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THE AFRICANIZATION OF HONEYBEES (*APIS MELLIFERA* L.) OF THE YUCATAN: A STUDY OF A MASSIVE HYBRIDIZATION EVENT ACROSS TIME

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Abstract.—Until recently, African and European subspecies of the honeybee (*Apis mellifera* L.) had been geographically separated for around 10,000 years. However, human-assisted introductions have caused the mixing of large populations of African and European subspecies in South and Central America, permitting an unprecedented opportunity to study a large-scale hybridization event using molecular analyses. We obtained reference populations from Europe, Africa, and South America and used these to provide baseline information for a microsatellite and mitochondrial analysis of the process of Africanization of the bees of the Yucatan Peninsula, Mexico. The genetic structure of the Yucatecan population has changed dramatically over time. The pre-Africanized Yucatecan population (1985) comprised bees that were most similar to samples from southeastern Europe and northern and western Europe. Three years after the arrival of Africanized bees (1989), substantial paternal gene flow had occurred from feral Africanized drones into the resident European population, but maternal gene flow from the invading Africanized population into the local population was negligible. However by 1998, there was a radical shift with both African nuclear alleles (65%) and African-derived mitochondria (61%) dominating the genomes of domestic colonies. We suggest that although European mitochondria may eventually be driven to extinction in the feral population, stable introgression of European nuclear alleles has occurred.

Key words.—Africanized bee, *Apis mellifera*, gene flow, honeybee, hybridization, microsatellites.

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Geographic isolation can lead to genetic differentiation of populations due to local selection and genetic drift, and this may eventually result in the development of groups that we recognize as subspecies. If such subspecies are brought together again in a new environment, the degree of genetic differentiation that has occurred while the subspecies were separated is crucial in determining whether hybridization is likely to take place. If substantial divergence has occurred, impediments to gene flow (e.g., divergent mating times or failure to recognize mates) are expected. If impediments to gene flow are asymmetrical between sexes, cytonuclear disequilibrium will develop (Arnold 1993). If subspecies or their hybrids have fitness differences in the new environment, competition between the two genotypes is expected to increase the frequency of one genotype relative to the other, or a hybrid zone may develop (Hewitt 1988). If there are no barriers to gene flow and no fitness differences of hybrids or their alleles, the linkage disequilibria of alleles strongly associated with the original populations is expected initially, but these are expected to break down rapidly over time (Falconer 1981).

Honeybees provide a good model system to obtain insights into processes of speciation and hybridization, because of their very great biodiversity and because their economic significance means that this biodiversity has been broadly sampled. In their natural range (across Europe, the Middle East, and Africa), more than 26 distinct subspecies have been named (Ruttner 1988), each adapted to a particular set of climatic and ecological conditions. The various honeybee subspecies can be assigned to four distinct evolutionary branches on the basis of morphology (Ruttner et al. 1978; Ruttner 1988) and molecular analyses (Garnery et al. 1992, 1993; Estoup et al. 1995; Arias and Sheppard 1996; Franck et al. 2000b). Subspecies from southeastern Europe (e.g., Italy, Yugoslavia, Austria) are classified as branch C; subspecies from northern and western Europe (e.g., France, Spain, Portugal) are classified as branch M; and subspecies from Africa are classified as branch A. The existence of branch O in the Middle East was recently confirmed by molecular analyses (Franck et al. 2000b; Palmer et al. 2000).

Extensive human-assisted migrations of some of these subspecies (predominantly European) has occurred since the 17th century in conjunction with European settlement of the New World (Cornuet 1986) and early in the 19th century in conjunction with settlement of Australia (Ruttner 1976; Oldroyd et al. 1992). This migration has resulted in a much broader modern distribution of the subspecies *Apis mellifera ligustica*, *A. m. carnica*, *A. m. caucasica*, *A. m. mellifera*, and *A. m. iberica* (Rothenbuhler 1979). These temperate subspecies are amenable to management for honey production and the pollination of crops.

In some parts of the New World the temperate subspecies of honeybee do not thrive, even several hundred years after their first introduction. In tropical southern and central South America, European subspecies can be maintained in apiaries (although honey production is poor) but do not exist as feral colonies (Kerr 1967; Rinderer et al. 1993). In an attempt to establish a population of honeybees that were better equipped for a tropical environment but still amenable to beekeeping, an African subspecies (*A. m. scutellata*) was introduced to Brazil in 1956 for experimental crossbreeding with European queens (Kerr 1967). Bees arising from this introduction led to the development of a vast population of feral Africanized bees that have subsequently migrated throughout South and Central America and into the southern United States at a rate of 100–300 km annually (Taylor 1977).

African and European subspecies of the honeybee have been geographically separated for around 10,000 years (Ruttner 1986). Striking differences exist between temperate and tropical subspecies in terms of their development, morphology, and behavior. Shorter developmental times (Winston 1991) and higher reproductive rates (Fletcher 1991) are characteristic of tropical subspecies, and these traits can lead to large population densities. Frequent swarming and absconding by African-derived bees (Fletcher 1991; Otis 1991) and their less exacting requirements for nesting sites compared with European-derived subspecies (Winston et al. 1981) allow very rapid dispersal of African-derived populations. African-derived bees are more prolific in drone production (Winston 1988), and workers forage at an earlier age (Winston 1988). The most publicized difference is that of defensive behavior. African-derived bees are up to six times as defensive (i.e., aggressive) as European-derived bees (Stort and Goncalves 1991).

The relative contributions of African and European subspecies to the gene pool of Africanized populations and the underlying mechanism for dispersal of these populations have been the subject of much controversy. In Neotropical regions of South America some populations are comprised nearly entirely of African-derived mitochondria (Hall and Muralidharan 1989; Smith et al. 1989). In some studies, however, markers of the nuclear genome have shown considerable hybridization (Lobo et al. 1989; Rinderer et al. 1991; Quezada-Euán 2000), whereas other studies have reported limited gene flow of European alleles into an essentially Africanized genome (Hall 1990; Muralidharan and Hall 1990; McMichael and Hall 1996; Suazo et al. 1998).

Hypotheses to explain the paucity of European mitochondria found in Africanized populations include subspecific differences in reproductive rates and other fitness parameters in the tropics (Rinderer 1988; Taylor 1988), large differences in colony densities (Rinderer et al. 1991), the possibility of inappropriate assumptions about fixation of mitochondrial DNA markers in Old World populations of bees (Schiff et al. 1994), and asymmetrical fitness of hybrids with European or African mitochondria (Harrison and Hall 1993).

A hybrid zone was found in Argentina that correlated with a change in environment (Sheppard et al. 1991). Parental genotypes were associated with ecotypes resembling those in which they evolved. Africanized bees are present in the tropical north of the country and European bees are found in the temperate south. In the transition zone, European-derived and African-derived mitochondria are associated with a range of morphological and allozymic phenotypes. The hybrid zone appears to be maintained by differences in climatic conditions, but there remains the possibility that hybrids are disadvantaged by selection.

Until the late 1980s, the Neotropical Yucatan Peninsula of Mexico contained an extremely high density (estimated at 17 colonies/km^2, Quezada-Euán and Hinsull 1995; Quezada-Euán et al. 1996) of managed colonies. These colonies were primarily derived from southeastern European subspecies (*A. m. ligustica* and *A. m. caucasica*) and smaller numbers of northern and western subspecies (*A. m. mellifera* and *A. m. iberica*; Clarke et al. 2001). The arrival of Africanized bees in 1986 (Fierro et al. 1987) provided an exceptional opportunity to study the population biology of a large-scale hybridization event between subspecies belonging to three distinct evolutionary branches. Prior to the arrival of Africanized bees in eastern Mexico, the northward-expanding population of Africanized bees had only encountered a tiny managed European population—they had been expanding into territory in which feralhoney bees were virtually absent (Roubik and Wolda 2001). Rinderer et al. (1991) showed that hybridization was occurring between European and Africanized bees in the Yucatan in 1989, and the level of hybridization appears to have increased with time (Quezada-Euán 2000). However, the possibility remained that late-generation hybrids were at a selective disadvantage (Hall 1991) and that over time Africanized genotypes would replace European ones.

This study set out to examine the extent of admixture between European and African subspecies over time and the processes involved in the Africanization of Yucatecan populations.

Materials and Methods

We wished to examine changes in the genetic architecture of the Yucatecan population over time. To analyze the process of Africanization of bees of the Yucatan Peninsula, it is necessary to have baseline information about the populations from which New World populations were derived and the populations likely to have been the direct ancestors of Yucatecan populations. To this end we obtained reference samples from Old World and New World populations (Clarke et al. 2001).

Samples from Italy (*A. m. ligustica*) and Yugoslavia (*A. m. carnica*) are of the C branch; samples from France (*A. m. mellifera*) Spain and Portugal (*A. m. iberica*) are of the M branch; and samples from South Africa (*A. m. scutellata*) and along the Cape of South Africa (*A. m. capensis*) are of the A branch (e.g., Ruttner 1988; Garnery et al. 1992; Estoup et al. 1995; Franck et al. 1998). New World reference populations included a pre-Africanized sample from the Yucatan Peninsula and an Africanized population from Venezuela. We hypothesized that our 1985 collection from the Yucatan Peninsula was representative of the population at that time. This population was then invaded by Africanized bees, which we assumed were similar to the bees we sampled in Venezuela in 1990. We characterized these two New World reference populations by comparing them to reference Old World populations.

Sampling Design

In May 1998, we retraced the 10 transects through the Yucatan and Quintana Roo states of the Yucatan Peninsula chosen by Rinderer et al. (1991) in 1989. Collections of adult worker bees and drones were made at four to 10 apiaries within each transect with approximately 10 colonies per apiary. The 1998 collection comprises 530 colonies, of which 31 were feral (i.e., not in a managed hive). On average, 30 bees were taken from the brood comb of managed hives or sweep netted from the entrance of feral hives. Samples were stored in absolute ethanol in plastic scintillation vials and transported back to the laboratory, where they were stored at −20°C. Samples were also obtained from T. E. Rinderer's extensive collection of *A. mellifera* from sites in Europe, Africa, and South America and from J. J. G. Quezada-Euán's collection from the Yucatan (Clarke et al. 2001).

Microsatellite Analysis

Total DNA was extracted from a hind leg of one bee per colony using a 5% Chelex solution (Walsh et al. 1991). DNA extracts were diluted 1:4 with distilled water. Six microsatellite loci (A8, A24, A113, A28, A88, A43; for primer sequences see Estoup et al. 1995; Franck et al. 1998) were scored for 921 individuals, each representing a distinct colony. DNA amplifications were carried out as described by Estoup et al. (1995), with minor modifications. The 10-μl reactions contained 2 μl of Chelex-extracted DNA as template, 1× reaction buffer, 20 μM of each dNTP, 1.2–1.7 mM $MgCl_2$, 400 nM of each primer, and 0.4 units of *Taq* DNA polymerase (Sigma, St. Louis, MO). Polymerase chain reaction (PCR) conditions consisted of one cycle of 3 min at 94°C; 35 cycles of 30 sec at 94°C, 30 sec at 53–58°C, 30 sec at 72°C; and one cycle of 10 min at 72°C. Reverse primers were fluorescently labeled with HEX (Gibco BRL, Bethesda, MD) to enable visualization of amplified fragments during electrophoresis. Samples were electrophoresed in a 6% denaturing polyacrylamide gel (for 25 ml: 10.5 g urea, 3 ml 5× TBE, 3.75 ml 40% acrylamide/bis (Astral Scientific, Gymea, Australia), 12.5 μl TEMED, 125 μl 10% APS), run on an automated DNA fragment analyzer (Corbett Research, Sydney) at 1200 V and 40°C, and analyzed using the software Onedscan (Scanalytics, Billerica, MA).

Statistical Analysis of Microsatellite Data

Unbiased estimates of gene diversity and their standard deviations were calculated according to Nei (1978). Exact tests for Hardy-Weinberg equilibrium, genotypic linkage disequilibrium, and population differentiation (genic and genotypic) were computed using GENEPOP (ver. 3.2; Raymond and Rousset 1995). We constructed a neighbor-joining tree based on Cavalli-Sforza and Edwards's (1967) chord distance, as described by Estoup et al. (1995). Bootstrap values were computed over 2000 replications (Hedges 1992) by resampling loci and individuals within populations.

The proportions of introgressed nuclear alleles from sources representing each of the evolutionary branches were estimated in populations from the New World using a program written by J.-M. Cornuet (unpubl. program). This program estimates the admixture proportion using a maximum-likelihood approach, inferring that the allele frequencies in the admixed population are a linear combination of the allele frequencies in the source populations (Franck et al. 2000a).

Individuals from the two New World reference populations (Yucatan 1985 and Venezuela 1990) were probabilistically assigned as either European (C, M branches) or African (A branch) according to their nuclear genotype for six microsatellite loci, using a model-based clustering method, computed using STRUCTURE (Pritchard et al. 2000).

The pre-Africanized (Y85) sample and the Venezuelan (Ven) sample were then used as reference populations to classify individuals of the Yucatan Peninsula over time. Africanized bees entered the Yucatan via Venezuela (Fierro et al. 1987), so presumably these bees and/or the pre-Africanized bees reflect the genetic make-up of subsequent Yucatecan populations (assuming, we think reasonably, that any human-assisted importations of bees had a minor contribution to the population).

An individual was classified as closer to the appropriate reference population (Y85 or Ven) if the parameter *Q* (the admixture proportions for each individual) was equal to or higher than 0.9, as calculated by the program STRUCTURE (Pritchard et al. 2000). Individuals with values of *Q* less than 0.9 were considered to be a hybrid of the two reference populations. To measure any cytonuclear disequilibrium within New World populations, we examined the associations of each individual's nuclear genotype with a mitochondrial *Dra*-I RFLP haplotype (Garnery et al. 1993), using the data of Clarke et al. (2001). Fisher exact tests were computed using a Monte Carlo sampling approach (Lewontin and Felsenstein 1965) to test the null hypothesis of random associations between nuclear and mitochondrial genotypes. Widespread nonrandom associations indicate a recent hybridization event or barriers to hybridization.

The *Dra*-I RFLP marker (Garnery et al. 1993; Franck et al. 1998) encompasses the intergenic region of the COI–COII genes and includes a size polymorphism due to the presence or absence of various insertions and deletions and variable arrangements of two sequence elements designated P and Q (Q for lineage C; PQ, PQQ, PQQQ, and PQQQQ for lineage M; P_oQ, P_oQQ, P_oQQQ, and P_oQQQQ for lineage A; P_oQ, P_oQQ, and P_oQQQ for lineage O; P_oQQ for a postulated lineage Y). The arrangement of these elements generates restriction fragment length polymorphisms for the enzyme *Dra*-I. Based on the restriction of the variable lengthed PCR product, the lineages are readily distinguishable. In all, 59 mitochondrial haplotypes have been described from various honeybee populations (Garnery et al. 1993, 1995, 1998; de la Rua et al. 1998; Franck et al. 1998, 2000a,b). Three of these belong to the C lineage, 25 to the M lineage, 24 to the A lineage, five to the O lineage, and two to the proposed Y lineage (formerly these were assigned to the A lineage). Many of these haplotypes differ from each other by either a small insertion or deletion and cannot be detected unless the PCR product is sequenced. However, to identify honeybees as being of European or African origin, it is not necessary to have such precision, so some of the subtleties of 22 of these haplotypes can be ignored.

Use of the *Dra*-I test for testing the origin of honeybees

TABLE 1. Allele frequencies, proportion of heterozygotes (H_p), and gene diversities (H_d) of populations from Europe, Africa, and South America. *n* refers to the number of alleles sampled in a population at each locus.

Locus A28	Allele (bp)	Italy (*n* = 142)	Yugoslavia (*n* = 54)	France (*n* = 38)	Spain (*n* = 172)	Portugal (*n* = 46)	South Africa (*n* = 106)	SA Cape (*n* = 34)	Venezuela (*n* = 64)	Yuc1985 (*n* = 50)	Yuc1989 (*n* = 384)	Yuc1998 (*n* = 644)	Feral (*n* = 62)
	124						0.019		0.031			0.019	0.016
	127				0.006		0.189	0.118	0.141	0.02	0.036	0.16	0.242
	129						0.038					0.011	
	130						0.028	0.118	0.078		0.042	0.068	0.032
	131						0.066	0.088	0.016		0.026	0.127	0.161
	132	0.007		0.921	0.948	0.957	0.047	0.147	0.266	0.34	0.302	0.16	0.129
	133						0.057	0.029				0.002	
	134		0.093		0.006		0.028		0.016	0.02	0.01	0.003	0.016
	135						0.217	0.118	0.297		0.057	0.216	0.242
	136						0.047	0.029	0.031		0.005	0.033	0.032
	137						0.009						0.016
	138	0.944	0.907			0.022	0.113	0.294	0.016	0.62	0.505	0.124	0.081
	139						0.009					0.002	
	140						0.057	0.029				0.039	
	141	0.028					0.066	0.029	0.078		0.016	0.034	0.032
	143						0.009						
	144	0.021		0.079	0.041	0.022			0.016				
	148								0.016			0.003	
H_p		0.113	0.185	0.158	0.081	0.087	0.943	0.765	0.719	0.6	0.62	0.879	0.871
H_d		0.109	0.171	0.149	0.101	0.086	0.89	0.865	0.819	0.509	0.648	0.863	0.843
Locus A88	Allele (bp)	Italy (*n* = 162)	Yugoslavia (*n* = 54)	France (*n* = 34)	Spain (*n* = 174)	Portugal (*n* = 44)	South Africa (*n* = 100)	SA Cape (*n* = 34)	Venezuela (*n* = 52)	Yuc1985 (*n* = 50)	Yuc1989 (*n* = 292)	Yuc1998 (*n* = 594)	Feral (*n* = 38)
	138						0.01			0.04		0.015	0.026
	140											0.002	
	142						0.01		0.019			0.056	0.053
	143		0.185		0.006		0.05	0.059	0.135	0.14	0.055	0.01	
	144						0.16	0.088	0.115		0.051	0.091	0.053
	146	0.025	0.148	1	0.989	1	0.14	0.147	0.288	0.32	0.298	0.249	0.316
	147						0.02	0.088	0.019			0.007	
	148						0.06					0.02	
	149						0.01		0.019		0.017	0.029	0.026
	150				0.006		0.03	0.029	0.038		0.007	0.03	0.079
	152	0.074	0.093				0.07	0.059	0.019	0.06	0.034	0.099	0.079
	153						0.08	0.118				0.066	
	154						0.11		0.096	0.44	0.476	0.125	0.184
	155	0.833	0.556				0.17	0.265	0.038		0.007	0.104	0.053
	156	0.068	0.019						0.154		0.045	0.049	0.079
	157						0.04	0.147	0.019		0.01	0.034	0.053
	158						0.02		0.038			0.013	
	159						0.02					0.002	
H_p		0.272	0.63	0	0.023	0	0.782	0.824	0.846	0.64	0.651	0.865	0.789
H_d		0.297	0.638	0	0.023	0	0.901	0.875	0.863	0.693	0.678	0.881	0.858

TABLE 1. Continued.

Locus A24	Allele (bp)	Italy (n = 156)	Yugoslavia (n = 54)	France (n = 34)	Spain (n = 180)	Portugal (n = 46)	South Africa (n = 106)	SA Cape (n = 34)	Venezuela (n = 52)	Yuc1985 (n = 46)	Yuc1989 (n = 354)	Yuc1998 (n = 590)	Feral (n = 52)
	96											0.002	
	97						0.009		0.038		0.028	0.042	0.019
	98	0.167	0.019	0.912	0.922	1	0.377	0.176	0.462	0.391	0.376	0.375	0.365
	100						0.075					0.002	
	102						0.085	0.118	0.173		0.025	0.081	0.096
	103						0.009						
	104						0.085	0.471	0.115		0.023	0.11	0.058
	105						0.009					0.003	
	106	0.308	0.556	0.059	0.072		0.208	0.088	0.058	0.152	0.093	0.066	0.077
	108	0.526	0.426	0.029	0.006		0.113	0.147	0.154	0.457	0.438	0.308	0.385
	110										0.017	0.01	
	112						0.009						
	114						0.019						
H_p		0.551	0.444	0.118	0.156	0	0.868	0.824	0.731	0.652	0.661	0.725	0.692
H_d		0.605	0.519	0.169	0.145	0	0.788	0.725	0.729	0.629	0.658	0.741	0.713

Locus A8	Allele (bp)	Italy (n = 158)	Yugoslavia (n = 46)	France (n = 34)	Spain (n = 184)	Portugal (n = 48)	South Africa (n = 106)	SA Cape (n = 32)	Venezuela (n = 54)	Yuc1985 (n = 32)	Yuc1989 (n = 366)	Yuc1998 (n = 640)	Feral (n = 62)
	160						0.009						
	164	0.07	0.152	0.941	0.962	0.917			0.111	0.5	0.309	0.191	0.226
	166						0.113	0.063	0.074		0.022	0.052	0.048
	168						0.179	0.594	0.167		0.036	0.13	0.145
	170						0.198	0.094	0.352		0.071	0.272	0.258
	172	0.013	0.087		0.038	0.083	0.302	0.094	0.185		0.063	0.1	0.097
	173						0.009						
	174	0.006	0.065	0.059			0.16	0.125	0.019	0.094	0.038	0.037	0.048
	175		0.043									0.011	
	176	0.196	0.391				0.009		0.037	0.063	0.074	0.066	0.065
	178	0.487	0.043				0.019	0.031	0.019	0.219	0.208	0.07	0.065
	180	0.215	0.217						0.037	0.125	0.18	0.072	0.048
	182	0.013											
H_p		0.658	0.696	0.118	0.076	0.167	0.868	0.688	0.852	0.75	0.814	0.797	0.871
H_d		0.677	0.778	0.114	0.074	0.156	0.806	0.629	0.808	0.696	0.814	0.846	0.85

Locus A113	Allele (bp)	Italy (n = 160)	Yugoslavia (n = 50)	France (n = 38)	Spain (n = 180)	Portugal (n = 48)	South Africa (n = 98)	SA Cape (n = 34)	Venezuela (n = 50)	Yuc1985 (n = 36)	Yuc1989 (n = 368)	Yuc1998 (n = 640)	Feral (n = 62)
	202			0.053	0.044		0.02		0.02		0.122	0.077	0.081
	204				0.017		0.01						
	206						0.01				0.003		
	208					0.042	0.112	0.235	0.06		0.016	0.036	0.048
	210	0.006			0.006		0.071		0.02			0.006	
	212						0.143	0.294	0.06	0.028	0.019	0.061	0.048
	214	0.269	0.94		0.006		0.133	0.118	0.06	0.111	0.098	0.13	0.065
	216		0.02		0.011		0.122	0.029	0.34	0.056	0.073	0.219	0.258
	218			0.053	0.028	0.021	0.071	0.088			0.005	0.013	0.016
	220	0.675		0.789	0.639	0.458	0.112	0.118	0.2	0.694	0.533	0.302	0.323
	222	0.031		0.053	0.078	0.104	0.102	0.029	0.12	0.056	0.033	0.072	0.065
	224	0.006		0.026	0.111	0.292	0.061	0.059			0.027	0.033	0.081
	226				0.039	0.021	0.031		0.1		0.019	0.034	
	228	0.006	0.02	0.026	0.011	0.042				0.028	0.008		
	230		0.02			0.021		0.029	0.02		0.003		
	232				0.006						0.003		

TABLE 1. Continued.

Locus A113	Allele (bp)	Italy (n = 160)	Yugoslavia (n = 50)	France (n = 38)	Spain (n = 180)	Portugal (n = 48)	South Africa (n = 98)	SA Cape (n = 34)	Venezuela (n = 50)	Yuc1985 (n = 36)	Yuc1989 (n = 368)	Yuc1998 (n = 640)	Feral (n = 62)
	234										0.008	0.003	
	236									0.028	0.027	0.014	0.016
	238	0.006			0.006						0.003	0.002	
H_p		0.525	0.12	0.263	0.511	0.542	0.837	0.882	0.76	0.389	0.679	0.828	0.839
H_d		0.474	0.118	0.377	0.572	0.704	0.905	0.841	0.824	0.511	0.685	0.827	0.816
Locus A43	Allele (bp)	Italy (n = 126)	Yugoslavia (n = 52)	France (n = 38)	Spain (n = 186)	Portugal (n = 50)	South Africa (n = 104)	SA Cape (n = 34)	Venezuela (n = 62)	Yuc1985 (n = 50)	Yuc1989 (n = 368)	Yuc1998 (n = 648)	Feral (n = 62)
	122						0.01						
	126		0.25				0.115	0.176	0.065	0.02	0.057	0.136	0.065
	127	0.087	0.019							0.44	0.063	0.015	0.032
	128	0.333		1	0.876	0.82	0.067		0.242	0.04	0.402	0.284	0.306
	132						0.096	0.147	0.048		0.003	0.015	0.048
	133										0.003		
	134				0.016	0.06	0.067		0.048		0.008	0.039	0.032
	136						0.077		0.032		0.008	0.011	
	138						0.144	0.412	0.081	0.02	0.008	0.066	0.032
	140		0.673		0.097	0.1	0.048	0.206	0.194	0.18	0.068	0.12	0.161
	141	0.579							0.016	0.02	0.196	0.059	0.048
	142		0.058		0.011		0.096	0.029		0.28	0.133	0.025	0.065
	143											0.012	0.016
	144						0.067		0.065		0.005	0.046	0.048
	146						0.029		0.065		0.016	0.043	0.016
	148					0.02	0.144	0.029				0.005	
	150						0.029		0.145		0.03	0.122	0.129
	152											0.002	
	156						0.01						
H_p		0.619	0.346	0	0.226	0.24	0.923	0.706	0.742	0.72	0.793	0.796	0.839
H_d		0.55	0.49	0	0.223	0.32	0.912	0.756	0.872	0.707	0.772	0.858	0.858
		Italy	Yugoslavia	France	Spain	Portugal	South Africa	SA Cape	Venezuela	Yuc1985	Yuc1989	Yuc1998	Feral
Mean H_p‡		0.456 ± 0.216	0.404 ± 0.232	0.11 ± 0.1	0.179 ± 0.178	0.173 ± 0.203	0.877 ± 0.048	0.782 ± 0.075	0.775 ± 0.059	0.625 ± 0.128	0.703 ± 0.08	0.815 ± 0.056	0.817 ± 0.068
Mean H_d‡		0.452 ± 0.213	0.452 ± 0.26	0.135 ± 0.139	0.19 ± 0.2	0.211 ± 0.27	0.867 ± 0.055	0.782 ± 0.1	0.819 ± 0.051	0.624 ± 0.093	0.709 ± 0.068	0.836 ± 0.05	0.823 ± 0.056

‡ Values are means ± standard deviations.

TABLE 2. Cavalli-Sforza and Edwards's (1967) chord distances provided by BIOSYS-1 (above diagonal) and pairwise multilocus unbiased estimates of F_{ST} provided by FSTAT (below diagonal). Values that are not significantly different from zero are in italics.

	Ita	Yug	Fra	Spa	Por	SAf	Cap	Ven	Y85	Y89	Y98	Fer
Ita		0.510	0.680	0.690	0.708	0.684	0.733	0.658	0.422	0.386	0.572	0.570
Yug	0.097		0.799	0.767	0.786	0.652	0.678	0.664	0.472	0.532	0.609	0.618
Fra	0.566	0.664		0.164	0.233	0.713	0.751	0.615	0.556	0.533	0.604	0.589
Spa	0.624	0.711	*−0.008*		0.167	0.680	0.732	0.574	0.545	0.520	0.574	0.561
Por	0.568	0.638	0.013	*−0.003*		0.689	0.726	0.596	0.576	0.544	0.598	0.586
SAf	0.276	0.235	0.342	0.423	0.323		0.370	0.367	0.617	0.504	0.315	0.380
Cap	0.366	0.310	0.500	0.602	0.475	*0.023*		0.473	0.647	0.573	0.439	0.476
Ven	0.329	0.301	0.329	0.430	0.307	*0.024*	0.093		0.583	0.408	0.251	0.280
Y85	0.194	0.272	0.400	0.488	0.392	0.158	0.219	0.115		0.353	0.504	0.491
Y89	0.114	0.226	0.257	0.297	0.258	0.128	0.181	0.081	*0.001*		0.313	0.316
Y98	0.209	0.222	0.247	0.268	0.235	*0.032*	0.084	*0.006*	0.067	*0.037*		0.175
Fer	0.268	0.271	0.316	0.422	0.305	*0.037*	0.099	*0.000*	0.051	*0.023*	*−0.005*	

is potentially complicated by the presence of both African and European mitochondrial genomes in Spain and Portugal (Sheppard et al. 1999; Clarke et al. 2001). Within the subspecies *A. m. iberica*, honeybees can have either M or A haplotypes but are clearly of European origin at the nuclear level, suggesting frequent introgression of genes of African lineage into this area (Franck et al. 1998). Early samples (1985) from the Yucatan suggest that 5% of the pre-Africanized population had African mitochondria derived from Iberia (Clarke et al. 2001).

RESULTS

Population Structure among Reference Populations

Microsatellite allele frequencies for each population at six loci, the proportion of heterozygotes, and gene diversity estimates are given in Table 1.

Each Old World reference population (Italy, Yugoslavia, France, Spain, Portugal, South Africa, and the Cape of South Africa) was tested for departures from Hardy-Weinberg equilibrium. Within these populations significant departures from Hardy-Weinberg expectations were detected in four instances, but these involved four different loci in four different populations, indicating departures are due to Type I error, and are of no consequence. Across all loci, no population showed significant departures from Hardy-Weinberg proportions, indicating the populations were panmictic and that gel scoring was satisfactory, with no significant errors arising from null alleles. Across all loci, the pre-Africanized Yucatecan population was in Hardy-Weinberg equilibrium, but significant departure from Hardy-Weinberg proportions was detected in the population from Venezuela ($P = 0.03$).

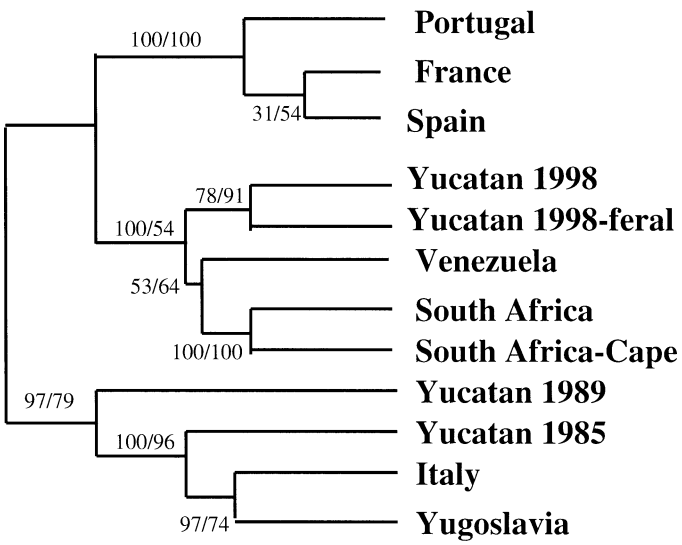

FIG. 1. Neighbor-joining tree based on the Cavalli-Sforza and Edwards's (1967) chord distance. Bootstrap values have been computed over 2000 replications by resampling individuals within populations (left values) and by resampling loci (right values) and are given as percentages.

Both Old World and New World (Yucatan 1985 and Venezuela 1990) reference populations were tested for linkage disequilibrium among the six microsatellite loci studied. Among the Old World reference populations, three instances of significant linkage disequilibrium were observed in 105 comparisons ($\alpha = 0.05$). However, after a sequential Bonferroni correction (Rice 1989), no two loci were in linkage disequilibrium in any of these populations, indicating long-term panmixia. Among New World reference populations, three cases of significant linkage disequilibrium were detected in 30 comparisons. Again, however, a sequential Bonferroni correction showed no cases of significant linkage disequilibrium, indicating the populations were panmictic.

Old World reference populations were tested to determine if they were genetically distinct. Pairwise Fisher's exact tests for genic and genotypic differentiation showed a high level of differentiation between each European and each African population ($P_{multilocus} < 10^{-4}$ for all sample pairs). F_{ST}-values between each European and each African population were significantly different from zero ($0.235 < F_{STmultilocus} < 0.602$; Table 2).

A neighbor-joining tree based on the chord distance of Cavalli-Sforza and Edwards (1967) showed that Old World populations are grouped by evolutionary lineage (Fig. 1). Populations from France, Spain, and Portugal are grouped, populations from South Africa and from along the Cape of South Africa are grouped, and populations from Italy and Yugoslavia form a third group (Fig. 1).

To understand changes in the genetic structure of the honeybees of the Yucatan Peninsula over time, we first determined the ancestry of the 1985 Yucatecan and Venezuelan populations by reference to the Old World populations. Fish-

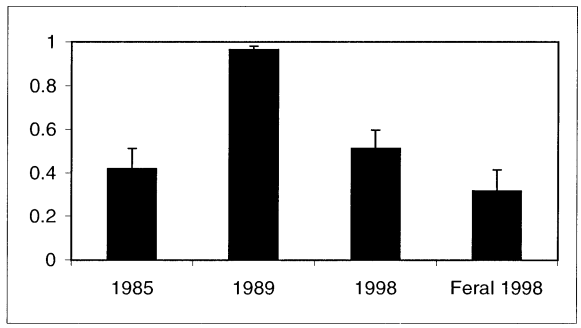

FIG. 2. A comparison of linkage disequilibrium among six microsatellite loci in the Yucatecan population across time. $1 - P$, where P is the mean value of Fisher exact tests for each locus pairs, and their standard errors are given for each population.

er's exact tests across all loci showed a high level of differentiation between the pre-Africanized Yucatecan population and all Old World reference populations ($P < 10^{-4}$ for genic and genotypic differentiation), and multilocus F_{ST}-values were significantly different from zero ($0.158 < F_{STmultilocus} < 0.488$; Table 2), but a neighbor-joining tree placed the population with populations from southeastern Europe (Fig. 1). The Africanized population from Venezuela showed a high level of differentiation from all Old World reference populations ($P < 10^{-4}$ for genic and genotypic differentiation), but the multilocus F_{ST}-value was not significantly different from zero between the Venezuelan population and the sample from South Africa ($F_{ST} = 0.024 \pm 0.035$; Table 2). A neighbor-joining tree also places the Venezuelan population with populations from South Africa (Fig. 1). These results strongly indicate that the pre-Africanized Yucatecan and Venezuelan populations had ancestry in southeastern Europe and South Africa, respectively.

Changes in Population Structure in the Yucatan Peninsula over Time

Striking changes in population structure occurred in the Yucatecan population after the arrival of Africanized bees. By 1989, three years after the arrival of Africanized bees, the population was in Hardy-Weinberg equilibrium ($P_{multilocus} = 0.6$), but there was significant linkage disequilibrium (Fig. 2) among the six loci studied. Eleven instances of significant linkage disequilibrium were observed in 15 comparisons ($\alpha = 0.05$). When a sequential Bonferroni correction (Rice 1989) was applied, seven of the 15 comparisons showed significant linkage disequilibrium within the sample from 1989, indicating recent mixing of populations. The 1989 population was significantly differentiated from the pre-Africanized sample ($P_{multilocus} < 10^{-4}$ for genic and genotypic differentiation), but only two loci showed significantly different allele frequencies between the two sampling times. The 1989 sample was also highly differentiated from all Old World reference samples ($P_{multilocus} < 10^{-4}$ for genic and genotypic differentiation). The multilocus F_{ST}-value between the pre-Africanized and the 1989 Yucatecan sample was not significantly different from zero ($P = 0.001 \pm 0.104$; Fig. 3). A neighbor-joining tree still placed the 1989 population with southeastern European and 1985 Yucatecan populations (Fig. 1).

The sample from 1998 showed significant departure from Hardy-Weinberg expectations ($P_{multilocus} < 10^{-4}$) within the managed population, indicating recent population mixing or subdivision. However, there was no significant departure from equilibrium in the 1998 feral Yucatecan population ($P_{multilocus} = 0.95$). A test for linkage disequilibrium (Fig. 2) among loci showed three of 15 (managed) and one of 15 (feral) comparisons showed significant departures from equi-

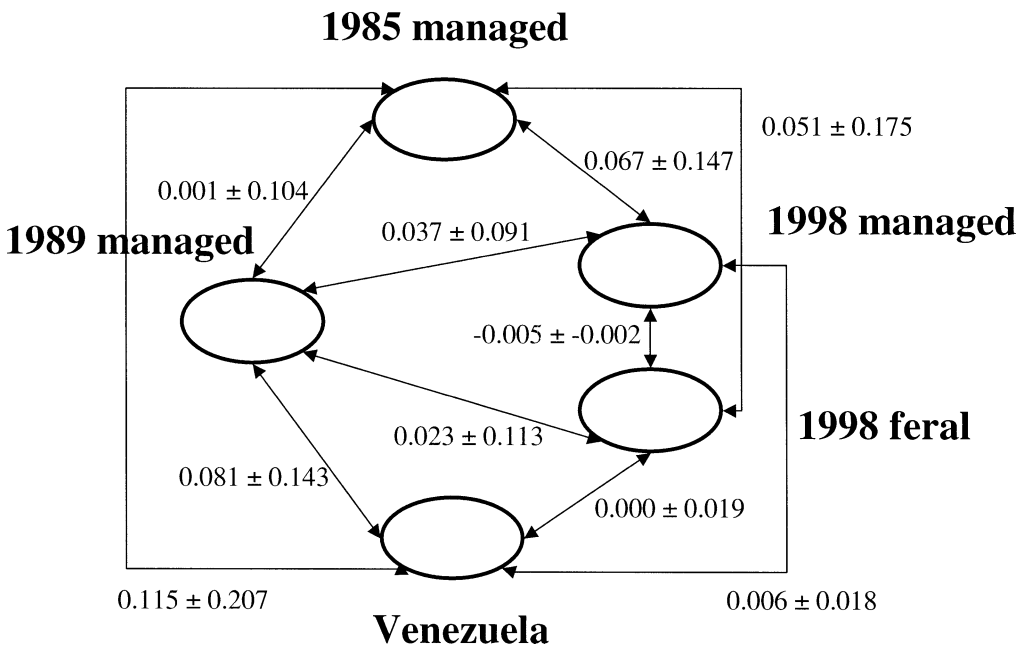

FIG. 3. Multilocus F_{ST}-values (Weir and Cockerham 1984) between pairs of New World populations. Samples from the Yucatecan population were taken before the arrival of Africanized bees (1985), during the arrival of Africanized bees (1989), and 12 years after the arrival of Africanized bees (managed and feral).

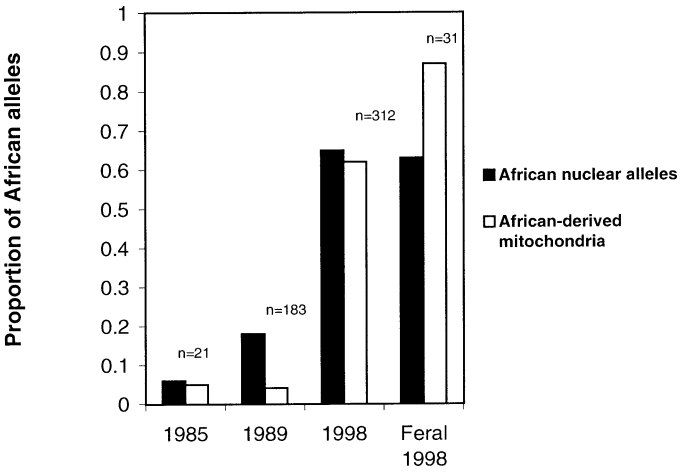

FIG. 4. The proportion of individuals having African-derived mitochondria (Clarke et al. 2001) and the proportion of introgressed African nuclear alleles in the Yucatecan population.

librium ($\alpha = 0.05$). However, none of these comparisons were significant after a sequential Bonferroni correction was applied. By 1998 both feral and managed Yucatecan populations were highly differentiated from Old World samples and from the 1985 and 1989 populations ($P_{multilocus} < 10^{-4}$ for genic and genotypic differentiation), and now are grouped with populations from South Africa and Venezuela (Fig. 1). Multilocus F_{ST}-values between the pre-Africanized population and the 1998 Yucatecan and Venezuelan populations were significantly different from zero (Fig. 3). The multilocus F_{ST}-value between the 1989 population and the Venezuelan population was significantly different from zero, but between the 1989 and 1998 (managed and feral) samples multilocus F_{ST}-values were not significantly different from zero (Fig. 3). The Venezuelan and 1998 populations had multilocus F_{ST}-values not significantly different from zero (Fig. 3).

The proportion of nuclear alleles defined as African increased over time in the Yucatecan population, from 6% in the pre-Africanized sample to 18% in 1989, 65% within the managed 1998 sample, and 63% within the feral 1998 population (Fig. 4). The increase in African nuclear alleles paralleled an increase in the proportion of African-derived mitochondria in the pre-Africanized sample and the 1998 managed sample but not in the 1989 sample or the 1998 feral sample (Fig. 4).

Characterization of the New World Reference Populations

We assigned individuals from the two New World reference populations (Yucatan 1985 and Venezuela 1990) to the two Old World reference populations (Europe or Africa) based on their nuclear genotype at six microsatellite loci using STRUCTURE (Pritchard et al. 2000). Individuals from the pre-Africanized Yucatecan sample were largely classified as having European nuclear genotypes and European-derived mitochondria (Fig. 5).

The Venezuelan sample includes individuals with African nuclear genotypes and African-derived mitochondria ($n = 12$), individuals with hybrid nuclear genotypes (where the European component is exclusively assigned to the M branch) and African-derived mitochondria ($n = 15$), and one individual with a hybrid nuclear genotype and European-derived mitochondria.

Associations of Nuclear and Mitochondrial Markers

Individuals from the various post-Africanization Yucatan Peninsula samples were then classified using STRUCTURE (Pritchard et al. 2000) according to their nuclear genotype using the pre-Africanized and Venezuelan populations as reference populations. In the 1989 Yucatecan population there were significant nonrandom associations between nuclear genotype and mitochondrial haplotype. In this 1989 sample bees classified as having pre-Africanized or hybrid nuclear genotype are not significantly different with respect to the proportion of European-derived or African-derived mitochondria ($P = 1.0$, Fig. 5), but both are more likely to have mitochondria of European origin (particularly southeast Europe) than mitochondria of African origin (Fig. 5). However, 1989 bees classified as having pre-Africanized and hybrid nuclear genotypes have significantly different associations with mitochondrial type compared to those classified as having Venezuelan nuclear genotypes ($P < 0.001$, Fig. 5).

In 1998, bees classified as Venezuelan based on their nuclear genotypes were more likely to carry African-derived mitochondria than European-derived mitochondria, within both domestic and feral colonies (Fig. 5). Within domestic colonies, individuals with hybrid nuclear genotypes were equally likely to have European-derived or African-derived mitochondria (Fig. 5). Bees classified as having Venezuelan or hybrid nuclear genotypes are significantly different with respect to the proportion of European-derived or African-derived mitochondria ($P = 0.003$, Fig. 5), but neither were significantly different from individuals having pre-Africanized nuclear genotypes ($P = 1.0$ pre-Africanized/Venezuela, $P = 0.361$ pre-Africanized/hybrid, Fig. 5), but the number of bees classified as having pre-Africanized nuclear genotypes was small ($n = 4$). Within feral colonies, bees with Venezuelan or hybrid nuclear genotypes are not significantly different with respect to the proportion of European-derived or African-derived mitochondria ($P = 0.602$, Fig. 5).

DISCUSSION

Africanization of the Yucatecan Population

Since the arrival of Africanized bees, the genetic architecture of the Yucatecan population of honeybees has changed dramatically. Prior to the arrival of Africanized bees, the population was primarily a mix of southeastern European subspecies probably imported from the United States relatively recently (Quezada-Euán and Hinsull 1995) and northern and western European subspecies (probably imported by the first European settlers). The majority of individuals were classified as having European nuclear genotypes (94%) and European-derived mitochondria (95%). A higher-than-expected proportion of pre-Africanized individuals (14%) were classified as having some African alleles. These individuals have a few nuclear alleles found at low frequencies among European populations but occurring at higher frequencies among African populations. We suggest that these individ-

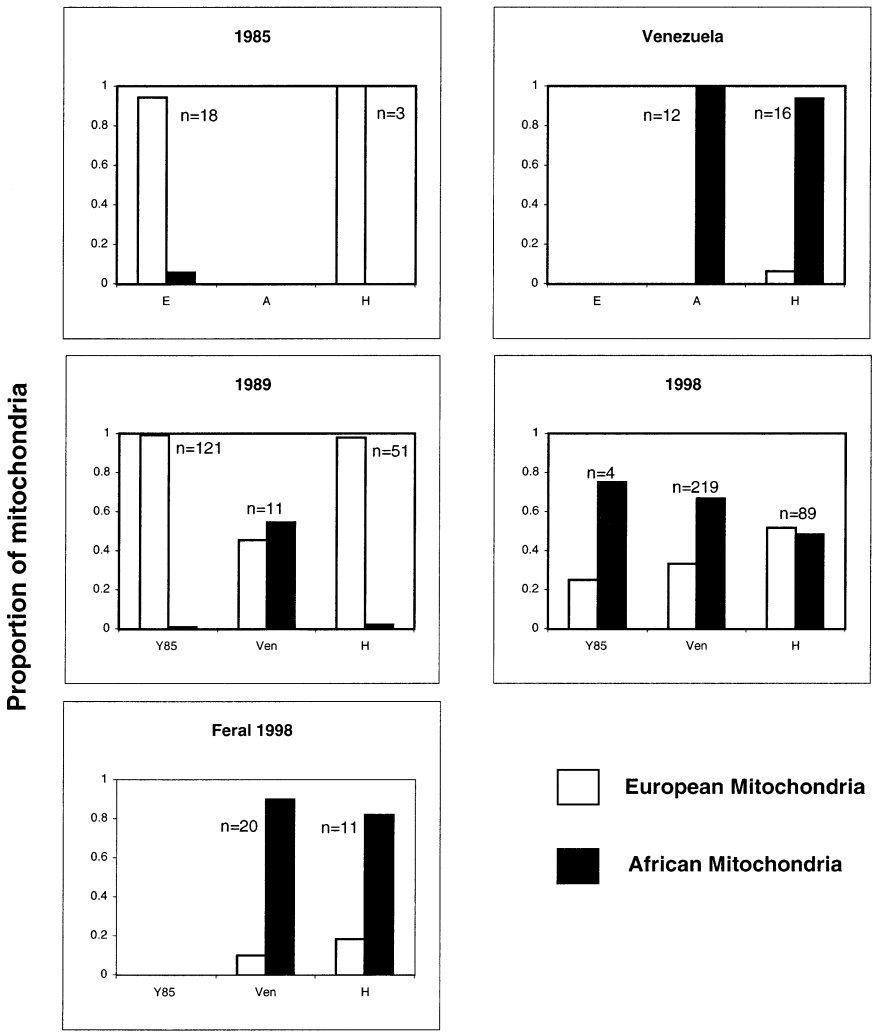

FIG. 5. Associations of nuclear and mitochondrial markers. The proportion of European-derived mitochondria and African-derived mitochondria that have European (E), African (A), hybrid (H), pre-Africanized (Y85), or Venezuelan (Ven) nuclear genotypes.

uals were actually European in origin and had rare European nuclear alleles derived from the Iberian Peninsula. However, it is possible that these individuals are descendants of honeybees imported into the United States from North Africa more than 100 years ago (Schiff et al. 1994). The single African mitochondrial haplotype that was detected (Clarke et al. 2001) is likely to have originated in the natural A-M hybrid zone of the Iberian Peninsula.

Africanized bees arrived in the Yucatan Peninsula in 1986 and three years later the presence of two genetically distinct populations is indicated by the significant nonrandom associations among nuclear genotypes and between nuclear genotypes and mitochondrial haplotypes. The proportion of nuclear genotypes classified as African increased to 18% and the number of individuals classified as having hybrid (pre-Africanized/Venezuelan) nuclear genotypes increased to 28%, although some of these individuals were not Africanized to a high degree. Similarly, morphometric analysis of the 1989 population (Rinderer et al. 1991) classified 22–25% of colonies as Africanized.

The proportion of African-derived mitochondria remained low (4%), indicating substantial introgression of nuclear but not of mitochondrial genes. In 1989 it appeared that Africanization of a predominantly European population occurred through matings of European queens with feral Africanized drones but that there was little or no takeover of domestic colonies by Africanized swarms. Feral populations of Africanized bees are reported to reach high numerical densities within two to three years of colonization (Michener 1975; Taylor 1985). Prolific drone production within Africanized colonies (Winston 1988) and subsequent migration of these drones in significant numbers into European colonies (Rinderer et al. 1985) mean that large numbers of Africanized drones would be present in drone congregation areas leading to Africanization of the nuclear genomes of managed colonies. Quezada-Euán (2000) showed that no detectable gene

flow from the European population into the feral population had occurred at this time because the population was morphologically Africanized and had mitochondria exclusively of African descent.

By 1998 (13 years after the arrival of Africanized bees) there had been a dramatic increase in the proportion of African nuclear alleles estimated in the managed Yucatecan population (65%) and, similarly, the gene pool of the feral population contained an estimated 63% African nuclear alleles. There was a reduction in the proportion of the population classified as having admixture of pre-Africanized and Venezuelan genotypes (28% and 36%, respectively). Furthermore, the increase in nuclear African genotypes occurred in concert with the increase of African-derived mitochondrial alleles among managed colonies (62%), but not in feral colonies (87%). Among managed colonies Africanization appears to have occurred by Africanized swarm takeover (Danka et al. 1992), beekeepers catching feral swarms, and matings with Africanized drones. However, minimal maternal gene flow took place from managed colonies into feral populations.

In the Venezuelan population there was a negligible proportion of European mitochondria but a significant proportion of the nuclear genome had both African and European alleles. A lack of maternal gene flow from European mitochondria (Smith et al. 1989; Hall and Smith 1991) into some Africanized populations was probably a function of population size differences (Page 1989), and this same phenomenon may be the cause of the apparent low maternal gene flow from the managed Yucatecan population into the feral Yucatecan population that we observed.

Nonrandom associations between nuclear genotypes and between nuclear genotypes and mitochondrial haplotypes within the managed 1998 Yucatecan population have greatly diminished since 1989 but have not disappeared. Although European-derived and African-derived mitochondrial haplotypes are associated with Venezuelan (highly Africanized) nuclear genotypes and hybrid (pre-Africanized/Venezuelan) nuclear genotypes, nearly half of the bees from managed colonies had Venezuelan (highly Africanized) nuclear genotypes and African-derived mitochondria.

There are several factors that could account for asymmetry favoring the African genome. First, some manipulation of the population by beekeepers (catching swarms from a steady influx of Africanized bees from areas outside the Yucatan; Quezada-Euán and Medina 1998), usurpation of European colonies by Africanized queens (Danka et al. 1992), or asymmetrical fitness of hybrids with European-derived or African-derived mitochondria (Harrison and Hall 1993) could all lead to a pronounced increase in African-derived mitochondria. Second, as previously mentioned, the sheer numbers of Africanized drones and their predisposition for parasitizing colonies (Rinderer et al. 1985) could account for a shift toward an Africanized nuclear genome in managed European colonies. Any positive assortative mating tendencies (Kerr and Bueno 1970) of Africanized bees would further enhance the Africanization process.

Africanized bees have a competitive advantage over European bees in the Neotropics, in terms of their colonizing abilities and rules of reproduction and dispersal. We suggest that, although the European mitochondrial genome may be lost over time, European nuclear alleles appear to have stably introgressed into the genome of Africanized bees.

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Literature Cited

Arias, M. C., and W. S. Sheppard. 1996. Molecular phylogenetics of honey bee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequence. Mol. Phylogenet. Evol. 5:557–566.

Arnold, J. 1993. Cytonuclear disequilibria in hybrid zones. Annu. Rev. Ecol. Syst. 24:521–554.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. 19:233–257.

Clarke, K. E., B. P. Oldroyd, J. J. G. Quezada-Euán, and T. E. Rinderer. 2001. Origin of honeybees (*Apis mellifera* L.) from the Yucatan peninsula inferred from mitochondrial DNA analysis. Mol. Ecol. 10:1347–1355.

Cornuet, J.-M. 1986. Population genetics. Pp. 235–254 *in* T. E. Rinderer, ed. Bee genetics and breeding. Academic Press, Orlando, FL.

Danka, R. G., R. L. I. Hellmich, and T. E. Rinderer. 1992. Nest usurpation, supersedure and colony failure contribute to Africanization of commercially managed European honey bees in Venezuela. J. Apic. Res. 31:119–123.

de la Rua, P., J. Serrano, and J. Galian. 1998. Mitochondrial DNA variability in the Canary Islands honeybees (*Apis mellifera* L.). Mol. Ecol. 7:1543–1547.

Estoup, A., L. Garnery, M. Solignac, and J.-M. Cornuet. 1995. Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and tests of the infinite allele and stepwise mutation models. Genetics 140:679–695.

Falconer, D. S. 1981. Introduction to quantitative genetics. Longman, New York.

Fierro, M. M., A. Barraza, D. L. Maki, and J. O. Moffett. 1987. The effects of the first year of Africanization on honey bee populations in Chiapas, Mexico. Am. Bee J. 127:845.

Fletcher, D. J. C. 1991. Interdependence of genetics and ecology in a solution to the African bee problem. Pp. 77–117 *in* M. Spivek, D. C. Fletcher, and M. D. Breed, eds. The ''African'' honey bee. Westview Press, Boulder, CO.

Franck, P., L. Garnery, M. Solignac, and J.-M. Cornuet. 1998. The origin of west European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data. Evolution 52:1119–1134.

Franck, P., L. Garnery, G. Celebrano, M. Solignac, and J.-M. Cornuet. 2000a. Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). Mol. Ecol. 9:907–921.

Franck, P., L. Garnery, M. Solignac, and J.-M. Cornuet. 2000b. Molecular confirmation of a fourth lineage in honeybees from the Near East. Apidologie 31:167–180.

Garnery, L., J.-M. Cornuet, and M. Solignac. 1992. Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. Mol. Ecol. 1:145–154.

Garnery, L., M. Solignac, G. Celebrano, and J.-M. Cornuet. 1993. A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure of *Apis mellifera*. Experientia 49: 1016–1021.

Garnery L., E. H. Mosshine, B. P. Oldroyd, and J-M. Cornuet. 1995.

Mitochondrial DNA variation in Moroccan and Spanish honey bee populations. Mol. Ecol. 4:465–471.
Garnery L., P. Franck, E. Baudry, D. Vautrin, J-M Cornuet, and M. Solignac. 1998. Genetic diversity of the west European honey bee (*Apis mellifera mellifera* and *A. m. iberica*). I. Mitochondrial DNA. Genet. Sel. Evol. 30:31–47.
Hall, H. G. 1990. Parental analysis of introgressive hybridisation between African and European honeybees using nuclear DNA RFLPs. Genetics 125:611–621.
———. 1991. Genetic characterization of honeybees through DNA analysis. Pp. 45–73 *in* M. Spivak, D. J. C. Fletcher, and M. D. Breed, eds. The ''African'' honey bee. Westview Press, Boulder, CO.
Hall, H. G., and K. Muralidharan. 1989. Evidence from mitochondrial DNA that African honeybees spread as continuous maternal lineages. Nature 339:211–213.
Hall, H. G., and D. R. Smith. 1991. Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. Proc. Natl. Acad. Sci. USA 88:4548–4552.
Harrison, J. F., and H. G. Hall. 1993. African-European honeybee hybrids have low non-intermediate metabolic capacities. Nature 363:258–260.
Hedges, S. B. 1992. The number of replications needed for accurate estimation of the bootstrap *P* value in phylogenetic studies. Mol. Biol. Evol. 9:366–369.
Hewitt, G. M. 1988. Hybrid zones: natural laboratories for evolutionary studies. Trends Ecol. Evol. 3:158–167.
Kerr, W. E. 1967. The history of the introduction of African bees in Brazil. S. Afr. Bee J. 39:33–35.
Kerr, W. E., and D. Bueno. 1970. Natural crossing between *Apis mellifera adansonii* and *Apis mellifera ligustica*. Evolution 24: 145–155.
Lewontin, R. C., and J. Felsenstein. 1965. The robustness of homogeneity tests in 2 x N tables. Biometrics 21:19–33.
Lobo, J. A., M. A. del Lama, and M. A. Mestriner. 1989. Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L.). Evolution 43:794–802.
McMichael, M., and H. G. Hall. 1996. DNA RFLPs at a highly polymorphic locus distinguish European and African subspecies of the honey bee *Apis mellifera* L. and suggest geographical origins of new world honey bees. Mol. Ecol. 5:403–416.
Michener, C. D. 1975. The Brazilian bee problem. Annu. Rev. Entomol. 20:390–416.
Muralidharan, K., and H. G. Hall. 1990. Prevalence of African DNA RFLP alleles in Neotropical African honeybees. Arch. Insect Biochem. Physiol. 15:229–236.
Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 145–163.
Oldroyd, B. P., W. S. Sheppard, and J. A. Stelzer. 1992. Genetic characterization of the bees of Kangaroo Island, South Australia. J. Apic. Res. 31:141–148.
Otis, G. W. 1991. Population biology of the Africanized honey bee. Pp. 213–234 *in* M. Spivak, D. J. C. Fletcher, and M. D. Breed, eds. The ''African'' Honey Bee. Westview Press, Boulder, CO.
Page, R. E. 1989. Neotropical African bees. Nature 339:181–182.
Palmer, M. R., D. R. Smith, and O. Kaftanoglu. 2000. Turkish honeybees: genetic variation and evidence for a fourth lineage of *Apis mellifera* mtDNA. J. Hered. 91:42–46.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.
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., and S. M. Hinsull. 1995. Evidence of continued European morphometrics and mtDNA in feral colonies of honey bees (*Apis mellifera* L.) from the Yucatan peninsula, Mexico. J. Apic. Res. 34:161–166.
Quezada-Euán, J. J. G., and L. M. Medina. 1998. Hybridization between European and Africanized honeybees (*Apis mellifera* L.) in tropical Yucatan, Mexico. I. Morphometric changes in feral and managed colonies. Apidologie 29:555–568.
Quezada-Euán, J. J. G., C. M. Echazarreta, and R. J. Paxton. 1996. The distribution and range expansion of Africanized honey bees (*Apis mellifera*) in the state of Yucatan, Mexico. J. Apic. Res. 35:85–95.
Raymond, M., and F. Rousset. 1995. Population genetics software for exact test and ecumenism. J. Hered. 86:248–250.
Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.
Rinderer, T. E. 1988. Evolutionary aspects of the Africanization of honey-bee populations in the Americas. Pp. 13–28 *in* G. R. Needham, R. E. Page, M. Delfinado-Baker, and C. E. Bowman, eds. Africanized honey bees and bee mites. Ellis Horwood, Chichester, England.
Rinderer, T. E., R. L. I. Hellmich, R. G. Danka, and A. M. Collins. 1985. Male reproductive parasitism: a factor in the Africanization of European honey-bee populations. Science 228:1119–1121.
Rinderer, T. E., J. A. Stelzer, B. P. Oldroyd, S. M. Buco, and W. L. Rubink. 1991. Hybridization between European and Africanized honey bees in the neotropical Yucatan peninsula. Science 253:309–311.
Rinderer, T. E., B. P. Oldroyd, and W. S. Sheppard. 1993. Africanized bees in the United States Sci. Am. 269:52–58.
Rothenbuhler, W. C. 1979. Semidomesticated insects: honeybee breeding. Pp. 84–92 *in* M. A. Moy and J. J. McKelvey, eds. Genetics and beneficial organisms. Rockefeller Foundation, Bellagio, Italy.
Roubik, D. W., and H. Wolda. 2001. Do competing honeybees matter? Dynamics and abundance of native bees before and after a honeybee invasion. Pop. Ecol. 43:53–62.
Ruttner, F. 1976. Isolated populations of honeybees in Australia. J. Apic. Res. 15:97–104.
———. 1986. Geographical variability and classification. Pp. 23–56 *in* T. E. Rinderer, ed. Bee genetics and breeding. Academic Press, Orlando, FL.
———. 1988. Biogeography and taxonomy of honeybees. Springer, Berlin.
Ruttner, F., L. Tassencourt, and J. Louveaux. 1978. Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. Apidologie 9:363–381.
Schiff, N. M., W. S. Sheppard, G. M. Loper, and H. Shimanuki. 1994. Genetic diversity of feral honey bee (Hymenoptera: Apidae) populations in the southern United States. Ann. Entomol. Soc. Am. 87:842–848.
Sheppard, W. S., T. E. Rinderer, J. A. Mazzoli, J. A. Stelzer, and H. Shimanuki. 1991. Gene flow between African- and European-derived honey bee populations in Argentina. Nature 349: 782–784.
Sheppard, W. S., T. E. Rinderer, L. Garnery, and H. Shimanuki. 1999. Analysis of Africanized honey bee mitochondrial DNA reveals further diversity of origin. Genet. Mol. Biol. 22:73–75.
Smith, D. R., O. R. Taylor, and W. M. Brown. 1989. Neotropical Africanized honey bees have African mitochondrial DNA. Nature 339:213–215.
Stort, A. C., and L. S. Goncalves. 1991. Genetics of defensive behavior. II. Pp. 329–356 *in* M. Spivak, D. J. C. Fletcher, and M. D. Breed, eds. The ''African'' honey bee. Westview Press, Boulder, CO.
Suazo, A., R. McTiernan, and H. G. Hall. 1998. Differences between African and European honey bees (*Apis mellifera* L.) in random amplified polymorphic DNA (RAPD). J. Hered. 89:32–36.
Taylor, O. R. 1977. The past and possible future spread of Africanized honeybees in the Americas. Bee World 58:19–30.
———. 1985. African bees: potential impact in the United States. Bull. Entomol. Soc. Am. 31:15–24.
———. 1988. Ecology and economic impact of African and Africanized honeybees. Pp. 29–41 *in* G. R. Needham, R. E. Page, M. Delfinado-Baker, and C. E. Bowman, eds. Africanized honey bees and bee mites. Ellis Horwood, Chichester, England.
Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex (R)100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:507.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.
Winston, M. L. 1988. The impact of a tropical-evolved honey bee in temperate climates of North America. Pp. 135–140 *in* G. R. Needham, R. E. Page, M. Delfinado-Baker, and C. E. Bowman, eds. Africanized honey bees and bee mites. Ellis Horwood, Chichester, England.
———. 1991. The inside story: internal colony dynamics of Africanized bees. Pp. 201–212 *in* M. Spivak, D. J. C. Fletcher and M. D. Breed, eds. The ''African'' honey bee. Westview Press, Boulder, CO.
Winston, M. L., J. Dropkin, and O. R. Taylor. 1981. Demography and life history characteristics of two honeybee races (*Apis mellifera*). Oecologia 48:407–413.

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