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Origin of honeybees (*Apis mellifera* L.) from the Yucatan peninsula inferred from mitochondrial DNA analysis

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Abstract

Honeybees (*Apis mellifera* L.) sampled at sites in Europe, Africa and South América were analysed using a mitochondrial DNA restriction fragment length polymorphism (RFLP) marker. These samples were used to provide baseline information for a detailed analysis of the process of Africanization of bees from the neotropical Yucatan peninsula of Mexico. Radical changes in mitochondrial haplotype (mitotype) frequencies were found to have occurred in the 13-year period studied. Prior to the arrival of Africanized bees (1986) the original inhabitants of the Yucatan peninsula appear to have been essentially of southeastern European origin with a smaller proportion having northwestern European ancestry. Three years after the migration of Africanized bees into the area (1989), only very low levels of maternal gene flow from Africanized populations into the resident European populations had occurred. By 1998, however, there was a sizeable increase in the proportion of African mitotypes in domestic populations (61%) with feral populations having 87% of mitotypes classified as African derived. The results suggest that the early stages of Africanization did not involve a rapid replacement of European with African mitotypes and that earlier studies probably overestimated the prevalence of African mitotypes.

Keywords: Africanized bee, *Apis mellifera*, gene flow, honeybee, hybridization, mitochondrial RFLP

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Introduction

The honeybee, *Apis mellifera* L., occurs naturally in Europe, the Middle East and Africa (Ruttner 1988). This diverse range of habitats has required adaptation to a variety of ecological and climatic conditions and historical separation has caused the evolution of over 24 named subspecies (Ruttner 1988). On the basis of morphology, these subspecies have been grouped into four distinct evolutionary branches, namely the African (A), western and northern European (M), southeastern European (C) and Near Eastern (O) branches (Ruttner 1988), and molecular analyses have broadly supported this classification (e.g. Garnery *et al*. 1992; Franck *et al*. 2000).

Honeybees have also been disseminated around the world by humans. Initial introductions by European settlers presumably resulted in a strong correlation between the racial origin of the bees and the nationality of the settlers. Hence, in the South American continent, we would expect that many of the early introductions would have been from Portugal and Spain, probably *A.m. iberica* and *A.m. mellifera*. More recent introductions have included commercially important subspecies from Italy (*A.m. ligustica*), Yugoslavia (*A.m. carnica*) and the Near East (*A.m. caucasica*). Infamously, the African subspecies, *A.m. scutellata*, was released in Brazil in the 1950s in an attempt to improve honey production. European honeybees do not flourish in the tropics, probably because of differences in environmental conditions to those in which they evolved (Kerr 1967; Rinderer *et al*. 1993). Populations of 'Africanized' honeybees have since expanded from Brazil at an estimated rate of 100–300 km annually. At present, African-derived populations can be found across south and central South America and in the southern USA (Rinderer *et al*. 1993). *A.m. scutellata* displays certain undesirable characteristics such as extreme defensive

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behaviour and excessive swarming (Rinderer *et al*. 1993). For this reason there has been considerable interest in the migration and hybridization of these Africanized bee populations.

Whether the European-derived and African-derived subspecies of honeybees are hybridizing on a massive scale, or whether the Africanized honeybees are essentially maintaining their genetic integrity and thereby largely displacing previously established European populations, remains controversial, as does the underlying means of dispersal of Africanized bees. Conflicting findings seem to be dependent on the nature of the markers used, the climatic and ecological conditions of the sampling region, and the numbers of European bees that are encountered by incoming Africanized bees.

Several researchers have reported populations of Africanized bees that are comprised almost exclusively of African-derived mitochondria (Hall & Muralidharan 1989; Smith *et al*. 1989; Hall & Smith 1991). Nuclear markers show either considerable mixing of European and Africanized populations (Lobo *et al*. 1989; Rinderer *et al*. 1991; Quezada-Euán & Hinsull 1995; Quezada-Euán *et al*. 1996) or limited admixture with only a very low frequency of European alleles detected (Hall 1990; Muralidharan & Hall 1990; McMichael & Hall 1996; Suazo *et al*. 1998).

Various hypotheses for the apparent asymmetry between mitochondrial and nuclear markers have been proposed. First, there may be selective advantages of Africanized bees over European bees in the tropics (Page 1989; Hall 1991, 1992; Rinderer *et al*. 1993) or large differences in population densities (Page 1989; Rinderer *et al*. 1991; Hall 1992). Second, fitness differences between European and African mitochondria have been postulated (Harrison & Hall 1993). Finally, it is possible that there have been inappropriate assumptions about the fixation of mitochondrial DNA (mtDNA) markers in Old World populations of honeybees (e.g. Schiff *et al*. 1994; Sheppard *et al*. 1999).

A hybrid zone between Africanized and European populations is present in Argentina that corresponds to a change in climatic conditions (Sheppard *et al*. 1991). African-derived bees were found in the tropical north of the country where Africanized bees could be expected to have a selective advantage. European-derived bees were found in the temperate south. In a transition zone between these two habitats, both European and African mitochondrial haplotypes (mitotypes) were associated with a range of morphometric and allozyme phenotypes, indicating that hybridization was occurring.

In 1989 the neotropical Yucatan peninsula in Mexico was chosen as an area of particular interest for the study of the hybridization between Africanized and European bees (Rinderer *et al*. 1991). In this region, in contrast to other parts of South America, European bees were first introduced early in the 20th century from the USA. Imported bees are thought to have been comprised largely of the subspecies *A.m. caucasica* and later *A.m. ligustica* (Quezada-Euán & Hinsull 1995). Due to the importance of the beekeeping industry in the area, a large population of domestic European bees has been established (17 colonies/km^2), although feral bees were apparently rare prior to the appearance of Africanized bees (Quezada-Euán *et al*. 1996). Thus in this region, low levels of European markers could not be due to unequal population sizes.

Africanized bees are considered to have arrived in the Yucatan region in 1986 (Fierro *et al*. 1987). Rinderer *et al*. (1991) showed that hybridization was occurring in the Yucatan in 1989, but that the process was by no means complete. The possibility remained that over time, postulated mitochondrial fitness differences (Harrison & Hall 1993) or selection against late generation hybrids (Hall 1991) could result in a much more Africanized population than was found in 1989. Furthermore, the 1989 study used a mitochondrial marker that is now known to be ambiguous for African mitotypes, because some bees of western and northern European origin could be classified as African (Smith *et al*. 1991).

Other studies in this region have revealed a consistent increase in the level of Africanization of managed colonies over time (Quezada-Euán *et al*. 1996; Quezada-Euán & Medina 1998). African- and European-derived mitotypes are now associated with a diverse array of morphotypes. In contrast, however, some degree of gene flow was initially detected from domestic European colonies to Africanized feral populations, but by 1994 feral populations were essentially Africanized (Quezada-Euán *et al*. 1996).

In this study, we analysed a collection obtained from the Yucatan in 1985 and reanalysed the 1989 collection of Rinderer *et al*. (1991) using a *Dra*I mtDNA restriction fragment length polymorphism (RFLP) test (Garnery *et al*. 1993). We report baseline haplotype frequencies for populations obtained from throughout the putative origins of Yucatecan bees. We then compare these data sets with a contemporary (1998) collection of Yucatecan bees obtained from identical transects to the 1989 study to ascertain the extent of maternal gene flow before, during and after the arrival of Africanized bees.

Materials and methods

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 comprised 530 colonies, of which 31 were feral (that is, not in a managed hive). An

Table 1 Details of the samples collected. The collection was assembled from samples taken across Europe, Africa and South America in the years shown. The sample numbers and inferred subspecies of *Apis mellifera* are indicated

Country (year of collection)	Subspecies	Number of colonies
Italy (1992)	*A.m. ligustica*	81
Yugoslavia (1989)	*A.m. caucasica*	11
Spain (1991)	*A.m. iberica*	101
Portugal (1991)	*A.m. iberica*	24
France (1991)	*A.m. mellifera*	18
Morocco (1989, 1991)	*A.m. intermissa*	22
South Africa (1984, 1991)	*A.m. scutellata*	57
South Africa (Cape) (1991)	*A.m. capensis*	17
Venezuela (1990)		28
Yucatan (1985)		20
Yucatan (1989)		208
Yucatan (1998)		331
Yucatan feral (1998)		28

average of 30 bees was taken from the brood comb of each hive, stored in absolute ethanol in plastic scintillation vials and transported back to the laboratory where they were stored at 4 °C. Samples were also obtained from T. E. Rinderer's extensive collection of *Apis mellifera* from sites in Europe, Africa and South America and from J. Quezada-Euán's collection from the Yucatan. A complete description of all samples is given in Table 1.

Analysis of mtDNA

Total DNA was extracted from one bee per colony using Chelex® resin. One hind leg was removed from each bee, placed in a sterile 1.5 mL microcentrifuge tube and chopped with sterile dissecting scissors on dry ice. Approximately 1 mL of boiling 5% Chelex® solution was then added. The samples were then floated in boiling water for 15 min, then stored at 4 °C until required (Walsh *et al*. 1991).

To assign bees to ancestral lineages, a mtDNA marker was selected that encompasses the intergenic region of the COI–COII genes, including the $tRNA^{LEU}$ gene and a noncoding sequence. The noncoding sequence has a size polymorphism owing to a combination of two sequences P and Q; of which the P element has two forms, P and P_o (Cornuet *et al*. 1991).

The various associations of the P and Q elements are indicative of the particular lineage from which they are derived (Q for lineage C; PQ, PQQ, PQQQ and PQQQQ for lineage M; P_oQ, P_oQQ, P_oQQQ and P_oQQQQ for lineage A). The arrangement of P and Q elements coupled with a polymorphism for the restriction enzyme *Dra*I and the presence or absence of various insertions and deletions make this a particularly useful marker for distinguishing between the lineages (Garnery *et al*. 1993; Franck *et al*. 1998). Twenty-six mitochondrial haplotypes have been described (Garnery *et al*. 1993; Franck *et al*. 1998), one of these belonging to the C lineage, 14 to the A lineage and 11 to the M lineage.

Primers E2 and H2 (Garnery *et al*. 1991) were utilized for the DNA amplification as described by Garnery *et al*. (1993), but with some modifications. The 25 μL reactions contained 5 μL of Chelex-extracted DNA as the template, 1× reaction buffer, 20 μM of each dNTP, 1 mM $MgCl_2$, 1 μM of each primer and 0.6 units of *Taq* DNA polymerase (Sigma). Polymerase chain reaction (PCR) conditions consisted of one cycle of 4 min at 92 °C; 30 cycles of 1 min at 92 °C, 45 s at 50 °C, 2 min at 62 °C; and one cycle of 10 min at 72 °C.

An aliquot of 10 μL of PCR product was analysed on a 1% agarose gel to calculate the size of the PCR fragment. The remainder of each reaction was digested with the restriction enzyme *Dra*I (Boehringer Mannheim). Restriction fragments were separated on 4% nondenaturing polyacrylamide gels [27 mL of 0.5× TBE, 3 mL of 40% acrylamide/bis (Astral Scientific Gymea), 150 μL of 10% ammonium persulphate (APS) and 15 μL of tetramethylethylenediamine (TEMED)], run on an automated DNA fragment analyser (Corbett Research) at 680 V and 40 °C, and analysed using the software ONEDSCAN (Scanalytics).

Colonies were classified according to their mtDNA haplotype. Each new haplotype was sequenced to confirm its uniqueness and identity with haplotypes published by Franck *et al*. (1998). To do this, the PCR products were purified using a Concert™ Rapid Purification System (Gibco BRL®), and sequenced using dye terminator chemistry on an automated DNA sequencer (Perkin-Elmer ABI Prism 377) at the University of Sydney's automated sequencing service, SUPAMAC. Both strands of each DNA fragment were sequenced at least once and conflicts were resolved by further sequencing.

Statistical analyses

Unbiased estimates of Nei's gene diversity and their standard deviations were calculated according to Nei & Tajima (1981) using POPGENE (version 1.21; Yeh *et al*. 1997). A distance matrix based on Nei's (1978) unbiased distance was prepared using BIOSYS (version 1.7; Swofford & Selander 1989). The populations were then clustered according to their haplotype similarities via a UPGMA tree produced from the distance matrix, again using BIOSYS.

The homogeneity of haplotype frequencies across the populations was tested using Fisher's exact test, calculated using GENEPOP (version 1.2; Raymond & Rousset 1995).

Results

*Dra*I RFLP mitochondrial analysis of the 949 colonies studied revealed 19 distinct mitotypes, all of which have previously been reported (Garnery *et al*. 1993, 1995; Franck

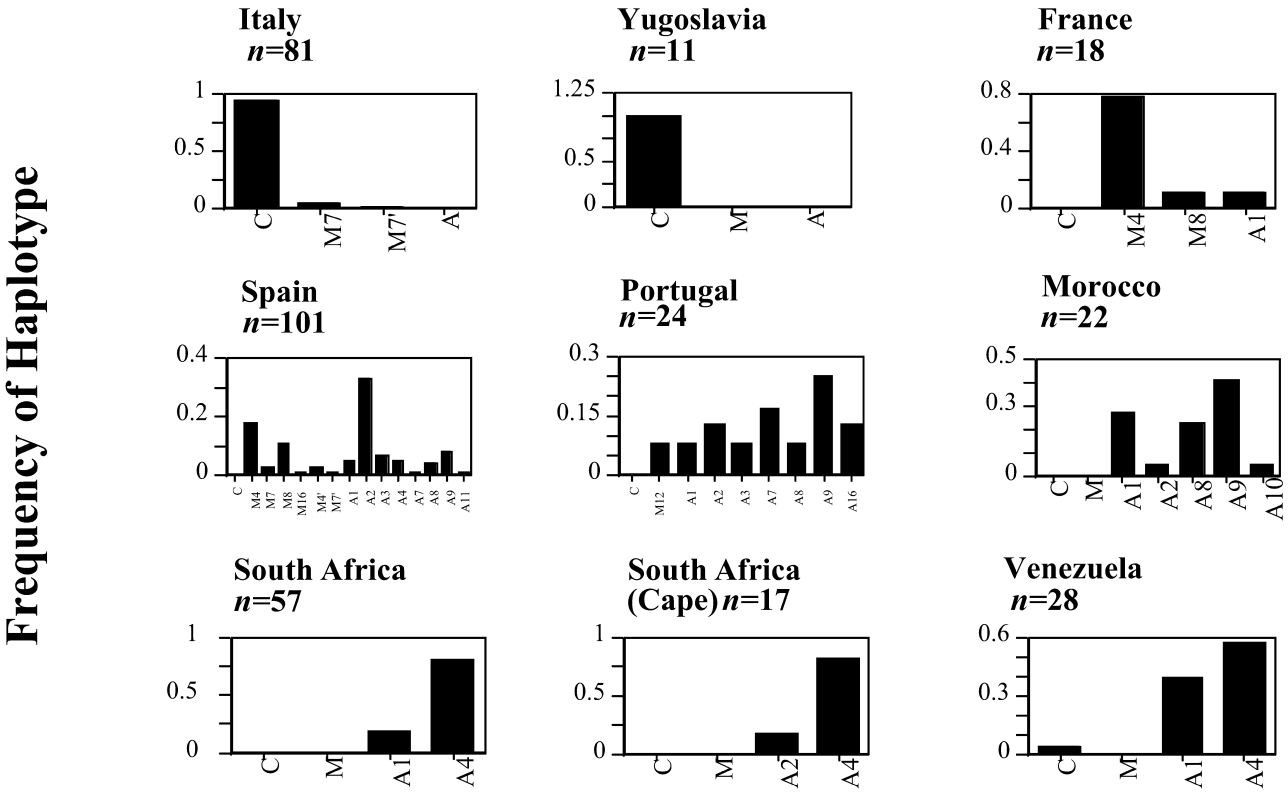

Fig. 1 Mitochondrial haplotype frequencies for reference populations of honeybees. *n* indicates the sample number for each collection.

Table 2 Unbiased estimates of Nei's gene diversity (H) and variance (s^2)

	Total population			Lineage M haplotypes*			Lineage A haplotypes†		
Populations	*n*	*H*	s^2	*n*	*H*	s^2	*n*	*H*	s^2
Italy	81	0.095	0.002	4	0.375	0.070			
Yugoslavia	11	0	0						
France	18	0.370	0.018	16	0.219	0.016	2	0	0
Spain	101	0.830	0.001	37	0.660	0.003	64	0.690	0.003
Portugal	24	0.851	0.001	2	0	0	22	0.831	0.002
Morocco	22	0.703	0.003				22	0.703	0.003
South Africa	57	0.312	0.004				57	0.312	0.004
South Africa (Cape)	17	0.291	0.015				17	0.291	0.015
Venezuela	28	0.518	0.003				27	0.483	0.002
Yucatan 1985	20	0.185	0.013	1	0	0	1	0	0
Yucatan 1989	208	0.566	0.001	65	0.698	0.002	11	0.298	0.024
Yucatan 1998	331	0.740	0.000	64	0.375	0.003	203	0.471	0.001
Yucatan feral 1998	31	0.497	0.009	4	0	0	27	0.359	0.010

*Estimates based only on haplotypes of the M lineage. †Estimates based only on haplotypes of the A lineage. *n* refers to the number of bees analysed.

et al. 1998). Striking differences in mitotype frequencies and diversity exist among the populations studied (Fig. 1, Table 2). Populations from southern Europe (Italy, Yugoslavia and France) and South Africa are characterized by uniformity of mitotype within each region (Fig. 1, Table 2). The Italian and Yugoslavian populations are characterized by the almost universal presence of the C lineage. As expected, populations in the natural A–M hybrid zone in Spain and Portugal (Smith *et al*. 1991; Garnery *et al*. 1993, 1995; Franck *et al*. 1998) are characterized by high haplotype diversities (Table 2).

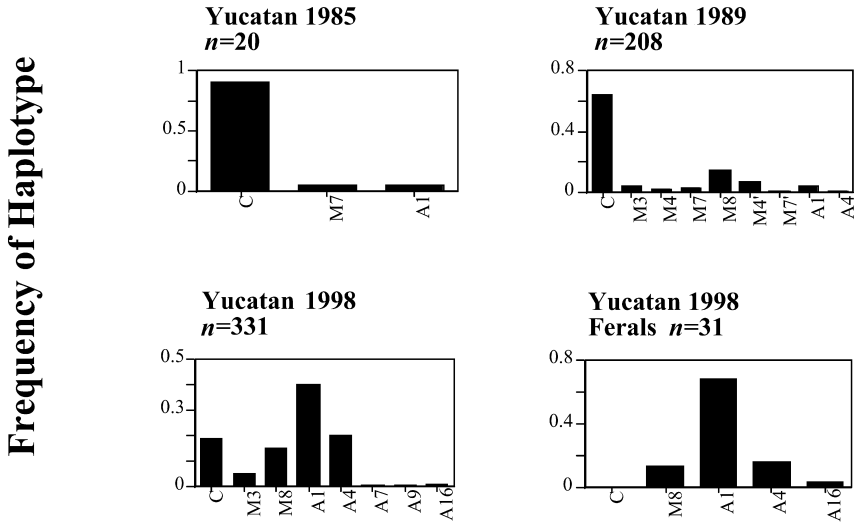

Fig. 2 Mitochondrial haplotype frequencies for Yucatecan populations of honeybees over time. The 1998 collection is separated into domestic and feral populations. *n* indicates the sample number for each collection.

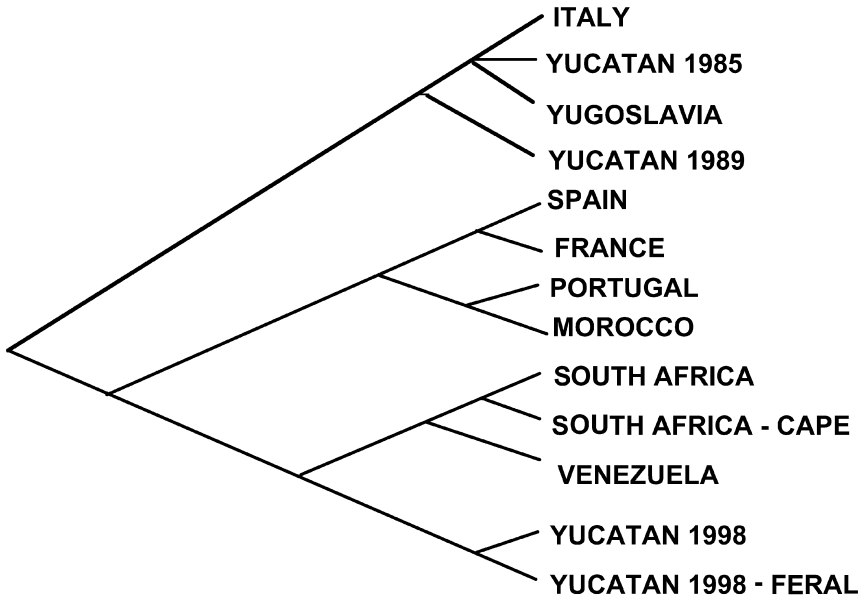

Fig. 3 UPGMA tree produced from a distance matrix based on Nei's (1978) unbiased distance.

Populations of the New World reflect repeated importations of bees from various localities in the Old World, and the rapid spread of Africanized bees. In Venezuela, 96.4% of bees collected in 1990 carried mitochondria of African descent (Fig. 2). A UPGMA cluster analysis of Nei's (1978) genetic distances among populations shows that Venezuelan populations are most similar in mitotype frequencies and type to South African populations (Fig. 3). However, a small proportion of these bees may be descendants of western European populations because the A1 and A4 mitotypes, most common in Venezuela, are also present in Spain, Morocco and probably Portugal (Fig. 1; Garnery *et al*. 1993, 1995; Franck *et al*. 1998).

Bees collected from the Yucatan in 1985, prior to the arrival of Africanized bees in 1986 (Fierro *et al*. 1987), show mitotypes and frequencies which cluster with southeastern European populations, and are indistinguishable from contemporary Italian and Yugoslav populations (Figs 1 and 3). Subsequent surveys reflect increasing Africanization of the area. In 1989, Yucatecan bees carried mitochondria which were still predominantly southeast European in origin (Fig. 2), with 5.3% of mitotypes originating in Africa or western Europe. However, by 1998, 61% of domestic bees carried mitochondria of the A lineage (Fig. 2). There was no significant difference in haplotype distributions of Yucatecan populations between 1985 and 1989 (Fisher's exact test;

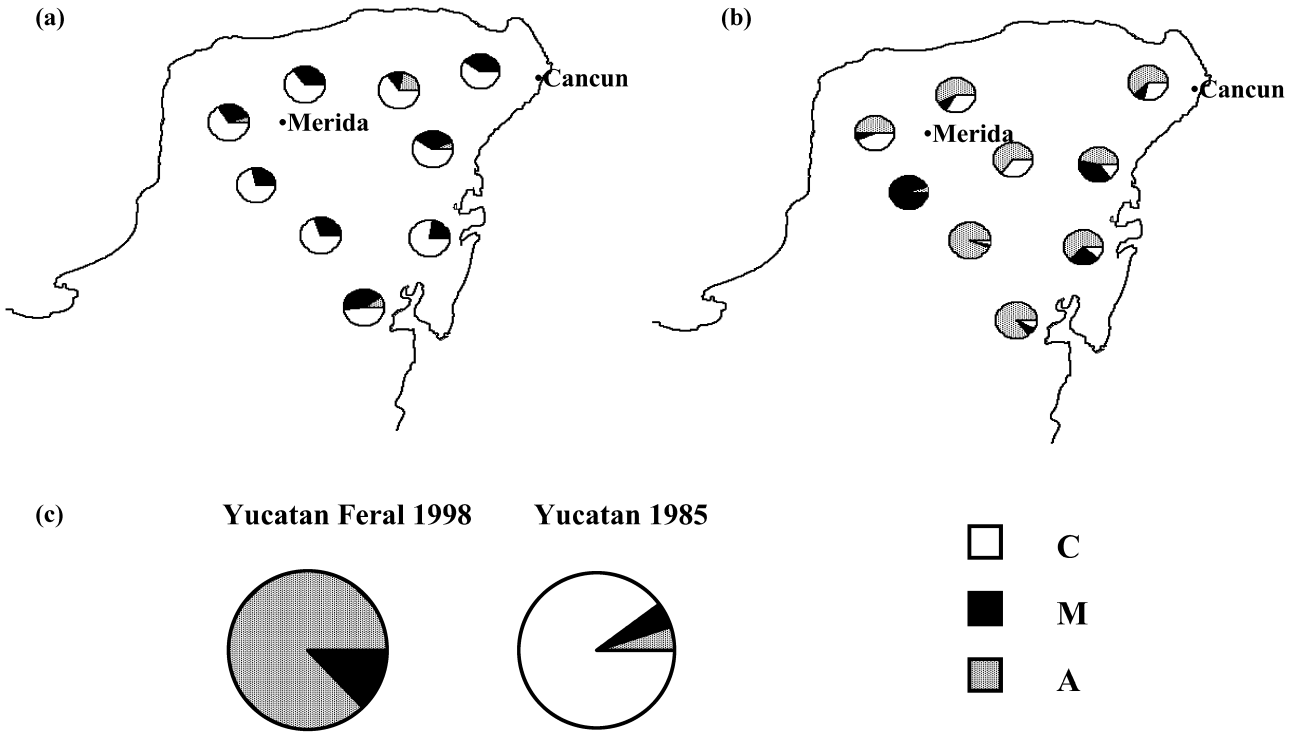

Fig. 4 Distribution of lineage C, M and A mitochondrial haplotypes from nine sampling sites across the Yucatan peninsula of Mexico in (a) 1989 and (b) 1998. (c) Pie charts, indicating the percentage of the population belonging to each lineage, are also included for the population collected in 1985 and for the feral population of 1998.

$P = 0.38$), but between 1989 and 1998, haplotype frequencies changed dramatically ($P < 0.001$). Given the diversity of haplotypes detected in Spain and Portugal, representative of both M and A lineages, it must be assumed that some of the A haplotypes found in Yucatecan populations are of Iberian origin and not African derived. Early samples (1985) from the Yucatan suggest that 5% of the pre-Africanized population had African mitochondria derived from Iberia. UPGMA cluster analysis shows that in 1998, the Yucatan population most closely resembled the South African and Venezuelan populations (Fig. 3).

During 1989, no feral bees were collected, and feral bees were extremely rare (Quezada-Euán *et al*. 1996). After Africanization, feral bees are now present in the area probably in similar densities to managed colonies (Quezada-Euán & Medina 1998), and these have higher frequencies of African mitotypes than domestic bees (Fig. 2, $P = 0.01$).

Examination of the 1998 data suggests that there is still considerable heterogeneity in the distribution of haplotypes in Yucatecan honeybees, although bees near Chetumal, closest to Guatemala and Belize, have a higher frequency of African mitotypes than bees in the north (Fig. 4b). By 1998, mitochondria of the A lineage were more abundant than the C and M lineages in the southeast of the Yucatan (Fig. 4b), the postulated place of entry for Africanized bees (Fierro *et al*. 1987).

Discussion

This study demonstrates that the honeybee populations of Spain, Portugal and Morocco have very high haplotype diversities relative to populations in Italy, Yugoslavia and France (Table 2). Franck *et al*. (1998) postulated that the high within- and between-lineage diversity of haplotypes found in western Europe reflects frequent introgression of genes of African lineage into this area. Alternatively, the low level of haplotype diversity in southeastern Europe may reflect population bottlenecks or even a selective sweep of a superior mitotype (Franck *et al*. 1998).

The heterogeneity of mitotypes in western Europe complicates the analysis of the origin of bees of the Yucatan peninsula. Of the bees sampled in 1985, 90% were of the C lineage, and these probably originated from commercial populations (primarily *Apis mellifera caucasica* and *A.m. ligustica*) imported from the USA (Quezada-Euán & Hinsull 1995). In this sample, a single bee of the M7 mitotype and a single A1 mitotype were identified. The M7 mitotype has been found in Spain (Fig. 1; Garnery *et al*. 1993, 1995;

Franck *et al*. 1998), France (Garnery *et al*. 1993; Franck *et al*. 1998) and Italy (Fig. 1). The A1 mitotype is at high frequency in Morocco, particularly in the south (Fig. 1; Garnery *et al*. 1993, 1995; Franck *et al*. 1998), western Africa (Franck *et al*. 1998), central Africa (Garnery *et al*. 1993) and South Africa, although not along the Cape (Fig. 1; Garnery *et al*. 1993), but is also present at low frequency in Spain (Fig. 1; Garnery *et al*. 1993, 1995; Franck *et al*. 1998), Portugal (Fig. 1; Franck *et al*. 1998) and France (Fig. 1). Given the known date of immigration of Africanized bees into the Yucatan, it seems most probable that this mitotype originated from Europe, and was present prior to the large-scale importation of commercial bees from the USA at the start of the 20th century (Calkins 1974). Alternatively, Schiff *et al*. (1994) demonstrated that a mitotype of African origin existed in feral bees of the southern USA, and speculated that this mitotype originated from an importation of Egyptian honeybees made over 100 years ago. Thus, it is possible, that this mitotype was imported from the USA into Mexico prior to 1985.

In 1989, 3 years after the invasion of Africanized bees (Fierro *et al*. 1987), African mitotypes remained at low frequency, and did not differ significantly from the pre-Africanization sample. A greater array of haplotypes from the M lineage were detected in 1989, but no doubt the diminutive sample available from 1985 was not sufficient to identify all mitotypes present in the Yucatan at that time. The low frequency of African mitotypes in samples from 1989 is surprising for three reasons. First, Rinderer *et al*. (1991) reported that 30% of the 1989 samples had mitochondria of African origin. The type of marker used can explain this discrepancy. Rinderer *et al*.'s (1991) study utilized an *Eco*RI RFLP mitochondrial marker which can classify M3 and M8 western European haplotypes as being of African origin, giving rise to an underestimate of European M haplotypes. Second, morphometric analysis confirms that Africanization was well advanced in 1989, with 22–25% of colonies classified as Africanized (Rinderer *et al*. 1991). Feral populations of Africanized bees are reputed to reach high population densities within 2–3 years of their arrival in a region (Michener 1975; Taylor 1985). It is conceivable, therefore, that at the time of the 1989 collection, feral populations of Africanized bees were not established in sufficient numbers for significant maternal gene flow into (predominantly European) managed colonies to be apparent. However, on the basis of Rinderer *et al*.'s (1991) morphometric study, it would appear that the European queens were mating with Africanized drones resulting in significant paternal gene flow from invading Africanized populations into the domestic colonies. Finally, Hall & Muralidharan's (1989), Smith *et al*.'s (1989) and Hall & Smith's (1991) studies revealed honeybee mitochondria to be almost exclusively of African descent.

By 1998, there was a dramatic increase in levels of African mitotypes (61%). The proportion of mitotypes of southeast European origin had markedly declined (19%) as had the proportion of mitotypes of northern and western European ancestry (19%). M3 and M8 were the only haplotypes of the M lineage still present. The M3 mitotype has been found in Spain only (Garnery *et al*. 1993, 1995; Franck *et al*. 1998) and the M8 mitotype has been detected in Spain (Fig. 1; Garnery *et al*. 1993, 1995; Franck *et al*. 1998) and France (Fig. 1; Franck *et al*. 1998). The high frequencies of A1 and A4 haplotypes are most probably indicative of mitotypes having African ancestry. The A1 mitotype has been discussed above. The A4 mitotype occurs at low frequencies in Morocco (Garnery *et al*. 1995; Franck *et al*. 1998) and Spain (Fig. 1; Garnery *et al*. 1993, 1995; Franck *et al*. 1998) and at a very high frequency in South Africa, including the Cape (Fig. 1; Garnery *et al*. 1993). Interestingly, this mitotype is absent in west Africa (Franck *et al*. 1998). It is also interesting to note that, of the African mitotypes, A4 is the most frequent mitotype (81%) amongst bees from South Africa. In Venezuela, this mitotype is less frequent (59% of African mitotypes are A4), and less frequent again in Yucatecan bees (33% in managed populations, 19% amongst feral bees), being replaced by A1, which is now the most frequent mitotype (65% in managed populations, 78% amongst feral bees). Presumably the change in haplotype frequencies from Africa to Mexico has arisen through founder effects.

The proportion of mitochondria of African origin is particularly high among populations sampled from Venezuela (96%), indicating a rapid spread of feral Africanized bees into areas that had hitherto been largely devoid of honeybees. However, feral populations of the Yucatan still carry mitochondria of European ancestry at moderate frequency (13%). These bees have the M8 mitotype that is present in Spain and France (Fig. 1; Garnery *et al*. 1993, 1995; Franck *et al*. 1998). (In Venezuela only 3.6% of bees have a mitotype associated with bees of southeast European origin.) The higher frequency of European mitotypes in the feral population of the Yucatan probably arises from the large population of European bees that existed prior to Africanization. The low density of feral bees prior to the arrival of Africanized bees and the much lower levels of European haplotypes found in feral populations after Africanized bees became established, are in agreement with predictions that Africanized bees are better adapted than European bees to tropical conditions (Michener 1975; Taylor 1977; Rinderer 1986). Gene flow from managed colonies must be occurring, but at a reduced rate to that seen from feral colonies to domestic colonies (Hall 1990; Quezada-Euán & Medina 1998).

In determining why there has been such a large shift towards mitochondria of African descent, despite the initially large European population, several factors probably favour African mitotypes over European mitotypes. First, swarm capturing by beekeepers may asymmetrically increase gene flow from (heavily Africanized) feral populations into

domestic populations (Quezada-Euán & Medina 1998). Second, Africanized queens usurping European colonies is an Africanized honeybee reproductive behaviour that favours the asymmetric spread of African mitotypes (Danka *et al*. 1992). Third, European mitotypes may be becoming diluted by a steady immigration of Africanized bees into the area from further south, as suggested by the larger proportions of A mitotypes in the south of the study area. Fourth, Africanized bees have a selective advantage over European bees, either in their superior colonizing ability or in their ability to survive in the neotropical environment (Rinderer 1988; Taylor 1988). Fifth, there is a possibility that mitochondria are not selectively neutral (Harrison & Hall 1993; Franck *et al*. 1998).

Conclusions

This study has shown that by 1998, 12 years after the arrival of Africanized bees, there was substantial gene flow from Africanized queens into domestic populations of European bees, and to a lesser extent from European queens into feral Africanized populations. Among managed populations, Africanized mitotypes are now at a higher frequency than their European counterparts (61%; Fig. 2). It is difficult to say if the hybridization process is complete, but we suggest that mitochondria of European descent are unlikely to be driven to extinction.

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