an advantage over European drones in mating queens); usurpation of European colonies by swarms of Africanized bees; advantages resulting from the process of natural selection such as their high reproduction rate and migration (swarming and absconding behaviors); dominance and reproductive advantages of subfamilies of the African genotype compared to those of European origins; and resistance and tolerance to disease, shorter developmental time, higher defensive and robbing behavior, etc. (Schneider *et al.*, 2004; Guzmán-Novoa *et al.*, 2011).

In Mexico, Africanized bees were first detected in 1986 in the state of Chiapas, and 20 years later, they had become well established in all states of the country (Quezada-Euan 2007; Guzmán-Novoa *et al.*, 2011). The Africanization of honey bee colonies is one of the main problems affecting the Mexican beekeeping industry (Uribe *et al.*, 2003). The negative impact of this phenomenon is a result of increased defensive, migratory and evasive behaviors of bees (Guzmán-Novoa and Uribe, 2004; Guzmán-Novoa *et al.*, 2011). This has resulted in the abandonment of beekeeping in some regions, significant changes in the management of these bees, the death of people and animals, increased production costs and a reduction in number of colonies and honey production relative to managing European bees (Uribe-Rubio *et al.*, 2003; Guzmán-Novoa and Uribe, 2004; Guzmán-Novoa *et al.*, 2011).

There is a considerable amount of information about the Africanization process of honey bee colonies in the southeast part of Mexico (Quezada-Euan, 2000; Clarke *et al.*, 2002), but little is known about this process in other regions of North America with different climatic conditions, beekeeping practices and densities of European bees, such as is the case in the state of Zacatecas, Mexico. Therefore, considering that there are no studies analyzing the Africanization process in honey bee colonies located at different altitudes and climates in northern Mexico, the objective of this study was to determine the frequency of managed honey bee colonies with African and European phenotypes and mitotypes in three different ecological environments in the state of Zacatecas.

## Materials and methods

### Location and sampling

Samples of bees were collected during two weeks in October of 2011, from 151 commercial honey bee colonies distributed in 25 commercial apiaries. Bee samples were collected from 20% of the total number of colonies of each apiary.

The sampled colonies belong to different producers and were located in 15 municipalities in three ecological regions of Zacatecas, Mexico (22°57'N, 102°42'W). Each sample consisted of approximately 40 worker bees that were stored in 90% ethanol. The samples were used to determine the African or European ancestry of the bees. The relevant climatic and floral characteristics of each region are described below and are presented in Fig. 1.

### Temperate semi-dry region

This is the largest region in the state of Zacatecas (60% of its area) and also the driest. The average annual precipitation, temperature and relative humidity are 469 mm, 15 °C and 54%, respectively, and the dominant type of vegetation is semi-open grassland (INEGI, 2005). The evaluation and sampling process were conducted in colonies located in the municipalities of Fresnillo, Villanueva, Villa García, Guadalupe, Ojo Caliente and Zacatecas at altitudes between 1800 and 2400 m above sea level.

### Temperate sub-humid region

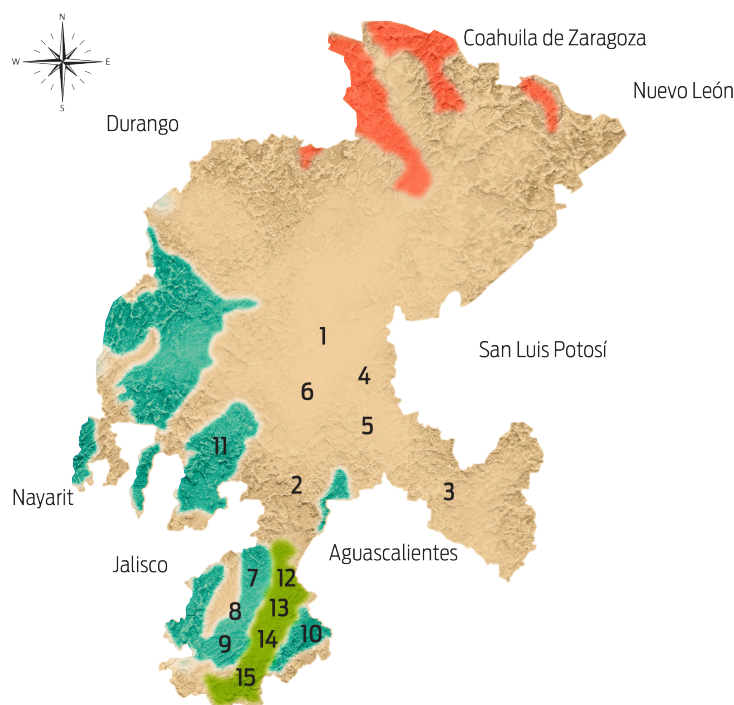
This region is characterized by average annual rainfall, temperature and relative humidity of 680 mm, 18 °C and 66.7%, respectively, and the dominant vegetation type is sclerophyllous broadleaf deciduous forest (INEGI, 2005). The samples were taken from hives located in the municipalities of Tepechitlán, Tlantenango, Momax, Nochistlán and Valparaíso at altitudes between 1200 and 2000 m above sea level.

### Semi-warm semi-dry region

This region is located in the southern part of the state and is characterized by average annual rainfall, temperature and relative humidity of 704 mm, 19.5 °C and 55%, respectively; the type of vegetation is tropical deciduous forest (INEGI, 2005). The colonies sampled were located in the municipalities of Tabasco, Jalpa, Juchipila and Moyahua at altitudes between 1000 and 1400 m above sea level.

### Morphotype and mitotype

To determine the morphological type of bees (morphotype), the Fast Africanized Bee Identification System (FABIS; Sylvester and Rinderer, 1987) was used. Briefly, the right forewing of 30 workers per colony were dissected and mounted on plastic slides and projected on a screen using a slide projector. Then, they were measured with a ruler and using the formula provided by Sylvester and Rinderer (1987), their actual length was calculated and an average of the 30 wings was obtained. The average values for wing length per colony were used to calculate the probability of Africanization. Colonies with an average above



**Figure 1.** Relief map of Zacatecas, with the location of sampling sites in regions: **temperate semi-dry**: Fresnillo (1), Villanueva (2), Villa García (3), Guadalupe (4), Ojo Caliente (5) and Zacatecas (6); **temperate sub-humid**: Tepechitlán (7), Tlantenango (8), Momax (9), Nochistlán (10) and Valparaíso (11); **semi-warm semi-dry**: Tabasco (12), Jalpa (13), Juchipila (14), and Moyahua (15).

9.001 mm were classified as European, while those with an average below 8.968 mm were classified as Africanized. Colonies with mean values between 8.968 and 9.001 mm were considered intermediate or mixed.

Additionally, 5 bees per colony were used to determine their mitochondrial DNA haplotype (mitotype). This was conducted by extracting DNA from bee samples and subjecting it to PCR (Polymerase Chain Reaction) amplification using specific primers. Additionally, the samples were further treated with restriction enzymes to produce and analyze RFLP (Restriction Fragment Length Polymorphism) markers on a gel as per [Nielsen \*et al.\* \(1999\)](#). The analyses of the markers allowed the classification of the samples as having an African or European mitotype.

The morphometric analyses of the bees were performed at the Veterinary Academic Unit, University of Zacatecas, Mexico. The molecular analyses to determine the mitotype of the bees were performed at the School of Environmental Sciences, University of Guelph, Canada.

## Statistical analyses

Colonies were classified according to their bees' morphotype and mitotype (African or European) for each of the regions that were sampled, and their frequencies were subjected to chi-square tests for comparisons. To more objectively assess the effect of region on the degree of Africanization, colonies were separately analyzed by morphotype, mitotype, or genotype (colonies that in both classifications were either European or African-derived). All statistical analyses were performed using Statistical Analysis System ([SAS Institute, 2002](#)).

## Results

Out of the total number of colonies sampled in all areas, the morphometric analysis classified 42.4% of the colonies as Africanized and 47% as European ( $\chi^2 = 35.6$ ,  $n = 151$ ,  $P < 0.0001$ ). The rest of the sampled colonies (10.6%) had bees with intermediate measurements which did not allow classification into the categories established by FABIS ([Sylvester, Rinderer, 1987](#)). The mtDNA analysis showed that the frequency of African maternal lineage was 22.5%, whereas the frequency of European maternal ancestry was 77.5% ( $\chi^2 = 45.6$ ,  $n = 151$ ,  $P < 0.0001$ ).

The frequency of colonies with African and European morphotypes varied significantly in each region and between the three regions, suggesting a greater degree of Africanization in the semi-warm semi-dry region, while the higher frequency of colonies with European morphotype occurred in the temperate semi-dry region ([Table 1](#)).

Regarding honey bee mitotypes in the three study areas, the frequency of European mitotypes was significantly higher than that of African mitotypes in all areas. However, there was a significantly lower frequency of colonies with African mitotype in the temperate semi-dry region compared to the temperate sub-humid and the semi-warm semi-dry regions, but there was no significant difference between these two regions ([Table 2](#)).

**Table 1.** Number and percentage of colonies with African and European morphotypes in temperate semi-dry, temperate sub-humid and semi-warm semi-dry regions in Zacatecas, Mexico.

Morphotype	Temperate semi-dry (n=50)	Temperate sub-humid (n=50)	Semi-warm semi-dry (n=51)
African (A)	7 (14%) <sup>a</sup>	22 (44%) <sup>b</sup>	35 (69%) <sup>c</sup>
European (E)	36 (72%) <sup>a</sup>	23 (46%) <sup>b</sup>	12 (24%) <sup>c</sup>
A vs E	$\chi^2 = 33.64$ , $P < 0.0001$	$\chi^2 = 12.28$ , $P = 0.002$	$\chi^2 = 30.47$ , $P < 0.0001$

Different letters on the same line represent significant differences based on  $\chi^2$  tests: temperate semi-dry region vs temperate sub-humid region ( $\chi^2 = 10.58$ ,  $n = 88$ ,  $P = 0.001$ ) vs semi-warm semi-dry region ( $\chi^2 = 30.54$ ,  $n=90$ ,  $P < 0.0001$ ); temperate sub-humid vs semi-warm semi-dry regions ( $\chi^2 = 6.38$ ,  $n = 92$ ,  $P = 0.011$ ).

**Table 2.** Number and percentage of honey bee colonies with African and European mitotypes in the temperate semi-dry, temperate sub-humid and semi-warm semi-dry regions in Zacatecas, Mexico.

Mitotype	Temperate semi-dry (n=50)	Temperate sub-humid (n=50)	Semi-warm semi-dry (n=51)
African (A)	6 (12%) <sup>a</sup>	14 (28%) <sup>b</sup>	14 (27%) <sup>b</sup>
European (E)	44 (88%) <sup>a</sup>	36 (72%) <sup>b</sup>	37 (73%) <sup>b</sup>
A vs E	$\chi^2 = 28.88$ , $P < 0.0001$	$\chi^2 = 9.68$ , $P = 0.0019$	$\chi^2 = 10.37$ , $P = 0.0013$

Different letters on the same line represent significant differences based on  $\chi^2$  tests: temperate semi-dry region vs temperate sub-humid region ( $\chi^2 = 3.8$ ,  $n = 100$ ,  $P = 0.05$ ) vs semi-warm semi-dry region ( $\chi^2 = 4.0$ ,  $n = 101$ ,  $P = 0.045$ ); temperate sub-humid vs semi-warm semi-dry regions ( $\chi^2 = 0.003$ ,  $n = 101$ ,  $P = 0.95$ ).

Only 12% of the samples (18 of 151) with African morphotype also had the African mitotype, while 41% (62 of 151) had both the European morphotype and mitotype. These 80 colonies with extreme genotypes were analyzed separately according to whether they were more African or more European genotypes, respectively. The frequency of colonies with extreme genotypes is shown in Table 3, indicating once again that the African genotype frequency was higher in the semi-warm semi-dry zone and null in the temperate semi-dry zone.

## Discussion

The presence of Africanized bees in the state of Zacatecas, Mexico, was first reported in 1991 (SAGARPA, 2005), although this study reveals for the first time the proportion of African phenotypes and genotypes in the most important bee-keeping regions, in three different climates and at different altitudes in the state of Zacatecas.

A comparison of the results of this study with those of the few previously published studies that were carried out in northern states of Mexico shows that the frequency of African mitotypes in Zacatecas (22.5%) is similar to that reported for Baja California Sur (21%) but lower than that reported for Sonora (48%) and Baja California Norte (50%) (Zamora *et al.*, 2008). However, in comparison with the high plateau of Mexico (Uribe *et al.*, 2003), the frequency of colonies with African <sub>mt</sub> DNA was 9% higher in Zacatecas. Recently, Domínguez-Ayala *et al.*



**Table 3.** Number and percentage of colonies with African and European genotypes (morphotype and mitotype of the same origin) in the temperate semi-dry, temperate sub-humid and semi-warm semi-dry regions in Zacatecas, Mexico.

Genotype	Temperate semi-dry (n=33)	Temperate sub-humid (n=22)	Semi-warm semi-dry (n=25)
African (A)	0 (0%) <sup>a</sup>	5 (22.7%) <sup>b</sup>	13 (52%) <sup>c</sup>
European (E)	33 (100%) <sup>a</sup>	17 (77.3%) <sup>b</sup>	12 (48%) <sup>c</sup>
A vs E	$\chi^2 = 0.00$ , $P = 0.000$	$\chi^2 = 6.54$ , $P = 0.01$	$\chi^2 = 0.040$ , $P = 0.84$

Different letters on the same line represent significant differences based on  $\chi^2$  tests: temperate semi-dry region vs temperate sub-humid region ( $\chi^2 = 8.25$ ,  $n = 55$ ,  $P = 0.0041$ ) vs semi-warm semi-dry region ( $\chi^2 = 22.11$ ,  $n = 58$ ,  $P < 0.0001$ ); temperate sub-humid vs semi-warm semi-dry regions ( $\chi^2 = 4.24$ ,  $n = 47$ ,  $P = 0.0394$ ).

(2015) reported that Zacatecas is the state in the Northern part of Mexico with the lowest proportion of African mitotypes, 12.5%, a result that is 10% lower than that found in the present study with a larger number of samples.

The proportion of colonies with bees of different morphotypes and mitotypes varied significantly between the study regions. A higher proportion of colonies with European-derived morphotypes or mitotypes was found in the temperate semi-dry region than in the temperate sub-humid and semi-warm semi-dry regions, where higher frequencies of African-derived morphotypes and mitotypes were observed. These results indicate a higher degree of Africanization in colonies of the semi-warm semi-dry and temperate sub-humid regions and a lower degree of Africanization in colonies of the temperate semi-dry area, suggesting environmental effects on the flow and introgression of African genes into commercial colonies of honey bees. This finding is in agreement with the results from Domínguez-Ayala *et al.* (2015), who indicated that a climatic gradient of African mitotype distribution exists in Mexico.

The differences in the frequency of colonies with predominantly European or African ancestry between regions may have resulted, at least in part, from differences in climate and the availability of floral resources and nesting sites in these areas. In the temperate semi-dry region that we studied, blossoms are scarcer, colder temperatures reduce colony reproduction during the winter, and its vegetation provides fewer nesting sites than in the subtropical regions. Thus, it is less likely for bee colonies to swarm frequently and to establish feral populations in temperate climates than in more tropical environments (Otis, 1991; Clarke *et al.*, 2002). Tropical and subtropical regions are more favorable for African gene introgression into commercial colonies because Africanized bees are better adapted to these environments than are European-derived bees. They show greater ability to reproduce and spread in the tropics than their European counterparts (Schneider *et al.*, 2004; Pinto *et al.*, 2005). The higher density of Africanized colonies of honey bees in tropical environments allows for increased mating of virgin queens from commercial colonies with African-derived drones (Rinderer and Hellmich, 1991). Additionally, a higher rate of usurpation of commercial colonies by Africanized bee swarms would be expected in tropical environments (Danka *et al.*, 1992). In managed apiaries such as those sampled, beekeepers had a European maternal bee lineage before the arrival of Africanized bees in Zacatecas. Therefore, most of the introgression of African genes in these populations may have occurred paternally, mainly through mating between virgin queens from commercial colonies and drones of African

origin that were produced by feral colonies (Clarke *et al.*, 2002). This process of Africanization of commercial colonies would explain why more colonies were classified as African-derived by morphometric analyses than by mitochondrial analysis, which only detects maternal inheritance.

In addition to the above and because the study was conducted in commercial colonies, the possible replacement of queens by beekeepers is another factor that may have influenced the frequency of morphotypes and mitotypes in different regions because this practice reduces the probability of introgression of African genes in commercial bee populations (Zamora *et al.*, 2008). However, this matter was discussed with the producers and there is no evidence to suggest that the practice of requeening in the temperate semi-dry zone was greater than in the other regions.

## Conclusion

In conclusion, these results suggest that environment affects the degree of Africanization of honey bee colonies in northern Mexico. It is expected that colonies established at higher altitudes and in more temperate climates will have more European genotypes than colonies established in tropical regions.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

## Author contributions

Carlos A. Medina Flores: conceived the study, performed all assays, and wrote part of the manuscript.

Ernesto Guzmán Novoa: conceived the study and wrote part of the manuscript.

M. M. Hamiduzzaman: performed the PCR-RFLP analyses and interpreted the results.

Jairo Aguilera Soto: collected the samples, analyzed the results and revised the manuscript.

Miguel A. López Carlos: helped with the morphometric analyses of bees and the statistical analyses.

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