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Gene flow between African- and European-derived honey bee populations in Argentina

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IN the Neotropics, introduced European honey bees (*Apis mellifera* L.)[1,2] have been largely supplanted by bees descended from an African race, *A. m. scutellata* Lepetier, which were introduced into Brazil in the 1950s. Recent restriction enzyme analyses indicate that mitochondrial DNA in some neotropical populations is almost entirely of African origin[3,4], and these data have been cited as evidence for asymmetrical gene flow between African- and European-derived populations[3,4]. Evaluation of the nature of hybridization in the Neotropics is, however, confounded by possible population size advantages for the African-derived group[5–7]. As an alternative approach, genetic interactions can be studied in transition areas between zones ecologically and climatically adaptive for both racial groups. We describe here results of a survey transecting regions populated by African- and European-derived honey bees in Argentina. Mitochondrial DNA, morphological and isoenzyme analyses show that substantial hybridization occurs between the two racial groups.

For several decades the northward expansion of African-derived (Africanized) honey bee populations has been tracked with behavioural, morphological and biochemical studies[8–13]. In general, behavioural studies have shown that certain characters of the African race, especially those with adaptive value in the tropics (including swarming and absconding propensities, defensive behaviour, and opportunistic use of resources), are predominant within the Africanized population. Alloenzyme evidence for varying degrees of hybridization between African- and European-derived honey bee populations in the Neotropics has recently been reported[14,15]. Although the frequency of malate dehydrogenase allele Mdh^{100} is >0.95 in African *A. m. scutellata* and >0.90 in some neotropical Africanized populations[3,12,13], the mean frequency of Mdh^{100} in 118 Africanized colonies within the Brazilian state of Sao Paulo was ~0.78, indicating sources other than *A. m. scutellata* for some genes[14,15].

We investigated the genetic interaction between European- and African-derived honey bee populations in an area of geographical overlap in the New World. We hypothesized that hybridization should be most evident between areas most suitable ecologically and climatically for the two racial groups. In the Russian steppes, for example, an area of natural introgression occurs between the endemic ranges of two honey bee races (*A. m. mellifera* and *A. m. carnica* Pollman)[16]. In the temperate New World, portions of Argentina provide the best current areas of interface between African- and European-derived populations[17,18]. The northern portion of the country, as far south as the sugar cane-growing region of Tucuman, is an area saturated by Africanized honey bees (AHB)[17] to the complete exclusion of commercial beekeeping with European-derived populations. From Buenos Aires province southward, the country is considered to be free of Africanized honey bees and, after the arrival of these bees in Argentina in 1968, has become the main area for the production of European-type queens for commercial use[17]. The region between these two areas is regarded as a transition zone between the two racial types[17] (Fig. 1).

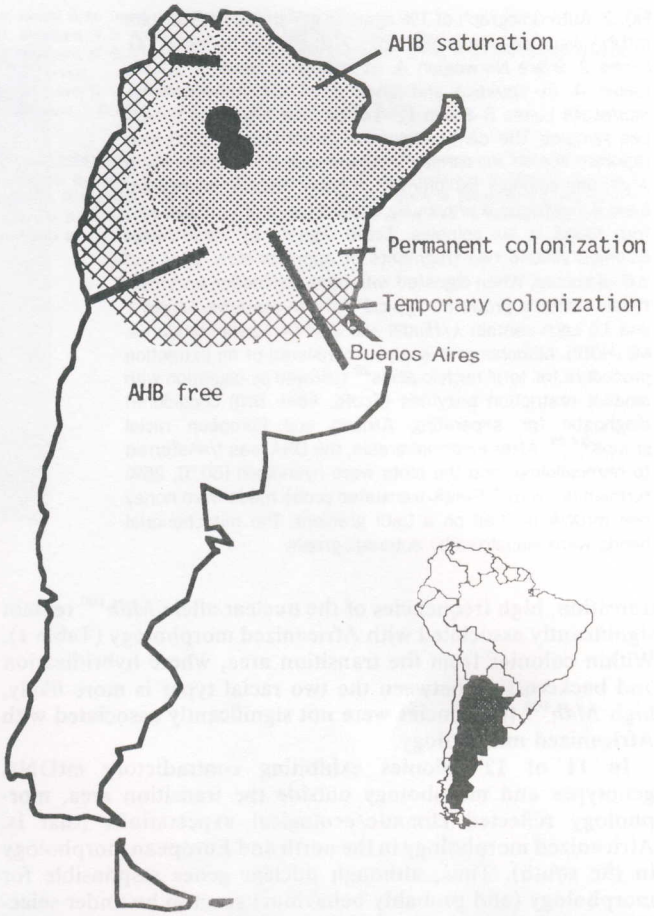

FIG. 1 Map of the collecting transects and published distribution of racial types in Argentina. Transects consisted of a northern Andean transect from Tucuman–Salta–Cachi, a southern Andean transect from Tucuman–San Juan–Uspallata and a southern transect from Tucuman–Cordoba–General Villejas–Chascamus. The major collections of African-derived populations were made near Tucuman, and are represented by the black circles. Honey bees taken from the 'permanent' and 'temporary' colonization regions constitute our transition zone samples. Samples of adult workers from 171 colonies and 44 locations were collected in liquid nitrogen from mtDNA analysis and ethanol for morphological analysis. Samples consisted entirely of feral honey bees to best reflect a measure of adaptation to local ecological conditions[27]. We defined as feral, colonies known to be free from requeening and other management techniques directly affecting the proportion of racial genotypes and included colonies living in bridges, trees, buildings, electric utility poles, fruit boxes and unmanaged beehives. Taken from ref. 17.

Mitochondrial DNA (mtDNA) genotypes of 106 feral colonies from the northern 'AHB-saturated' region and the southern 'AHB-free' region were consistent with expectations (Table 1). That is, 59 of 67 colonies in the north and 33 of 39 colonies in the south exhibited mtDNA restriction patterns indicative of African and European races, respectively (Fig. 2). Morphological analyses of the same colonies yielded similar results: 63 of 67 colonies in the AHB-saturated region have Africanized morphology and 30 of 39 colonies in the AHB-free zone display European morphology (Table 1). In the area of transition both mtDNA types were detected in relatively high frequency. The predominance of Africanized morphology in samples from this region suggests that the main interface may be south of previous estimates[17]. Additional samples are, however, required to determine the current position, shape and width of the transition zone and to allow estimation of the relative contributions of selection and dispersal to its maintenance[19].

In 28 colonies, including 16 of 65 sampled from the transition zone, mtDNA and morphological analyses gave contradictory indications. That is, colonies exhibited Africanized morphology and European mtDNA, or vice versa. In addition, isoenzyme analysis of these colonies showed that, outside the area of

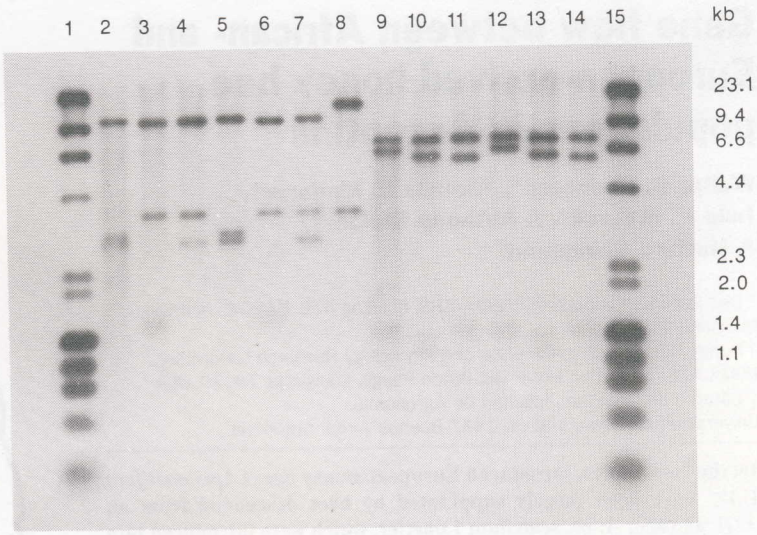

FIG. 2 Autoradiograph of 1% agarose gel showing honey bee mtDNA digested with *Eco*RI (lanes 2–8) and *Bcl*I (lanes 9–14). Lanes 2, 9 are Norwegian *A. m. mellifera*, lanes 3, 10 are Italian *A. m. ligustica* and lanes 4, 11 are Kenyan *A. m. scutellata*. Lanes 5–8 and 12–14 are feral Argentine honeybee samples. The 'carnica/ligustica' pattern (lanes 3, 10; also reported for *A. m. carnica*[3]) constituted 67% of the feral Argentine colonies exhibiting 'European' mtDNA haplotypes. Lane 8 illustrates a previously unreported *Eco*RI mtDNA pattern found in six colonies. *Eco*RI digests of these latter colonies yielded two fragments of approximately 14.0 and 3.8 kilobases. When digested with other restriction enzymes, these samples produced 'typical' African patterns. Lanes 1 and 15 each contain λ/*Hind*III and φX174/*Hae*III standards. METHODS. Mitochondrial analysis consisted of an extraction procedure for total nucleic acids[28] followed by digestion with several restriction enzymes (*Eco*RI, *Xba*I, *Bcl*I) considered diagnostic for separating African and European racial groups[3,4,29]. After electrophoresis, the DNA was transferred to nitrocellulose and the blots were hybridized (50 °C, 25% formamide) with ^{32}P-nick-translated probe made from honey bee mtDNA purified on a CsCl gradient. The mitochondrial bands were visualized by autoradiography.

transition, high frequencies of the nuclear allele Mdh^{100} remain significantly associated with Africanized morphology (Table 1). Within colonies from the transition area, where hybridization and backcrossing between the two racial types is more likely, high Mdh^{100} frequencies were not significantly associated with Africanized morphology.

In 11 of 12 colonies exhibiting contradictory mtDNA genotypes and morphology outside the transition area, morphology reflected climatic/ecological expectations (that is, Africanized morphology in the north and European morphology in the south). Thus, although nuclear genes responsible for morphology (and probably behaviour) seem to be under selection outside the transition area, no evidence was detected to support the speculation that European mtDNA interacts suboptimally with African nuclear DNA.

Natural selection against feral European maternal descendants is a reasonable assumption in tropical climates[7,20,21] given the known fitness and reproductive advantages of the Africanized population[10,22] and, together with population size disparities[6], may best explain the predominance of African mtDNA in Neotropical honey bees[3,4]. Further studies using markers to detect both maternal and paternal genetic contributions[23] may resolve the issue of mitochondrial–nuclear genotype interactions. But the possibility that Africanized populations originally dispersed in the Neotropics by occupying a virtually *Apis mellifera*-free niche, necessitates caution in interpreting putative changes in allele (marker) frequencies in the Neotropics, unless relative sizes of the interacting populations can be estimated.

These results demonstrate that a zone of complex hybridization exists between European- and African-derived racial types of honey bees in temperate Argentina. Gene flow between the racial types occurs with sufficient frequency in this zone for both mtDNA genotypes to be associated with a range of nuclear genotypes. Through backcrossing, mtDNA genotypes move with the 'opposite' morphology (nuclear genotype) into the latter's geographic region. These results are reminiscent of other subspecies and species hybrid zones, insofar as both mtDNA genotypes occur within geographically structured classes of nuclear genotype[24,25], although analysis of additional samples from the transition zone is required to determine the structure of the cline and classify genotype fitness relationships[26]. Further data from this established zone of hybridization in temperate Argentina may help predict the spread of Africanization in temperate North America. □

TABLE 1 Mitochondrial and morphological classification of Argentine honey bee colonies

Geographical zone cf. racial type*	Mitochondrial pattern		Morphology†		
			Africanized	Intermediate‡	European
Northern	African	59	58	1	—
'AHB concentration area'	European	8	5	—	3
Transition	African	46	31	13	2
	European	19	14	4	1
Southern	African	6	—	—	6
'AHB-free'	European	33	1	8	24

Twenty-eight colonies where mtDNA and morphological indications were contradictory were assayed also for variation in *Mdh* allele frequencies (20 workers per colony) using starch gel electrophoresis[27]. Colonies were classified as Africanized or European based on morphology, and the distribution of Mdh^{100} was tested by the Mann–Whitney *U*-test. Analysis of the 12 colonies from the AHB concentration area and AHB-free area revealed a significantly higher Mdh^{100} frequency in colonies with Africanized morphology (AHB Mdh^{100} frequency = 0.69, European Mdh^{100} frequency = 0.36; $P = 0.015$). However, there was no significant association of Mdh^{100} frequency with morphology when all 28 colonies were tested.

* From ref. 17.

† Morphological analysis was preceded by dissection, staining and slide-mounting 10 bees from each sample, according to the methods of Daly and Balling[8]. Slides were then digitized and the data analysed using two different programs written for the morphological determination of Africanization[8,30]. These programs use discriminant analysis, with functions derived from (1) South American Africanized populations; (2) 'European' populations from US apiaries; and (3) F_1 hybrids of African- and European-derived populations produced in Venezuela. Colonies were scored Africanized or European on the basis of a 95% probability of group membership as ascertained by both programs. In cases of less than 95% probability or discordant results among the programs, samples were scored as Intermediate, indicating noninclusion in the discriminated groups.

‡ Nondiscriminated, see †.

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