lines to enter the migrating feral population. The migrating front, by its very nature, would represent the furthest flying African bees. African bees fly much further than European bees[31]. It is tempting to speculate that European mitochondria may limit a metabolic capability needed for long distance dispersal. The African bee's shorter developmental period and lifespan suggest metabolic differences[8,21,32]. Interspecies incompatibility is sometimes manifested as impaired mitochondrial function[4,33–35]. It is conceivable that interactions between mitochondrial and nuclear factors, from widely divergent subspecies, could be suboptimal and thus contribute a disadvantage to hybrids. The dynamics of African honey bee nuclear and mitochondrial gene spread will probably be quite different in the temperate United States and may point to underlying selective processes. □

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Neotropical Africanized honey bees have African mitochondrial DNA

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NON-INDIGENOUS African honey bees have invaded most of South and Central America in just over 30 years[1]. The genetic composition of this population and the means by which it rapidly colonizes new territory remain controversial. In particular, it has been unclear whether this 'Africanized' population has resulted from interbreeding between African and domestic European bees, or is an essentially pure African population. Also, it has not been known whether this population expanded primarily by female or by male migration. Restriction site mapping of 62 mitochondrial DNAs of African bees from Brazil, Venezuela and Mexico reveals that 97% were of African (*Apis mellifera scutellata*) type. Although neotropical European apiary populations are rapidly Africanized by mating with neotropical African males, there is little reciprocal gene flow to the neotropical African population through European females. These are the first genetic data to indicate that the neotropical African population could be expanding its range by female migration.

New World domesticated honey bees are descendants of introduced Old World subspecies[2], primarily *A. m. mellifera*, *ligustica* and *carnica*[3–5]. In 1956, 47 queens of African *A. m. scutellata* were imported from South Africa to Brazil as part of a breeding programme to develop hybrid strains better suited to the South American climate. Twenty-six queens escaped and

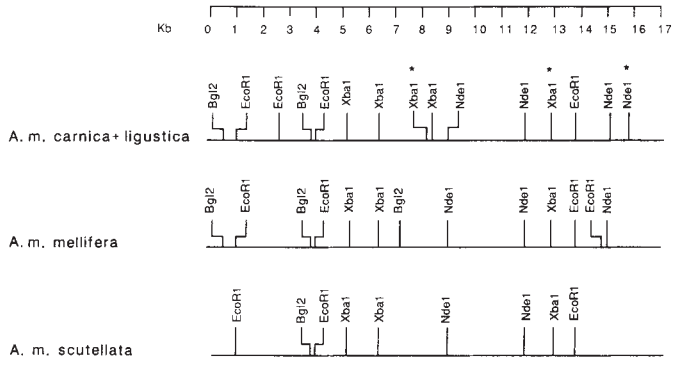

FIG. 1 Maps of *Xba*I, *Eco*RI, *Bgl*II and *Nde*I cleavage sites in mtDNA of European and African honey bee subspecies. The restriction fragments generated by each of these enzymes distinguish African *A. m. scutellata* from European *A. m. carnica, ligustica* and *mellifera*. The *Xba*I fragment from approximately 5.2–6.4 (kilobases) (kb) is 80–250 base pairs larger in *A. m. mellifera* than in *A. m. scutellata*. *Xba*I and *Eco*RI were selected for use in a survey of neotropical African bees because they produce multiple restriction fragments in the mtDNA of each subspecies and the fragments they generate end-label reliably. MtDNA was prepared[27,28] and digested with the restriction enzymes *Acc*I, *Ava*I, *Bcl*I, *Bgl*II, *Eco*0109, *Eco*RI, *Eco*RV, *Hin*cII, *Hind*III, *Nde*I, *Pst*I, *Pvu*II, *Spe*I, *Xba*I and *Xho*I, generating a total of 40 cleavage sites (29–33 per subspecies) which were mapped by means of double digests[27–29]. No consistent differences were found between the mtDNA restriction maps of *carnica* and *ligustica*. Per cent sequence divergences among mitochondrial genotypes were calculated[33] from non-degenerate recognition sites. Per cent sequence divergences (standard deviations) within populations are *A. m. carnica/ligustica*: 0.33% (0.33) to 0.97% (0.58); *A. m. mellifera*: 0.34% (0.34) to 0.71% (0.51); *A. m. scutellata*: 0.78% (0.56). Per cent sequence divergences between subspecies are *A. m. carnica/ligustica* versus *A. m. mellifera*: 2.91% (1.12) to 4.05% (1.34); *A. m. carnica/ligustica* versus *A. m. scutellata*: 2.23% (0.96) versus 3.04% (1.16); *A. m. mellifera* versus *A. m. scutellata*: 2.3% (1.01) to 3.44% (1.01). Restriction maps are based on 17 hives of *A. m. carnica* from West Germany, Austria and Yugoslavia; nine hives of *A. m. ligustica* from Italy; 22 hives of *A. m. mellifera* from Norway, Sweden, Denmark and France; and 19 hives of *A. M. scutellata* from South Africa.

established a feral neotropical African population[6,7]. These bees subsequently spread across South and Central America at the rate of as much as 500 km per year[1,8], and are expected to arrive in the United States in 1989–90 (ref. 1). Their habits of frequent swarming and absconding make them undesirable for commercial bee keeping, and their aggressive nest defence, which has resulted in numerous human fatalities, is viewed as a serious threat to public health[1,9–12].

Gene flow from feral neotropical African bees to managed European apiaries is well documented. Feral neotropical African bees attain high population densities within 2–3 years after arrival in a region, followed by Africanization of local apiaries[12,13]. Africanization entails appearance of behavioural[11,13–15] and morphological[16] characteristics typical of *A. m. scutellata*, as well as changes in allozymes[17,18] and nuclear DNA[19,20]. Because neotropical African colonies produce more drones than European colonies, and in some circumstances suppress production of drones by European colonies[21], Africanization of apiaries occurs primarily through mating of domestic European queens with abundant neotropical African drones[21,22], and less commonly by take-over of hives by African swarms[13,21].

Because young queens return to their colonies after mating flights, the rapid spread of neotropical African bees is often attributed to the dispersal of drones, which may fly several kilometres from their colonies to join mating congregations. But queens do disperse later in their life-cycle. After producing a daughter queen, who will inherit the nest, the older queen plus a retinue of workers swarm, leaving the old nest and establishing a new colony elsewhere. African and European/African hybrid queens swarm more frequently[8,23–25] and travel further[8,26] than typical European queens. Thus, whenever apiaries become Africanized, large numbers of queens carrying European mitochondrial (mt) DNA enter the feral population.

A key question is the fate of these swarms. If they survive in the feral population, the Africanized apiaries become sources of swarms which contribute European mtDNA to the feral neotropical African population. Alternatively, if few of these swarms survive in the feral population, then little European mtDNA will be introduced to the African population.

We tested these hypotheses by examination of maternally inherited mitochondrial genomes in the neotropical African population. We constructed mtDNA restriction enzyme cleavage maps for the European subspecies *A. m. ligustica*[27], *A. m. carnica*[27,28] (which had similar restriction maps), and *A. m. mellifera*[27,28], and for the African subspecies *A. m. scutellata*[27,29] (Fig. 1). The restriction enzymes *Eco*RI and *Xba*I were selected for a survey of neotropical African populations because each produces unique, multi-fragment length polymorphisms in the mtDNAs of *A. m. mellifera*, *scutellata* and *carnica*/*ligustica* (Figs 1, 2).

MtDNA was prepared (Fig. 3) from ten neotropical African hives from Ribeirão Preto, Brazil (near the point of escape of *A. m. scutellata*); twelve hives from Acarigua, Venezuela; 39 feral swarms from Tapachula, Chiapas, Mexico, (near the current front of neotropical African bee expansion); and from five hives from Ribeirão Preto, Brazil (near the point of escape of with European queens. Samples were digested with *Xba*I and *Eco*RI and the resulting restriction fragments compared with those of European and African subspecies. Nine of ten Brazilian, all twelve Venezuelan, and 38 of 39 Mexican mtDNAs were of African (*scutellata*) type (Fig. 3). Only one Brazilian and one feral Mexican mtDNA were of European (*carnica* or *ligustica*) type. In the apiary sample, four hives had European mtDNA and one hive had African mtDNA, indicating a colony take-over by an African queen.

Thus, the feral neotropical African population does not include a high frequency of swarms with European mtDNA, showing that there is little gene flow from European queens into this population. Hall and Muralidharan (see accompanying paper) arrive at the same conclusions in their study of North American and neotropical African honey bee mtDNA.

These data show that the mitochondrial genomes of a small number of African females have rapidly colonized most of a

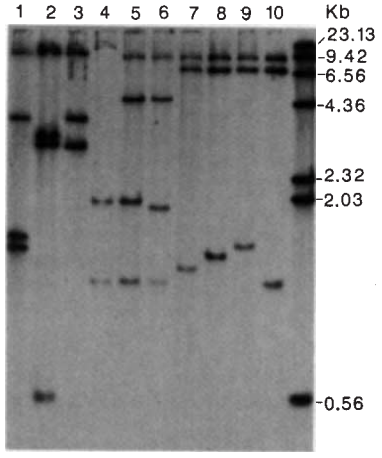

FIG. 2 Autoradiograph of 1% agarose gel showing honey bee mtDNA digested with *Eco*RI (lanes 1–3) and *Xba*I (lanes 4–10). The *Eco*RI restriction fragment pattern in lane 1 is found in *A. m. carnica* and *ligustica*; the pattern in lane 2 is found in *A. m. mellifera*; the pattern in lane 3 is found in *A. m. scutellata*. The *Xba*I restriction fragment patterns in lanes 4–6 are found in *A. m. carnica* and *ligustica*; the pattern and size variants in lanes 7–9 are found in *A. m. mellifera*; the pattern in lane 10 is found in *A. m. scutellata*. The last lane is a size standard (*Hin*dIII-digested lambda phage DNA). MtDNA samples were prepared, digested and end-labelled as described[27–29].

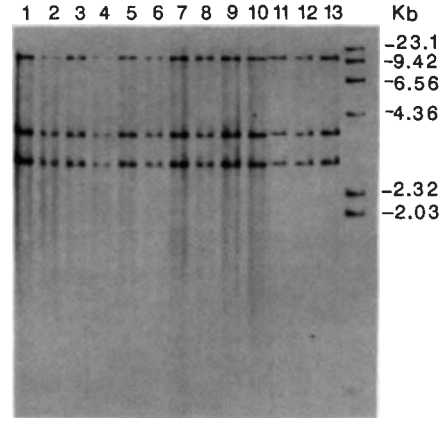

FIG. 3 Autoradiograph of 1% agarose gel showing honey bee mtDNA digested with *Eco*RI. Lanes 1–9, nine Mexican Africanized swarms; lanes 10–11, two Venezuelan Africanized swarms; lane 12, a Brazilian Africanized hive; lanes 13–14, two South African *A. m. scutellata* hives. The last lane is a size standard (see Fig. 2). Mexican samples were collected in January 1988; Venezuelan samples in 1987; Brazilian and South African samples in 1986.

METHODS. MtDNA for population surveys was prepared as follows: 15 thoraces from adult worker hive mates were powdered in liquid nitrogen, suspended in 10 ml of 10 mM Tris, 10 mM NaCl, 200 mM EDTA, pH 7.7, and ground for 30 s with a Tekmar Tissumizer; 1.3 ml 20% sodium dodecyl sulphate was added and the mixture incubated at 23 °C for 15 min; 2 ml of CsCl-saturated 10 mM Tris, 10 mM EDTA, pH 7.7, was added and the mixture incubated on ice for 15 min; cellular debris and precipitated SDS were removed from the supernatant by centrifugation for 10 min at 17,000 *g*, 4 °C; 0.5 ml of 2 mg ml^{-1} propidium iodide was added to the supernatant and the density adjusted to 1.52–1.53 g ml^{-1} with solid CsCl. Samples were loaded into a Beckman Vti 65.2 vertical rotor and centrifuged for 10–15 hours at 55,000 r.p.m.. Sample collection and analysis have been described elsewhere[27–29].

TABLE 1 Mean allele frequencies for Mdh^{100} and Hk^{100} in five honey bee populations

Population	$f(Mdh^{100})$	N	$f(Hk^{100})$	N
European subspecies	0.18 (a)	20	1.00 (d)	27
New World Europeans	0.23 (a)	84	0.95 (d)	40
Neotropical Africans				
Mexican	0.70 (b)	56	0.63 (e)	56
non-Mexican	0.92 (c)	21	0.45 (f)	191
African *A. m. scutellata*	0.99 (c)	25	0.29 (f)	15

Mean allele frequencies (f) for Mdh^{100} and Hk^{100} in five honey bee populations, weighted by number of hives sampled (N). Frequencies of Mdh^{100} and Hk^{100} are significantly independent among populations (Mdh: G, the likelihood ratio chi-square, =137.21; Hk: $G=146.6$; 4 d.f., 1 $P<0.001$); frequencies with the same letter (a–f) are not significantly different ($P>0.05$, simultaneous procedure for test of independence[34]). Frequencies presented include original and previously published data. New data: Mdh in European *A. m. mellifera* and *A. m. carnica* (collected 1987 by O.R.T. and D.R.S.); Mdh and Hk in European populations from Costa Rica (1986), in neotropical African populations from Mexico (1988) and in South African *A. m. scutellata* (1986). Previously published data: Mdh in *A. m. ligustica*[35], New World Europeans from the United States[36] and Brazil[30], neotropical Africans from Brazil[17], and *A. m. scutellata*[17]; Hk in European *A. m. ligustica* and *A. m. carnica*[32] and New World Europeans from the United States and Mexico[32]; Mdh and Hk in New World Europeans from the United States[31] and neotropical African populations from Costa Rica[31]. Samples from Africanized apiaries were avoided where possible. There is no significant difference in allele frequencies between European subspecies and New World Europeans, nor between long-established (non-Mexican) neotropical African populations and *A. m. scutellata*. There are significant differences between Mexican and long-established (non-Mexican) populations of neotropical African bees. Although there may be gene flow from the European to the African population (through drones) when the African population initially invades new territory, allele frequencies in long-established neotropical African populations are not different from those of South African *A. m. scutellata*.

continent. In addition, allele frequencies of biparentally inherited allozymes (malate dehydrogenase (Mdh), and hexokinase (Hk) distinguish African *A. m. scutellata* from European subspecies[18,30–32] and do not differ significantly between South African *A. m. scutellata* and long-established neotropical African populations (Table 1). Thus, an essentially African population is expanding in neotropical habitats through migration and colonization of new territory by African females. The paucity of European mtDNA in the feral neotropical African populations may be due to selection against some trait(s) of European or European/African hybrids in tropical habitats, to competitive interactions between European and African bees or to other factors.

The invasion of the New World by African bees provides an unusually clear example of asymmetrical gene flow between two conspecific populations. There is no evidence that the feral neotropical African population has become extensively 'Europeanized'. Future research into the management of neotropical African honey bees should emphasize the factors that limit gene flow from European to African populations and the potential competitive interactions between neotropical African bees and the large North American feral European population. □

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Associative long-term depression in the hippocampus induced by hebbian covariance

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A BRIEF, high-frequency activation of excitatory synapses in the hippocampus produces a long-lasting increase in synaptic strengths called long-term potentiation (LTP)[1]. A test input, which by itself does not have a long-lasting effect on synaptic strengths, can be potentiated through association when it is activated at the same time as a separate conditioning input[2–4]. Neural network modelling studies have also predicted that synaptic strengths should be weakened when test and conditioning inputs are anti-correlated[5–8]. Evidence for such heterosynaptic depression in the hippocampus has been found for inputs that are inactive[2,9] or weakly active[3] during the stimulation of a conditioning input, but this depression does not depend on any pattern of test input activity and does not seem to last as long as LTP. We report here an associative long-term depression (LTD) in field CA1 that is produced when a low-frequency test input is negatively correlated in time with a high-frequency conditioning input. LTD of synaptic strength is also produced by activating presynaptic terminals while a postsynaptic neuron is hyperpolarized. This confirms theoretical predictions[8] that the mechanism for associative LTD is homosynaptic and follows a hebbian covariance rule[7].

We searched for conditions under which the stimulation of a hippocampal pathway, rather than its inactivity, could produce, depending on the pattern of stimulation, either long-term

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