Apidologie 39 (2008) 388–396
© INRA/DIB-AGIB/ EDP Sciences, 2008
DOI: 10.1051/apido:2008016

Available online at:
www.apidologie.org

Original article

Frequency of European and African-derived morphotypes and haplotypes in colonies of honey bees (*Apis mellifera*) from NW Mexico*

Omar ZAMORA[1], Ricardo DOMINGUEZ[1], Luis ALANIZ-GUTIERREZ[2], J. Javier G. QUEZADA-EUÁN[1]

[1] Departamento de Apicultura, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Apdo. postal 4-116, Mérida, Yucatán, 97100, Mexico
[2] Universidad Autónoma de Baja California, Ensenada BC, Mexico

Received 29 March 2007 – Accepted 30 January 2008

Abstract – Africanized honey bees (AHBs *Apis mellifera*) have been reported in NW Mexico since the mid 90s, but no study on the process of admixture with local European honey bees has been conducted. Morphometrics and haplotype analyses were used to investigate the frequency of African markers in honey bees from Sonora (SON), the north and south of Baja California (BCN and BCS). Morphometrics identified 42% of the samples from SON, 44% from BCN and 15% of BCS as Africanized. Honey bees from BCS had larger body size and formed a separate cluster from BCN and SON which were similar to each other. The molecular analysis revealed a higher frequency of African-derived haplotypes in SON (48%) and BCN (50%) compared to BCS (21%). The morphometric and molecular evidence suggests that the colonization of BCS by AHBs may be recent. Nest and food availability in desert areas and beekeeping practices are evoked to explain the reduced introgression of African genes into honey bee populations from this region of Mexico.

***Apis mellifera* / Africanized honey bee / Baja California / hybridization / haplotype / morphometrics**

1. INTRODUCTION

African honey bees (*Apis mellifera scutellata* Lepeletier) were introduced 50 years ago to Brazil (Kerr, 1967). The escape and further genetic admixture of their descendants with resident European honey bees across the Neotropics, has resulted in an event regarded as one of the biological phenomena with the most transcendental impact on the ecology and economy of the Americas (Winston, 1992; Schneider et al., 2004).

The first report of AHBs in Mexico occurred in 1986 in the state of Chiapas (Moffet et al., 1987). The following year, AHBs were reported in the Yucatan Peninsula, one of the EHB most densely populated areas in the neotropics. So far, this remains as the only area in Mexico where an extensive documentation of the process of Africanization of European honey bees has been conducted (Quezada-Euán, 2000; May-Itzá et al., 2001; Quezada-Euán and May Itzá, 2001; Clarke et al., 2002). Evidence from studies in the Yucatan Peninsula suggest that both, the feral and the managed population of AHBs are a composite of diverse degrees of introgression of African/European genes possibly as a consequence of the high densities of European honey bees (EHBs) prior to Africanization in this area (Quezada-Euán, 2000; Clarke et al., 2002). Recent analysis of managed honey bees from the tropical regions of Tabasco and Chiapas has revealed a

Corresponding author: J.J.G. Quezada-Euán, qeuan@tunku.uady.mx
* Manuscript editor: Watter S. Sheppard

significantly low frequency of European derived haplotypes (Quezada-Euán, 2006). In contrast, only limited information exists about the Africanization of other honey bee populations, especially in the non tropical areas of Mexico (Labougle et al., 1989; Labougle and Yarce-Salazar, 1990; Trejo-Cruz et al., 1990; Becerra-Guzmán et al., 2000).

Africanized bees were first reported in the northern part of Sonora (SON) and the northern part of the Peninsula of Baja California (BC) near the border with the United States, between 1993 and 1994 (Guzmán-Novoa and Page, 1994). Later, Alaniz-Gutierrez (2002), conducted a study in northern Baja California (BCN), to evaluate the frequency of the AHB and EHB morphotypes and detected a high percentage of colonies with intermediate morphology. However, morphometrics alone may only provide a limited picture of the process given that it may underestimate subtle levels of Africanization, (Guzmán-Novoa et al., 1994).

The southern part of Baja California Sur (BCS) remains officially as an Africanized free zone (SAGARPA NOM- 002-200-1994; Modified 25-04-2001). This status could have important repercussions on the honey bee population and beekeeping industry of Mexico since BCS annually provides ca. 5000 European queens (basically breeding stock) that are distributed to various breeding apiaries within the country (May-Itzá et al., 2001; Zamora and Quezada-Euán, 2005) with the aim of ameliorating the effect of Africanized honey bees in beekeeping.

The North West part of Mexico is predominantly an arid zone with the lowest density of managed EHBs in the country: 0.13 colonies per km^2 (SAGARPA, 2005). The relative importance of the population size of resident European honey bees plus the environmental and beekeeping characteristics in different localities on the process of Africanization is controversial (Smith et al., 1989; Rinderer et al., 1991; Sheppard et al., 1991). One of the main problems in elucidating the relative importance of different factors and the mechanisms involved in the process is the lack of long term detailed studies before, during and after the arrival of colonizing swarms (Quezada-Euán, 2000; Pinto et al., 2005). The study of the expansion and establishment of AHBs in NW Mexico can provide additional information to test hypothesis regarding the relative effect of the size of the resident EHB population and that of climatic and geographic barriers (Quezada-Euán et al., 2003) different from the ones that AHBs have encountered during their expansion history across tropical and subtropical Mexico.

2. MATERIALS AND METHODS

2.1. Sample collection

The Peninsula of Baja California is the second longest Peninsula in the world (ca. 1220 km), extending from the latitude of 32° 30' to 22° 52'. It is surrounded by the Gulf of Baja California (Sea of Cortes) to the East and the Pacific Ocean to the West (Fig. 1). Sonora (SON) is the second largest state of Mexico, together with BCN and BCS they cover a surface of approximately 336 000 km^2. The climate in such area of México ranges from semi to extremely arid with areas of virtually no rain in years (BW h-like or BS h-like; Garcia, 1998). Accordingly, the vegetation is scarce and xerophytes are dominant (Dominguez-Cadena and Leon de la Luz, 1992).

Honey bee workers were sampled from managed colonies across SON (50 colonies) and the north (BCN) and south (BCS) of the Peninsula of Baja California (50 and 100 colonies respectively) between 2003 and 2004 (Fig. 1). All samples were labeled and maintained in ethanol (95%) and stored at –20 °C until further analyses were conducted.

2.2. Morphometric analyses

Ten worker bees were randomly selected from each sample in ethanol. The right forewing, hind wing, and third leg were dissected in each specimen and mounted on plastic slides. Forewing length and femur length were measured in honey bees from the 200 colonies collected. The average values of wing and femur length per colony were used to calculate the probability of Africanization per colony using the coefficients in the FABIS method (Sylvester and Rinderer, 1987) and colonies with a result equal or below –0.56 were considered Africanized with a probability of 0.99. Colonies with a result between

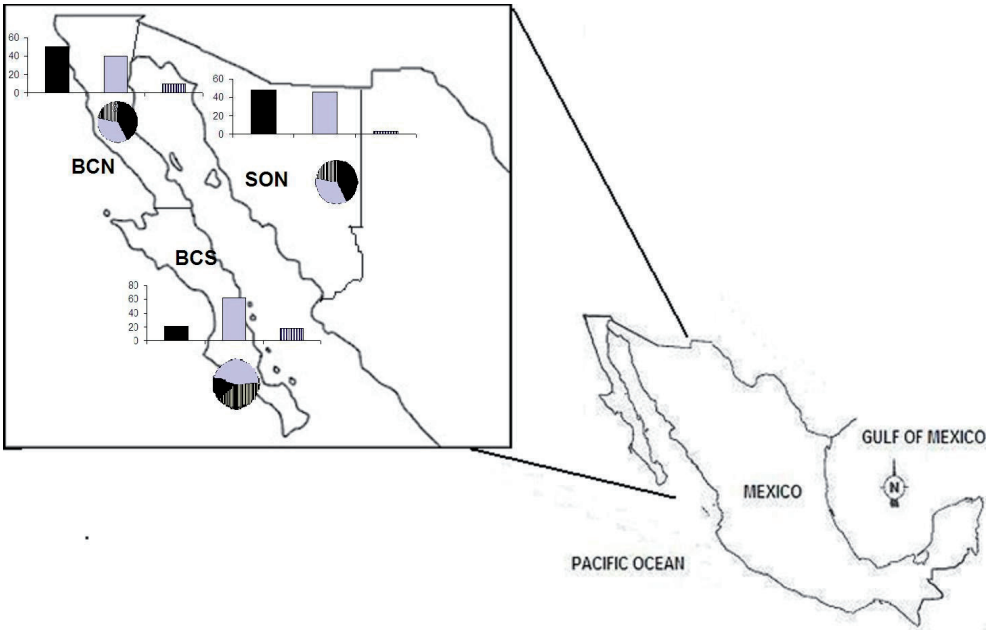

Figure 1. Map of NW Mexico showing the frequencies of haplotypes (in bars) and morphotypes (in pies) in each of the three areas studied, Sonora (SON), Baja California Norte (BCN) and Baja California Sur (BCS). In black are the frequencies of African morphotypes and haplotypes, in grey the frequency of East European haplotype and European morphotype and in stripes the frequency of West European haplotype and Intermediate morphotypes respectively.

–0.212 and 0.56 were considered intermediate morphotypes and colonies with values above 0.56 were considered European.

In a second approach, 25 colonies were selected randomly from each state to measure six additional morphometric characters (see Tab. II) in 10 worker honey bees. To measure the characters, we used an inverted microscope which projected the images on a digitizing pad. The limiting points of each structure were converted to microns with by means of the software AFUSDA (Rubink, unpubl. data). Morphometric characters were compared amongst populations by means of ANOVA and significant differences between means were detected by Duncan multiple tests using the general linear models (GLM) procedure of SAS (SAS Institute, 1990). Additionally, a Principal Component Analysis was used in order to evaluate differences at the multivariate level between populations. Colony component scores were calculated for each of the derived components as an indicator of overall body size and shape for each colony. Colony component scores were compared by means of ANOVA between populations and Duncan multiple tests as for the individual characters. Plots were produced for the distribution of Colony component scores of the three populations using components 1 and 2.

2.3. Analysis of haplotypes

For the analysis of haplotypes, total DNA was isolated from the thoracic muscles of a single adult worker bee per colony, using the high salt extraction protocol by Paxton et al. (1999). Haplotype identification was conducted by PCR analyzing the 485-bp section of the cytochrome *b*gene (Crozier et al., 1991) and the 783-bp of the large ribosomal subunit (ls rRNA) gene (Hall and Smith, 1991). The fragments were amplified using a modified protocol of Saiki et al. (1988) and the PCR conditions were those of Nielsen et al. (1999, 2000). Restriction digests of the amplified segments were performed with 3 IU of either *Bgl* II (cytochrome *b*) or *Eco* RI (ls rRNA), 2.5 μL buffer 10 X and 2.66 μL of deionized water. The digestion products were electrophoresed on 1.5% agarose gels stained with ethidium bromide and photographed under UV

Table I. Numbers and frequencies (in brackets) of colonies within each category of morphotype (after FABIS) and haplotype in the three areas studied. AM = Africanized morphotype, EM = European morphotype, IM = intermediate morphotype.

	Sonora				fBaja California Norte				Baja California Sur			
Haplotype	AM	EM	IM	Total	AM	EM	IM	Total	AM	EM	IM	Total
A	12	8	4	24	7	11	7	25	15	4	2	21
	(24)	(16)	(8)	(48)	(14)	(22)	(14)	(50)	(15)	(4)	(2)	(21)
EE	6	10	7	23	13	5	2	20	0	28	34	62
	(12)	(20)	(14)	(46)	(26)	(10)	(4)	(40)		(28)	(34)	(62)
WE	3	0	0	3	2	2	1	5	0	13	4	17
	(6)			(6)	(4)	(4)	(2)	(10)		(13)	(4)	(17)
Total	21	18	11	50)	18	10	22)	50	15	45	40	100
	(42)	(36)	(22)	(100)	(44)	(36)	(20)	(100)	(15)	(45)	(40)	(100)

light. The lack of a *Bgl* II cleavage site at the cytochrome *b*fragment identified bees with African haplotype. An *Eco* RI cleavage site on the ls rRNA segment identified the bees with haplotypes of east-European origin from the bees of western European origin (Hall and Smith, 1991; Nielsen et al., 1999, 2000).

The association between haplotype and morphotype was tested by means of a G-test.

3. RESULTS

The results of FABIS showed that 42% of the samples from SON, 44% from BCN and 15% from BCS were Africanized (Fig. 1). It was interesting that in all states there were a high proportion of colonies that had intermediate probabilities of Africanization and couldn't be assigned to the Africanized or European morphotypes: 40% in BCS, 20% in BCN and 22% in SN (Fig. 1; Tab. I). The results of the *G* test showed that the proportion of morphotypes and haplotypes varied significantly between BCS with both BCN and SON (χ^2 = 72.76, df = 4). However, the proportions of the different morphotypes were not significantly different between the latter two.

The results of the second approach showed that seven morphometric characters were significantly different between the honey bees from BCS and both, SON and BCN (Tab. II). Only the basitarsus width was not different between the three populations. Forewing length was significantly different amongst the three populations with BCS reporting the highest values, BCN the lowest and SON intermediate.

The results of PCA showed that the first 4 components included ca. 73% of the variation in the data (Tab. III). Component 1 included 48.3% of the total variation. Usually component 1 is related to size and other components are linked to shape (Wiley, 1981). Including all eight characters, the 3 populations were not different for components 2 and 3. Nevertheless, there were significant differences between populations for component 1 and 4. The populations from SON and BCN were not significantly different for components 1 and 4; however, the honey bees from BCS had significantly larger values compared with the other two populations (Tab. III). Moreover, the honey bees from BCS formed a different cluster from its BCN and SON counterparts when plotted against components 1 and 2 (Fig. 2). These results showed that honey bees from BCS were comparatively larger than those from SON and BCN.

The molecular results confirmed the presence of African genes in NW México. The African haplotype was detected in 48% of the samples from SON, 50% from BCN and 21% in BCS (Fig. 1). A *G* test with Yates correction showed that the proportion of colonies with African haplotype was significantly higher in BCN and SON compared with BCS (Fig. 1)

Table II. Comparison of means of eight morphometric characters between colonies from the three areas studied (s. dev. in brackets).

	Population			
Character	Sonora (n = 25)	Baja California Norte (n = 25)	Baja California Sur (n = 25)	F Value
Forewing length	9.01 ± 0.21 a	8.90 ± 0.18 b	9.1 ± 0.17 c	64.78**
Forewing width	3.06 ± 0.08 a	3.04 ± 0.07 a	3.12 ± 0.07 b	57.12**
Hind wing length	4.16 ± 0.07 a	4.14 ± 0.05 a	4.23 ± 0.04 b	61.22**
Hind wing width	1.68 ± 0.04 a	1.7 ± 0.06 a	1.76 ± 0.06 b	59.83**
Femur length	2.53 ± 0.13 a	2.50 ± 0.17 a	2.60 ± 0.08 b	45.24**
Tibia length	3.22 ± 0.07 a	3.23 ± 0.06 a	3.31 ± 0.07 b	84.31**
Basitarsus length	1.95 ± 0.06 a	1.95 ± 0.07 a	1.99 ± 0.05 b	41.71**
Basitarsus width	1.17 ± 0.02 a	1.15 ± 0.02 a	1.17 ± 0.02 a	3.9 ns

** Significant at $P < 0.01$; ns = not significant.

Table III. Comparison of means of colony scores for principal components 1 to 4 (s. dev. in brackets).

		Population			
Component	% variation explained	Sonora (n = 25)	Baja California Norte (n = 25)	Baja California Sur (n = 25)	F value
1	48.3	−0.13 ± 0.7 a	−0.05 ± 0.8 a	1.32 ± 1.1 b	110.3**
2	12.4	0.32 ± 0.5 a	0.38 ± 0.6 a	0.45 ± 0.7 a	4.4 ns
3	8.2	0.27 ± 0.7 a	0.35 ± 0.8 a	0.31 ± 1.1 a	3.3 ns
4	4.2	0.22 ± 0.9 a	0.29 ± 0.7 a	0.44 ± 0.8 b	45.6**

** Significant at $P < 0.01$; ns= not significant.

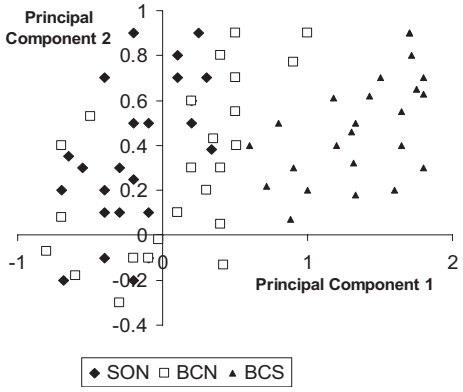

Figure 2. Plot of colony scores from Sonora (SON), Baja California Norte (BCN) and Baja California Sur (BCS) against principal components 1 and 2.

but that there were no significant differences between the former two (χ^2 = 41.3; df = 4; $P < 0.01$). Both European haplotypes were found in the three states, the most frequent was the East European type (46% in SON, 40% in BCN and 62% in BCS) (Tab. I). These results together with the analysis of morphotypes showed that the frequency of African derived honey bees was comparatively lower in BCS compared with BCN and SON (Fig. 1).

The percentage of colonies with mixed morphotype and haplotype (combination of European/African or vice versa) was 34% in SON, 52% in BCN and 4% in BCS (Tab. I). Colonies with intermediate morphotypes were significantly associated with European haplotypes in SON (22%) and BCS (38%) but not in BCN (6%) where there was a higher association of this type of colony with the African haplotype (14%) (χ^2 = 16.7; df = 4; $P < 0.01$).

4. DISCUSSION

In this study we confirmed the presence of AHB in NW México by both morphometrics and molecular markers. We also found a high frequency of colonies with European markers in the managed population and evidence of colonies with mixed morphotype and haplotype. These results are in contrast with the high frequency of African-derived markers found in tropical populations in México (Quezada-Euán, 2006). These results could be explained by differences in beekeeping practices and/or differences in climatic conditions between the NW and the tropical regions in southern México. At first glance, it could be suggested that the process of hybridization with the resident population has not resulted in a rapid reduction of European markers, compared to the tropical areas of Mexico and elsewhere (Clarke et al., 2002; Quezada-Euán, 2006). In NW México, population densities of resident EHB were small before Africanization and they cannot be evoked to explain the reduced spread of African-derived characteristics in the managed population (Dominguez-Cadena and Leon de la Luz, 1992; Alaniz-Gutierrez, 2002). We suggest that in this area, the possibility of hybridization with the resident European stocks is probably related to both, the low numbers of colonies in the feral population of AHB and the requeening of managed colonies with European stock.

In a study in Texas, Baum et al. (2005) reported that brush and grass land areas register the lowest numbers of available nests for honey bees, so desert areas (deprived of large trees) could offer less possibilities for the establishment of a large feral population. Additionally, there may have been characteristics in the colonizing front that were selected upon during their expansion across tropical America (such as the build up of large or multiple swarms) that were no longer advantageous in the reduced nest and resource environment typical of the northern arid zones (Labougle et al., 1989; Labougle and Yarce-Salazar, 1990; Aguirre and Demedio, 2005).

In 1993, AHB were officially reported in the state of Sonora, the most northern state on the Pacific Coast of Mexico. There are no data on the numbers or size of arriving swarms to this area, but high numbers of large swarms such as those found on the tropical Gulf region (there were over 3000 swarms in Veracruz in 1989, Trejo-Cruz et al., 1990) could have been less adaptive in the desert where availability of nest and food is comparatively low. Nest sites are referred as some of the most important factors influencing, the survival, growth and reproduction of feral colonies (Seeley, 1985). Thus, the lower resource predictability together with the reduced availability of nesting sites compared with the tropics could have been the most important factors limiting the opportunities of establishing an important feral population. Other factors, such as parasitism of *Varroa destructor* and the predation of the fire ant *Solenopsis invicta* may have played an additional role in limiting the growth of a feral population since small ground nesting colonies are probably more vulnerable in desert areas (Cox, 1994; Villa et al., 2002). The reduced opportunities for nesting alone could perhaps have restrained the establishment of a feral Africanized population to a large extent. The desert areas of Sonora are regarded as the ones with the lowest feral populations of honey bee colonies in México even before Africanization (Salvador Cajer, unpubl. data).

Requeening of managed colonies also could have an important effect on the spread of AHB in managed apiaries. We have no report of the number of queens used annually in NW México but annual requeening in these areas is a common practice compared to the tropical areas of Mexico (SAGARPA, 2000). It is therefore likely that Africanized queens were more frequently replaced in managed apiaries in NW México compared to those in tropical areas, thus reducing the probability of African gene introgression.

The climatic conditions in desert zones could also be less suitable for Africanized feral colonies, especially at night when environmental temperatures may fall below 0 °C. However, AHB have been found at high altitudes where cold conditions are extreme (Quezada-Euán et al., 2003). Therefore we suggest that nest availability may be the main factor reducing the numbers of feral colonies.

In the United States, the spread of AHB across the southern areas has been reduced, probably reflecting a similar situation to that found when tropical originating swarms first entered in contact with the desert areas, as we found in our study of NW México. The slow expansion across the United States has been attributed to differences in factors such as more extreme temperature regimes, rainfall, changes in photoperiod and a large European managed and feral populations compared to those found in the tropics (Visscher et al., 1997; Villa et al., 2002; Schneider et al., 2004). Similar climatic conditions and reduced resources in the desert areas along the northern part of the Peninsula of Baja California could also explain the persistence of European haplotypes and the reduced presence of African haplotype in NW Mexico. Under this scenario, European colonies, although in reduced numbers, may not be reproductively out competed by high densities of feral AHB colonies as those commonly found in tropical and subtropical Mexico.

The honey bees from BCS showed larger body size (diagnostic between European and Africanized honey bees) coupled with a significantly lower frequency of African haplotype compared with their BCN and SON counterparts which are geographically better connected with the mainland. Thus, these results indicate that the insular situation of BCS may have restrained the movement of Africanized swarms from the mainland and that honey bees from BCS remained European to a great extent. It may be possible that swarms find it more difficult to travel to the southern part of the Peninsula given the extreme climatic conditions and the mountain barriers found across the area (Pérez-Castro et al., 2002). However, evidence of AHB colonization such as a high number of migrating swarms has been recently reported in BCS (Aguirre and Demedio, 2005). We suggest that the EHB from this area had not experienced substantial hybridization with AHB given the significant differences in morphology and haplotype frequencies found with surrounding areas. However, the status as an Africanized honey bee free zone should be revised since there is evidence that hybridization is now occurring and soon the genetic composition of European breeding stock may include African genes.

We are presently conducting studies on migration patterns and feral swarm densities and analyzing their genetic composition across time and space in Baja California. This will further help to understand the role of resource and nest availability coupled with the size of resident and feral bee populations on the outcome of the Africanization process in the arid zones of Northern Mexico and southern United States.

ACKNOWLEDGEMENTS

To CONACYT-SAGARPA Project “Rescate, conservación y mejoramiento genético de los recursos apícolas en México”. To Programa Nacional para el Control de la Abeja Africanizada and the regional delegates in BCN, BCS y Sonora for the support during sampling of apiaries in the three states. We are thankful to two anonymous reviewers for their comments on earlier versions of the ms.

Fréquence des morphotypes et des haplotypes européens et africains dans les colonies d’abeilles (*Apis mellifera*) du nord-ouest du Mexique.

***Apis mellifera* / abeille africanisée / hybridation / haplotype / morphométrie / Mexique**

Zusammenfassung – Häufigkeit von europäischen und afrikanischen Morphotypen und Haplotypen in Honigbienen-Völkern (*Apis mellifera*) von NW Mexiko. Über afrikanisierte Honigbienen (AHB *Apis mellifera*) wird in NW Mexiko (Sonora und der nördliche Teil der Halbinsel von Baja California) seit Mitte der 90er Jahre berichtet, es gibt aber keine Untersuchungen zum Einfluss der lokalen europäischen Honigbienen auf den Verlauf der Afrikanisierung. Bienenarbeiterinnen wurden in Bienenständen in SON (50 Völker) und dem Norden (BCN) und Süden (BCS) der Halbinsel Baja California (50 bzw. 100 Völker) in den Jahren 2003 und 2004 gesammelt (Abb. 1).
Anhand der durchschnittlichen Flügel- und Femurlängen von 10 Arbeiterinnen pro Volk wurde unter Verwendung der Koeffizienten in der FABIS-Methode (Sylvester and Rinderer, 1987) der Grad der Afrikanisierung beurteilt. In 25 Völkern von jedem Standort wurden sechs zusätzliche morphometrische Eigenschaften gemessen und über ANOVA und einer Hauptkomponentenanalyse

verglichen. Aus den Messwerten wurden morphometrische Eigenschaften für die einzelnen Bienenvölker ermittelt und die Populationen mit ANOVA und Duncan multiple Tests verglichen.
Die Haplotyp-Analysen wurden ebenfalls dazu benutzt, die Häufigkeiten der afrikanischstämmigen Marker in den drei imkerlich gehaltenen Honigbienen-Populationen zu untersuchen. Die Haplotypen wurden über PCR und Analyse der 485-bp-Sektion des Cytochrome *b*Gens (Crozier et al., 1991) und der 783-bp-Sektion der großen ribosomalen Untereinheit (ls rRNA, Hall and Smith, 1991) bestimmt.
Die Längen von Flügel und Femur unterschieden sich signifikant zwischen den drei Untersuchungsarealen ($F = 45{,}2$ df = 2.197 und $F = 64{,}7$ df = 2.197; $P < 0,001$). Die Ergebnisse des Duncan Tests zeigten, dass die Bienen von BCS mit 9,12 mm ± 0,17 die höchsten Flügellängen im Vergleich zu SON (9,00 mm ± 0,21) und BCN (8,90 mm ± 0,18) aufwiesen. Bei der Femurlänge waren die Werte in BCS (2,60 mm ± 0,08) signifikant höher als in SON (2,53 mm ± 0,13) und BCN (2,50 mm ± 0,17). FABIS identifizierte 42 % der Proben von SON, 44 % von BCN und 15 % von BCS als afrikanisiert. Die Ergebnisse der Hauptkomponentenanalyse zeigten, dass die Honigbienen von BCS größer waren und einen von BCN und SON getrennten Cluster bildeten. BCN und SON unterschieden sich dagegen kaum. Die molekulargenetische Analyse ergab eine höhere Häufigkeit der afrikanischstämmigen Haplotypen in SON (48 %) und BCN (50 %) im Vergleich zu BCS (21 %). Es gab eine höhere Frequenz von Völkern mit gegensätzlichen Haplotypen und Morphotypen in den drei Populationen; dies spricht für eine symmetrische Hybridisierung zwischen Honigbienen mit afrikanischem und europäischem Ursprung in NW Mexiko.
Die Besiedlung der Wüstengebiete wird durch Mangel an Futter und Nestmöglichkeiten sowie durch andere imkereispezifische Faktoren erschwert. Dies könnte die geringe Einwanderung afrikanischer Gene in die europäische Honigbienen-Population dieser Region in Mexiko erklären.

***Apis mellifera* / Afrikanisierte Bienen / Baja California / Hybridisierung / Haplotyp / Morphometrie**

REFERENCES

Aguirre J.L., Demedio J. (2005) Un fenómeno apícola único en Baja California Sur, México, Apitec 54, 4–7.

Alaniz-Gutierrez L. (2002) Factores Potenciales y Limitantes para el Desarrollo de la Apicultura en el Norte de Baja California, Tesis de Maestría, Facultad de Ciencias, Universidad Autónoma de Baja California, Ensenada, Baja California, México, 119 p.

Baum K.A., Rubink W.L., Pinto A., Coulson R.N. (2005) Spatial and temporal distribution and nest site characteristics of feral honey bee (Hymenoptera: Apidae) colonies in a coastal prairie landscape, Environ. Entomol. 34, 610–618.

Becerra-Guzmán F.J., Contreras-Escareño F., Echazarreta G.C.M., Quezada-Euán J.J.G. (2000) Cambios Morfológicos de las Abejas (*Apis mellifera*) a Través del Proceso de Africanización en el Estado de Jalisco, En: Memorias del XIV Seminario Americano de Apicultura, Tampico, Tamaulipas, 2000.

Clarke K.E., Rinderer T.E., Franck P., Quezada-Euán J.J.G., Oldroyd B.P. (2002) The Africanization of honey bees (*Apis mellifera* L.) of the Yucatan: a study of a massive hybridization event across time, Evolution 56, 1462–1474.

Cox B. (1994) USDA interagency meeting on Africanized honey bees, Am. Bee J. 134, 666–668.

Crozier Y.C., Koulianos S., Crozier R.H. (1991) An improved test for Africanized honey bee mitochondrial DNA, Experientia 47, 968–969.

Dominguez-Cadena R., Leon de La Luz J.L. (1992) Avance del estudio para la estimación de la capacidad de carga apícola en el matorral Sarcocaule, de el Comitán, Bahia de la Paz BCS. VI Seminario Americano de Apicultura Oaxtepec Morelos México, pp. 71–74.

Guzmán-Novoa E., Page. R.E. (1994) The impact of Africanized bees on Mexican beekeeping, Am. Bee J. 134, 101–106.

Guzmán-Novoa E., Page R.E., Fondrk M.K. (1994) Morphometric techniques do not detect intermediate and low levels of Africanization in honey bees (Hymenoptera: Apidae) colonies, Ann. Entomol. Soc. Am. 87, 507–515.

Hall H.G., Smith D.R. (1991) Distinguishing African and European honey bee matrilines using amplified mitochondrial DNA, Proc. Natl. Acad. Sci. USA 88, 4548–4552.

Kerr W.E. (1967) The history of the introduction of Africanized bees in Brazil, S. Afr. Bee J. 39, 3–5.

Labougle J.M., Yarce-Salazar E. (1990) Caracteristicas biológicas de los enjambres de dos zonas del Istmo de Tehuantepec. IV Seminario Americano de Apicultura, Mazatlán, México, 1990, pp. 53–57.

Labougle J.M., Mancera M., Taylor O.R. (1989) Morphometric and ellectrophoretic study of the African honey bee in southern Mexico, Am. Bee J. 129, 817.

May-Itzá W.J., Quezada-Euán J.J.G., Iuit L., Echazarreta C.M. (2001) Do morphometrics and allozymes reliably distinguish Africanized

and European *Apis mellifera* drones in subtropical México? J. Apic. Res. 40, 17–23.

Moffet J.O., Maki D.L., Andre T., Fierro M.M. (1987) The Africanized bee in Chiapas, México, Am. Bee J. 127, 517–520.

Nielsen D.I., Ebert P.R., Hunt G.J., Guzman-Novoa E., Kinee S.A., Page R.E. Jr. (1999) Identification of Africanized honey bees (Hymenoptera: Apidae) incorporating morphometrics and an improved polymerase chain reaction mitotyping procedure, Ann. Entomol. Soc. Am. 92, 167–174.

Nielsen D.I., Ebert P.R., Page R.E. Jr., Hunt G.J., Guzman-Novoa E. (2000) Improved polymerase chain reaction-based mitochondrial genotype assay for identification of the Africanized honey bees (Hymenoptera: Apidae), Ann. Entomol. Soc. Am. 93, 1–6.

Paxton R.J., Weibschuh N., Quezada-Euán J.J.G. (1999) Characterization of dinucleotide microsatellite loci for stingless bees, Mol. Ecol. 8, 690–691.

Pérez-Castro E., Quezada-Euán J.J.G., May-Itzá W. de J. (2002) Thirty years after: a survey on the distribution and expansion of Africanized honey bees (*Apis mellifera*) in Peru, J. Apic. Res. 41, 69–73.

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 honey bees (*Apis mellifera* L.) by an African hybrid swarm, Genetics 170, 1653–1665.

Quezada-Euán J.J.G. (2000) Hybridization between European and Africanized honey bees in tropical Yucatan, Mexico. II. Morphometric, allozymic and mitochondrial DNA variability in feral colonies, Apidologie 31, 1–10.

Quezada-Euán J.J.G. (2006) The present status of African-derived honey bees in tropical México, in: Hartfelder K., Santana W.C. (Eds.), Memorias VII Encontro sobre Abelhas, Sao Paulo Brasil 12–15 Julio 2006.

Quezada-Euán J.J.G., May-Itza W. de J. (2001) Partial seasonal isolation of African and European-derived *Apis mellífera* (Hymenoptera: Apidae) drones at congregation areas from subtropical Mexico, Ann. Entomol. Soc. Am. 94, 540–544.

Quezada-Euán J.J.G., Perez-Castro E., May-Itzá W. de 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., Stelzer J.A., Oldroyd B.P., Buco S.M., Rubink, W.L. (1991) Hybridization between European and Africanized honey bees in the Neotropical Yucatan Península, Science 253, 309–311.

SAGARPA (2000) Situación actual y perspectiva de la producción apícola 1990–1998. Centro de Estadística Agropecuaria.

SAGARPA (2005) Servicio de información y estadística agroalimentaria (SIAP) [online] http://www.siap.sagarpa.gob.mx (accessed on 31 January 2008).

Saiki R.K., Gelfand D.M., Stoffel S., Scharf S.J., Higuchi R., Horn G.T., Mullis K.B., Erlich H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase, Science 239, 487–491.

SAS Institute Inc. (1990) SAS/STAT User's guide, version 6, 4th ed., Cary, SAS Institute Inc., North Carolina.

Schneider S.S., DeGrandi-Hoffman G., Smith D.R. (2004) The African honey bee: factors contributing to a successful biological invasion, Annu. Rev. Entomol. 49, 351–376.

Seeley T.D. (1985) Honey bee ecology, Princeton University Press, Princeton, NJ.

Sheppard W.S., Soares A.E.E., DeJong 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., Taylor O.R., Brown W.M. (1989) Neotropical Africanized honey bees have African mitochondrial DNA, Nature 339, 213–215.

Sylvester H.A., Rinderer T.E. (1987) Fast Africanized bee identification system (FABIS), Am. Bee J. 127, 511–516.

Trejo-Cruz R.I., Quintero E., Mondragon-Muñoz L., Gonzalez-Orona N., Martinez-Solís M. (1990) Una comparación morfométrica entre abejas (*Apis mellifera*; Apoidea: Hymenoptera) provenientes del Golfo de México y de la costa Pacífica de Oaxaca, México, IV Seminario Americano de Apicultura, Mazatlán, México 1990, pp. 94–110.

Villa J.D., Rinderer T.E., Stelszer J.A. (2002) Answers to the puzzling distribution of Africanized bees in the United States, Am. Bee J. 142, 480–483.

Visscher P.K., Vetter R.S., Baptista F.C. (1997) Africanized bees, 1990–95: Initial rapid expansion has slowed in the U.S., Calif. Agric. 51, 22–25.

Wiley E.O. (1981) Phylogenetics, John Wiley and Sons, New York.

Winston M.L. (1992) Killer bees: The Africanized honey bee in the Americas, Harvard Univ. Press. Cambridge, Massachusetts.

Zamora O., Quezada-Euán J.J.G. (2005) Análisis molecular y morfométrico en poblaciones de *Apis mellifera* de Baja California Sur, XIX seminario Americano de apicultura. Campeche México, Julio 2005, pp. 99–105.