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A population genetic analysis of honey bees of the Mexican neotropical Yucatan peninsula shows that the range expansion of Africanized bees there has involved extensive introgressive hybridization with European bees. Yucatan honey bee populations now include many colonies with intermediate morphologies. Genotypes of mitochondria have disassociated from historically correlated Africanized or European morphology, producing diverse phenotypic associations. This suggests that the size of resident European populations may be important in explaining previously reported asymmetrical hybridization. Evidence of natural hybridization is encouraging for the use of genetic management to mitigate the effects of Africanized bees in the United States.

AFRICANIZED HONEY BEES (*Apis mellifera*) now range from central Argentina to southern Texas. They will soon confront U.S. agriculturalists with their notorious abilities to disrupt both beekeeping and crop pollination (*1*) and the general public with a health nuisance (*2*). African bees (*A. m. scutellata*) were imported to Brazil to improve honey production through crossbreeding with European honey bees (*3*). They have founded populations of bees that are infamous as "killer bees" in the popular press and considered objectionable by bee specialists because of poor honey production, excessive stinging, difficult handling characteristics, and inferior value as commercial pollinators. (*1, 4*).

Whether or not Africanized bees are essentially "African" bees that remain undiluted by hybridization with European bees is controversial (*5–8*). Africanized bees in Argentina hybridize extensively with European bees in a temperate climate (*8*). There, mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs) from both African and European origins are associated with a full range of morphological and allozyme phenotypes (*8*). In the neotropics, populations of Africanized bees are hybrids but to a lesser degree for morphology (*8*) isozymes (*9*), and nuclear DNA RFLPs (*7*). European-derived mtDNA RFLPs are rare in some populations (*5, 6*). One interpretation is that although interbreeding occurs, hybrids may be disadvantaged by maladaptive genes from European parents, or genetic incompatibilities (*5–7*). The origins of the asymmetry of nuclear and mitochondrial markers in these neotropical populations are central to both understanding the Africanization process and developing optimal recommendations to mitigate problems caused by Africanized bees.

We studied a population of honey bees undergoing Africanization in the neotropical Yucatan peninsula of Mexico. The peninsula has the largest concentration of commercial colonies of honey bees in the world (about 17 colonies per square kilometer) (*11*). This population is the first large population of European bees encountered by expanding populations of Africanized bees in the neotropics. Consequently, it is an ideal site to evaluate three competing hypotheses concerning the asymmetrical parentage of neotropical Africanized populations. This asymmetry may arise from (i) asymmetrical hybridization producing essentially "pure" African bees, possibly caused by mitochondrial-nuclear incompatibilities (*5–7*), (ii) colony usurpation by Africanized queens (*12*), or (iii) population size advantages for African-derived bees (*13*).

Colonies were surveyed along transects through the peninsula (Fig. 1). The transects were divided into nine equal-sized sampling regions. Each area was represented among samples taken from 163 colonies. Colonies were sampled by collecting 20 to 30 worker bees from inside hives, usually from the surface of combs containing brood. The survey was of rustic commercial colonies owned by beekeepers who stated that they did not requeen colonies except that some killed old queens causing colonies to raise replacements. This practice may accelerate but will not alter the processes of Africanization since colonies naturally replace queens, often several times a year in the tropics.

An additional 28 colonies from four apiaries near Merida were sampled which had been requeened during the previous year with European stock obtained through the local beekeeper cooperative from reported non-Africanized areas of northern Mexico. These colonies were compared to 34 colonies from seven nearby apiaries that had not been requeened according to their owners.

Colonies were assessed for matriline origin using mitochondrial RFLPs (*14*). Two restriction patterns resulting from digestion with Eco RI are associated with sub-Saharan *A. mellifera* and two patterns with European subspecies (*5, 8*). The samples were also measured morphologically (*15*) to produce 25 length and angle measures of the wings, hind-legs and sternites of ten bees per colony. Morphological data were analyzed by two multivariate discriminate analysis procedures (*15–17*) developed for the classification of bees according to their morphological similarities to reference populations. The first procedure (*15*) (DF-AE) compares colonies to strongly Africanized colonies collected from South America and European colonies from the United States and classifies them by their most probable group membership. A second discriminant analysis (DF-AEHF) was developed specifically for this study (*16*). In addition to reference collections of Africanized and European colonies, DF-AEHF includes reference data from known F_1 hybrid colonies (*19*) and from feral European bees from "pre-African-

Table 1. Mitochondrial (A, African; E, European) and morphological classification by two discriminant functions (A, Africanized; E, European; H, hybrid similar to F_1 colonies; and F, feral European bees common to central and northeastern Mexico before Africanization) of honey bee colonies of the Mexican Yucatan.

Discriminant function	Mitochondrial classification	Colonies (*n* and %) by morphological classification			
		A	E	H	F
DF-AE (*17*)	A	21 (42.9)	28 (57.1)		
	E	19 (16.7)	95 (83.3)		
DF-AEHF (*18*)	A	0 (0)	17 (34.7)	14 (28.6)	18 (36.7)
	E	2 (1.8)	32 (28.1)	20 (17.5)	60 (52.6)

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ized" central and northeastern Mexico and southern Texas. These bees tend to be small, and their use as an additional reference population reduces the chance that a European colony would be misclassified as Africanized or hybrid.

Mitochondrial RFLP analysis revealed that Africanized bees have spread throughout the Yucatan peninsula. Only one sampling region had no African-derived RFLP patterns (Fig. 1A). Thirty percent of the colonies displayed African-derived mtDNA genotypes (Table 1), indicating that substantial maternal gene flow has occurred from expanding Africanized populations into the resident European honey bee population.

Morphological analyses also revealed substantial intrusion of African-derived genes into resident honey bee populations throughout the peninsula (Fig. 1B and Table 1). Both discriminant analyses suggested consistent decreases in Africanization from south to north and from east to west, confirming reports (*18*) that Africanized bees first entered the peninsula near Chetumal. By the DF-AE procedure, 40 (25%) of the colonies were classified as Africanized. However, probabilities of group membership were usually lower than 0.9 for these Africanized colonies (Fig. 2), indicating that many of them were more nearly intermediate between the two groups (or different from both groups) rather than members of them. Confirming results were obtained by the DF-AEHF procedure. Although many colonies (78 or 48%) were classified as being most similar to the feral European population, 36 (22%) remained classified as Africanized or hybrid with the vast majority classified as hybrid (34 or 21%). Thus, both analyses indicate that while African-derived genes are influencing the morphology of the bees of the Yucatan, very few colonies are morphologically Africanized to a high degree.

The distribution of mitochondrial genotypes across morphological classifications (Table 1 and Fig. 2) provides strong evidence that introgressive hybridization is a major component of the Africanization process in the Yucatan peninsula. Colonies displaying mtDNA forms in association with the "opposite" morphology are most likely derived through hybridization and then repeated backcrossing to males carrying alleles for morphological traits predominantly of the "opposite" population. African- and European-derived mtDNA were widely distributed across morphological classifications. By the DF-AE procedure, more colonies with African-derived mtDNA were morphologically European (57%) whereas fewer of the colonies with European-derived mtDNA were morphologically Africanized (17%) (Table 1) [*P* values from Fisher's exact test (*19*), $P = 0.0006$]. With the DF-AEHF procedure, mtDNA RFLPs were equally distributed among morphological classifications (Table 1) ($P = 0.16$). Interestingly, with the DF-AEHF procedure only two colonies were classified as Africanized and both had mtDNA of European origins. These and the other 69 colonies displaying extensive hybridization and backcrossing are evidence against the hypothesis of asymmetrical hybridization arising from sub-optimal interactions between mtDNA and nuclear DNA or other genetic incompatibilities. Although environment modifies morphological phenotypes of honey bees, its influence is quite small compared to that of genotype (*20*). It is unlikely that the range of morphological classifications from Africanized to European would be found with mtDNA of both types simply as a consequence of special environmental effects in the Yucatan peninsula. Selection is an equally poor explanation because the production of African-like European bees and European-like African bees in the same area simultaneously is improbable. Extensive hybridization due to the presence of a significant population of European bees is the most parsimonious explanation of the results.

The comparison of requeened colonies with those not requeened (Table 2) reveals additional characteristics of the Africanization process. First, the appearance of Afri-

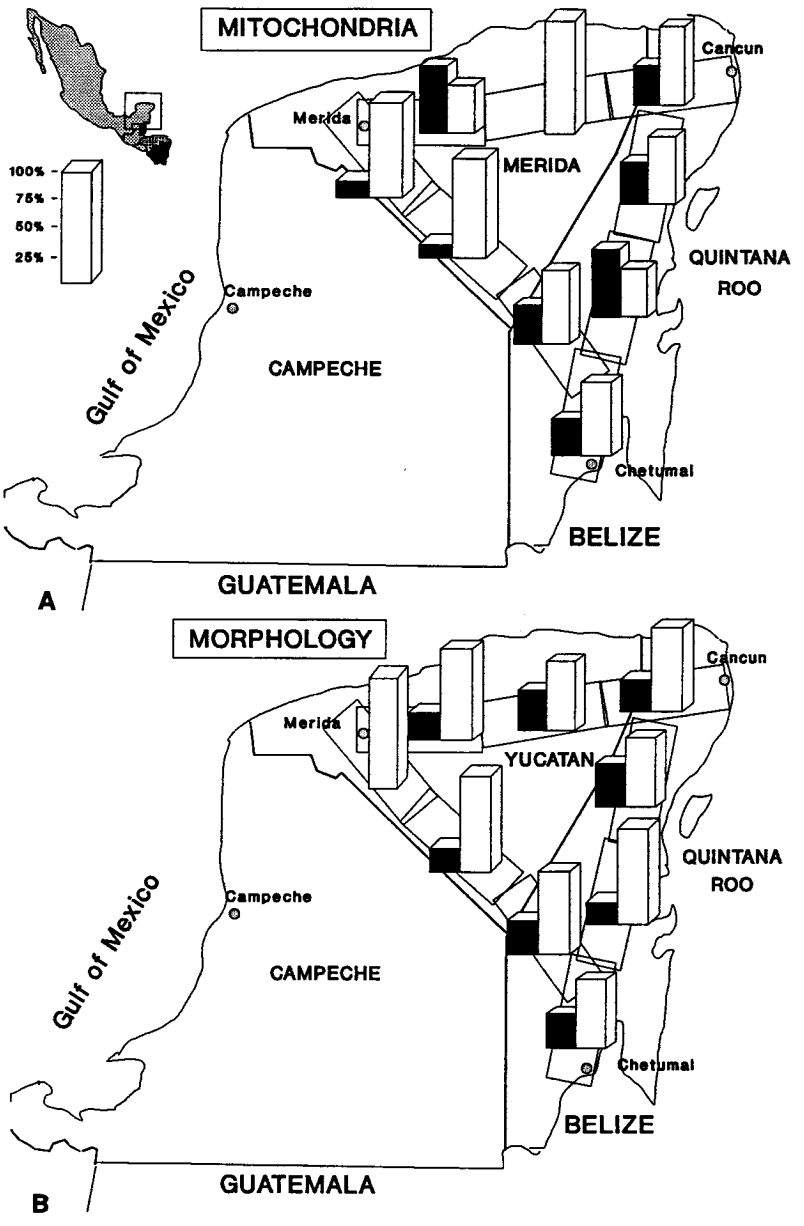

Fig. 1. Percentages of colonies from each of nine sampling regions across the Yucatan peninsula of Mexico that have African or Africanized (black bars) or European (white bars) (**A**) mitochondrial RFLP patterns (*16*) and (**B**) morphology according to the multivariate analysis DF-AE (*17*).

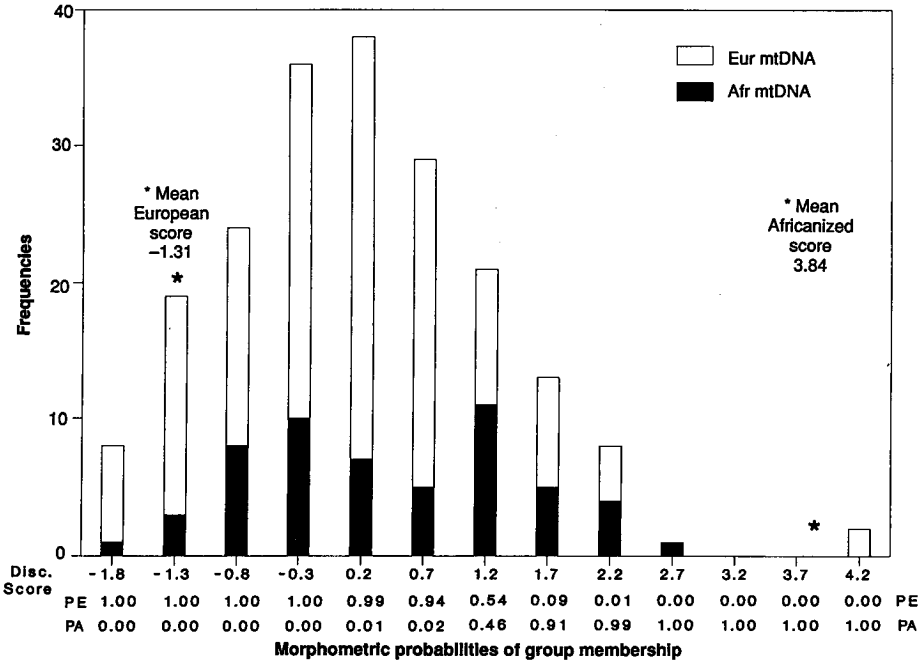

Fig. 2. The proportions of mtDNA RFLP forms found across the distribution of morphological variation of 163 colonies from the Yucatan peninsula of Mexico. Morphological classification is distributed as classes of discriminant scores according to DF-AE (*17*) and ranges from colonies that are typically European (discriminant score = −1.8) to those that are typically Africanized (discriminant score = 4.2). PE and PA values indicate the probability that a colony with the associated discriminant score is European or Africanized. Colonies having mtDNA patterns of African origin (black bars) and European origin (white bars) are both widely distributed across morphological classifications.

can-derived mitochondria in colonies established from European sources reflects the tendency of Africanized queens to invade European colonies and replace their queens (queen usurpation) (*12*). The requeened apiaries had fewer colonies with African-derived mtDNA evidencing queen usurpation ($P = 0.0003$). Second, when usurped colonies replace their queens with daughter queens, mating with the resident European drone population produces colonies having African-derived mtDNA and hybrid morphology. Additional generations of backcrossing produce colonies with bees having African-derived mtDNA and European morphology. Both types of apiaries have examples of such colonies. Third, natural queen replacement in colonies with European-derived mtDNA may result in hybridization through mating with Africanized drones (*21*). Requeened and non-requeened apiaries have similar numbers of such colonies ($P = 0.77$).

Thus, asymmetrical hybridization of honey bee populations in the neotropics can be explained primarily by a lack of breeding populations of European bees (*13*) and secondarily by usurpation of European colonies by Africanized bees (*14*). Future gene flow will probably further change Yucatecan honey bees. However, the extensive hybridization of the honey bee populations of the Yucatan suggests an optimistic outcome for U.S. agriculture. Areas having large populations of European bees such as subtropical northeast Mexico and southern Texas (*22*) are likely to show a "Europeanization" of expanding Africanized bee populations. "Europeanization" would be further enhanced through the genetic management tools of requeening and the production of European drones by beekeepers in their apiaries. This hybridization will presumably produce bees more desirable for commercial applications, more amenable to selection because of wider genic variance, and less likely to cause public health problems.

Table 2. Mitochondrial (A, Africanized; E, European) and morphological classification (E, European; H, hybrid similar to F_1 colonies; and F, feral European bees common to central and northeastern Mexico before Africanization) according to DF-AEHF (*18*) for colonies requeened within a year with mated European queens from a non-Africanized area (requeened) and for colonies not requeened.

Mitochondrial classification	Colonies (*n*) by morphological classification		
	E	F	H
Requeened			
A	1	1	0
E	11	11	4
Not requeened			
A	2	7	5
E	3	15	2

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16. Evaluating the morphology of Yucatecan bees required developing new discriminant functions by standard methods (*15, 17*). We included samples from 460 feral European colonies collected from central Mexico, northeastern Mexico and southern Texas before Africanization with previous data (*17*) from European, Africanized, and F_1 colonies to produce the new functions. Thirty-six rustic Yucatecan colonies collected in 1985 before the arrival of Africanized bees were analyzed. These analyses assessed the new multivariate discriminant analysis procedures with an independent sample from the study area and also provided a pre-Africanized baseline of Yucatecan honey bees. These analyses demonstrated that the small European bees of the Yucatan may be occasionally misclassified by DF-AE but almost never by DF-AEHF. DF-AE misclassified one colony as Africanized. DF-AEHF correctly classified all colonies as European or feral European at probabilities of group membership greater than 0.91. The DF-AEHF functions are available from T.E.R.
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