Subspecific hybridization between populations of *Apis mellifera* in the neotropics

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Introduction

Africanized honey bees (*Apis mellifera*) now range from central Argentina to southern Texas in the United States. They will soon confront United States agriculturalists with their notorious abilities to disrupt both beekeeping and crop pollination (Danka *et al.* 1987, Michener 1975, Taylor 1985, Rinderer 1986) and the general public with a health nuisance (Taylor 1986).

Whether or not Africanized bees are essentially "African" bees that remain undiluted by hybridization with European bees has been controversial (Smith *et al.* 1989, Hall and Muralidharan 1989, Hall 1990, Sheppard *et al.* 1991, Rinderer *et al.* 1991). Africanized bees in Argentina hybridize extensively with European bees in a temperate climate (Sheppard *et al.* 1991). There, mtDNA restriction fragment length polymorphisms (RFLPs) from both African and European origins are associated with a full range of morphological and allozyme phenotypes. In the neotropics, populations of Africanized bees still show hybridization but to a lesser degree for morphology (Buco *et al.* 1987, Sheppard *et al.* 1991a,1991b) isozymes (Lobo *et al.* 1989, Sheppard *et al.* 1991a; 1991b), and nuclear DNA RFLPs (Hall 1990). European-derived mtDNA RFLPs are rare in some populations (Smith *et al.* 1989, Hall and Muralidharan 1989, Sheppard *et al.* 1991b). One interpretation is that although interbreeding occurs, hybrids may be disadvantaged by maladaptive genes from European parents, or genetic incompatibilities, (Smith *et al.* 1989, Hall and Muralidharan 1989, Hall 1990). The origins of African-like characteris-

tics in these neotropical populations are central to both understanding the Africanization process and developing optimal recommendations to mitigate problems caused by Africanized bees.

Some answers to these questions can be found in a study of a population of honey bees undergoing Africanization in the neotropical Yucatan peninsula of Mexico (Rinderer *et al.* 1991). The peninsula has the largest concentration of commercial colonies of honey bees in the world (about 17 colonies/km^2) (Labougle and Zozaya 1986). 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 parentage of neotropical Africanized populations. These populations may arise: i) from limitations to hybridization producing essentially "pure" African bees, possibly caused by mitochondrial-nuclear or other genetic incompatibilities (Smith *et al.* 1989, Hall and Muralidharan 1989, Hall 1990), ii) from colony usurpation by Africanized but not by European queens (Vergara 1992), or iii) from population size advantages for African-derived bees (Page 1989).

Materials and Methods

Colonies were surveyed along transects through the peninsula two years after the area was first reported to contain Africanized honey bees (Figure 1). The transects were divided into 9 equal-sized sampling regions. Each area was represented among samples taken from 163 colonies. Colonies were sampled by collecting 20-30 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. In effect, the area's beekeepers are providing nesting sites to a genetically feral population of honey bees. Queens and drones that fly from boxes owned by beekeepers and mate cannot be expected to detect and avoid mating with queens and drones that fly from "feral" nesting sites. The critical criterion for a colony to be genetically "feral" is whether or not its contribution to an area's gene frequencies are skewed by beekeepers changing queens.

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 then 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 (Maniatis *et al.* 1982). Two restriction patterns resulting from digestion with *Eco*RI are associated with sub-saharan *A. mellifera* and two patterns with European sub-species (Smith *et al.* 1989, Sheppard *et al.* 1991). The samples were also measured morphologically (Daly *et al.* 1978, 1982) to produce 25 length and angle measures of the wings, hind legs and sternites of 10 bees/colony. Morphological data were analyzed by two multivariate discriminate analysis procedures developed for the classification of bees according to their morphological similarities to reference populations. The first procedure (Daly *et al.* 1978; 1982) (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. Samples from 460 feral European colonies collected from central Mexico, north-eastern Mexico and southern Texas prior to Africanization were included with previous data (Rinderer *et al.* 1990) from European, Africanized and F_1 colonies to produce the new functions. Thirty-six rustic Yucatecan colonies collected in 1985 prior to 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 as Africanized 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.

Results and Discussion

Mitochondrial RFLP analysis revealed that Africanized bees have spread throughout the Yucatan peninsula. Only one sampling region had no African-derived RFLP patterns (Figure 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 (Figure 1b,

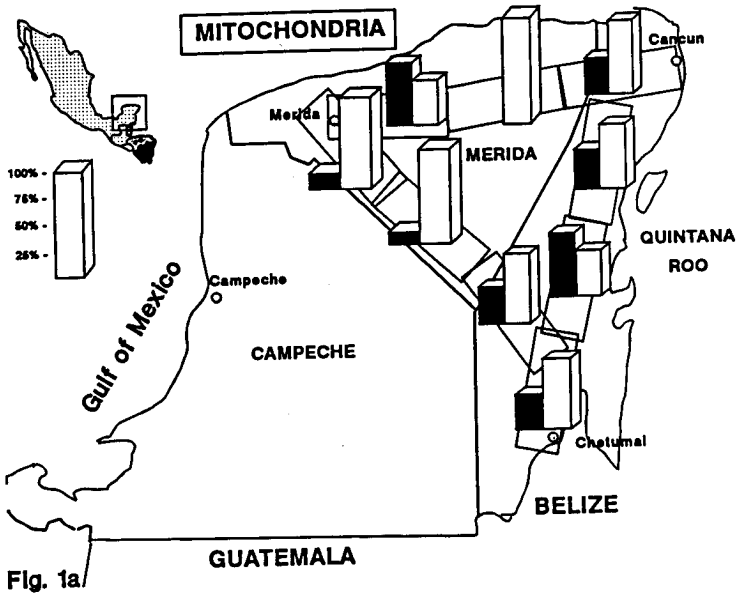

Fig. 1a

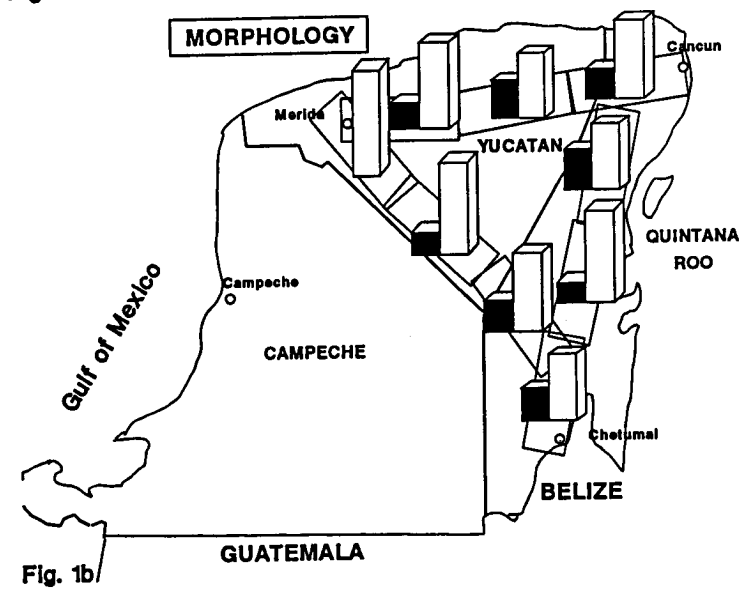

Fig. 1b

Figure 1. Percentages of colonies from each of 9 sampling regions across the Yucatan peninsula of Mexico that have African(ized) (black bars) or European (white bars) mitochondrial RFLP patterns following digestion with EcoR I (1a) and morphology according to the multivariate analysis DF-AE (1b).

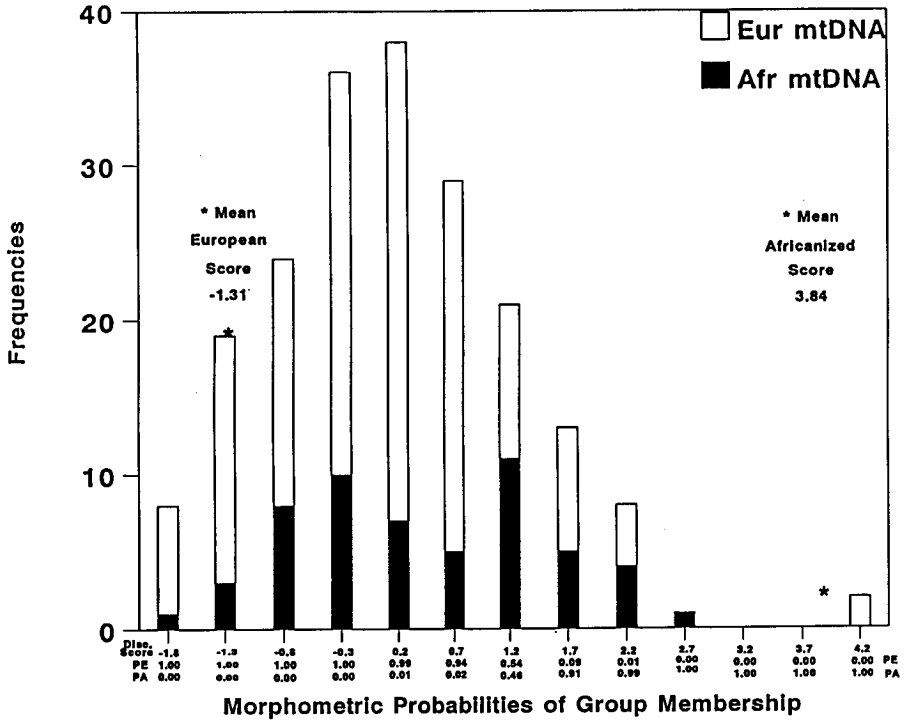

Figure 2. The proportions of mtDNA RFLP forms found across the distribution of morphological variation of 163 colonies from the Yucatan peninsula of Mexico. Morphological classification is distributed as classes of discriminant scores according to DF-AE and ranges from colonies that are typically European (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.

Table 1). Both discriminant analyses suggested consistent decreases in Africanization from south to north and from east to west, confirming reports (R. Iwamoto, Personal Communication) that Africanized bees first entered the peninsula near Chetumal. DF-AE classified 40 (25%) of the colonies as Africanized. However, probabilities of group membership were usually lower than 0.9 for these Africanized colonies (Figure 2), indicating that many of them were more nearly interme-

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 north-eastern Mexico prior to Africanization) of honey bee colonies of the Mexican Yucatan.

Discriminant Function	Mitochondrial Classification	Numbers (and row percentages) of Colonies Morphological Classification			
		A	E	H	F
DF-AE (Daly and Balling 1978)	A	21(42.9)	28(57.1)		
	E	19(16.7)	95(83.3)		
DF-AEHF (This study)	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)

diate between the two groups (or different from both groups) rather than members of them. Confirming results were obtained using DF-AEHF. 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 continued immigration of Africanized colonies to the Yucatan from Guatemala and Belize, can be expected to cause the honey bees of the Yucatan to become more Africanized unless the beekeepers provide a counter "immigration" of European genes through requeening efforts.

The distribution of mitochondrial genotypes across morphological classifications (Table 1, Figure 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

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 north-eastern Mexico prior to 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.

Colony Type	Mitochondrial Classification	Numbers of Colonies: Morphological Classification E	F	H
Requeened	A	1	1	0
	E	11	11	4
Not Requeened	A	2	7	5
	E	3	15	2

across morphological classifications. Using DF-AE, more colonies having African-derived mtDNA were morphologically European (57%) while fewer of the colonies having European-derived mtDNA were morphologically Africanized (17%) (Table 1, P=0.0006, Fisher's Exact Test (FET) (Metha and Patel 1983). When using DF-AEHF, mtDNA RFLPs were equally distributed among morphological classifications (Table 1, FET, P=0.16). Interestingly, DF-AEHF only classified two colonies as Africanized and both had mtDNA of European origins. These and the other 69 colonies displaying extensive hybridization and backcrossing are irrefutable evidence against the hypothesis of asymmetrical hybridization arising from sub-optimal interactions between mt- 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 (Daly *et al.* 1988, Herbert *et al.* 1988, Rinderer *et al.* 1986, Ruttner *et al.* 1978, Oldroyd *et al.* 1991). 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 since 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 three additional characteristics of the Africanization process. First, the appearance of African-derived mitochondria in colonies established from European sources may reflect the tendency of Africanized queens to invade European colonies and replace their queens (queen usurpation). Vergara 1992 found that about 10 percent of 500 colonies in central Mexico had been subject to queen usurpation in 18 months. In our study, the requeened apiaries had fewer colonies with African-derived mtDNA evidencing queen usurpation (FET, P=0.0003) than did the apiaries that had colonies which had not been requeened.

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 mt-DNA and European morphology. Both types of apiaries have examples of such colonies. Of course, beekeepers may contribute to the Africanization of their apiaries by using captured Africanized swarms, which will behave genetically like colonies that have been subject to usurping queens.

Third, natural queen replacement in colonies with European-derived mtDNA may result in hybridization through mating with Africanized drones (Rinderer *et al.* 1985, Rinderer *et al.* 1987). Requeened and non-requeened apiaries have similar numbers of such colonies (FET, P=0.77).

After our study was completed, Moritz and Meusel (1992) conducted a survey of the feral honey bees of the Sao Paulo valley in Brazil, in areas near the original point of release for African bees in 1956. They found apparently stable populations that showed clear hybridization. Morphological measurements indicated bi-racial origins for the Africanized bees of Brazil. They also found that 17% of the colonies had European-derived mtDNA. Thus, due to small sample sizes or some other cause, the first surveys of the mitochondrial DNA (Smith *et al.* 1989, Hall and Muralidharan 1989) seem to have underestimated the frequency of European mtDNA in neotropical Africanized populations. In addition to their field study, Moritz and Meusel (1992) modeled the effects of the intensive swarming of Africanized bees and conducted a computer simulation of them for several generations. The population size advantages gained by Africanized bees through frequent swarming accounted for the comparatively low frequencies of European

mtDNA. It appears that both these effects and selection of tropically adapted traits in tropical areas from an initial hybrid population (in unknown but probably African skewed proportions) lead to the detectable but reduced levels of European morphology and isozymes in Africanized bees.

Thus, asymmetrical hybridization of honey bee populations in some areas of the neotropics can be explained primarily by a lack of breeding populations of European bees (Page 1989) and secondarily by usurpation of European colonies by Africanized bees (Danka and Rinderer 1988, Rinderer 1986, Vergara 1992). Future gene flow resulting from continual immigration of Africanized bees, perhaps requeening efforts, and selection 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 northeastern Mexico and southern Texas (Rubink *et al.* 1990) 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 less undesirable for commercial applications, more amenable to selection due to wider genic variance, and less likely to cause public health problems. Although this study was done in North America with *Apis mellifera*, it provides a message for Asian beekeeping. As in *A. mellifera, A. cerana* has great variation across its species range. Some ecotypes or subspecies are clearly more suitable to beekeeping than others. More commercial ecotypes can be expected to be imported and used in apiculture in areas thought to have less commercially desirable ecotypes. Hybridization will predictably alter local gene pools. This prediction probably is not a good reason to prohibit importations. It is a good reason to establish reserves for the maintenance of local ecotypes that may one day prove to be valuable.

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Acknowledgment

In cooperation with Louisiana Agricultural Experiment Station.

Abstract

A population genetic analysis of honey bees of the Mexican neotropical Yucatan peninsula shows that the range expansion of Africanized bees has involved extensive introgressive hybridization with European bees. Yucatan honey bee populations now include many colonies having 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.

Key Words:

Apis mellifera, Africanized honey bees, European honey bees, hybridization, colony replacement, South America, Central America, Mexico, Yucatan peninsula, mitochondria DNA, restriction fragment length polymorphisms, United States.

Tom Rinderer was the program chair of the First Symposium on Asian Honey Bees and Bee Mites because of his interests in biodiversity of honey bees and germplasm preservation. Ben Oldroyd is a honey bee population geneticist and honey bee behaviorist from Australia. He was a visiting scientist at the USDA Baton Rouge laboratory from 1990 to 1992. Steve Buco is a statistical consultant who has worked on various projects with the Baton Rouge USDA staff on the morphological assessment of honey bee populations.