0014-4754/93/490530-10$1.50 + 0.20

Priority Papers

Mitochondrial DNA evidence for the 19th century introduction of African honey bees into the United States

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Received 10 March 1993; accepted 17 March 1993

Abstract. Since the introduction of an African subspecies into Brazil in the mid-1950's[1], descendent 'Africanized' honey bees (*Apis mellifera* L.) have spread throughout the Neotropics and into temperate North America. Restriction enzyme analysis of 422 feral honey bee colonies collected from non-Africanized areas in the southern United States revealed that over 21% of them had mitochondrial DNA (mtDNA) derived from a European race established in North America by the 17th century, 77% of them had mtDNA common in honey bees maintained by beekeepers and about 1% exhibited African mtDNA. Further analysis revealed that the African mtDNA was derived from a north African subspecies imported to the US in the 19th century.
Key words. Honey bee; *Apis mellifera*; mitochondrial DNA; colonization; subspecies; population genetics.

The geographic dispersion of Africanized honey bees (AHB) in the New World has been followed using a variety of behavioral, morphological, biochemical and molecular techniques[2–11]. Collectively, these studies suggest that in neotropical areas containing relatively few European bees, AHB spread rapidly and maintained many of the behavioral traits and biochemical and molecular markers of the African *A. m. scutellata* subspecies. In contrast, in the more temperate regions of Uruguay and Argentina, transitions from AHB to temperate-adapted bees of European ancestry occur[12]. These transition zones exhibit many of the characteristics of a hybrid zone, including morphological intermediates and evidence of backcrossing based on allozyme and mtDNA evidence[4,13,14]. As the Africanized bees move northward in the United States, they will encounter large populations of commercial and feral temperate-adapted European bees, and it is expected that they will also reach climatic limits. However, the potential limits of the AHB expansion in the US are very much a matter of conjecture[15–17].

There are 25 recognized geographic races of honey bees (*Apis mellifera* L.) endemic within Europe, Africa and the Middle East[18]. Historical records support the successful introduction of *A. m. mellifera* into Virginia in the 17th century[19] by settlers from England. During the period 1861–1922 seven additional races were introduced into North America[20]. These included: *A. m. ligustica* from Italy, *A. m. carnica* from Carniola (Hungary, former Yugoslavia, Bulgaria, Rumania), *A. m. cypria* from Cyprus, *A. m. syriaca* from what was then Palestine, *A. m. caucasica* from the Caucasus mountain region and two from Africa, *A. m. intermissa* from northwestern African (Morocco, Tunisia, Algeria and Libya) and *A. m. lamarckii* from Egypt[20,21]. There is also some indication that the honey bee race of Spain, *A. m. iberica*, may have been introduced[22]. Despite these early introductions, only three races (*ligustica*, *carnica*, and *caucasica*) are generally available and presumably constitute the current commercial population. Allozyme differences between feral and commercial gene pools have been reported[23], although the feral population is not well known.

To investigate the dynamics of AHB population spread in the US, it is necessary to characterize the genetic diversity of feral populations extant in the path of this expansion. In view of the establishment of AHB in southern Texas in late 1990, we initiated an investigation of genetic variation among feral populations in the southern US.

Materials and methods

We initially examined mitochondrial DNA (mtDNA) fragment patterns generated with the restriction endonuclease *Eco*R1 from 422 feral colonies collected from Alabama, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina and Texas (north of AHB intrusion) (fig. 1). Feral colonies were defined as colonies occurring in unmanaged homesites (trees, caves, bridges, buildings) or colonies collected by beekeepers from unmanaged sites and placed in hives without further management, such as queen replacement. Colonies exhibiting typical African *Eco*R1 haplotypes[2–5] were further analyzed using the enzyme *Hin*f1. Mitochondrial DNA analysis consisted of a total nucleic acid extraction[24] from several workers followed by digestion with *Eco*R1 or *Hin*f1 according to the manufacturer's instructions. After electrophoresis, the DNA was

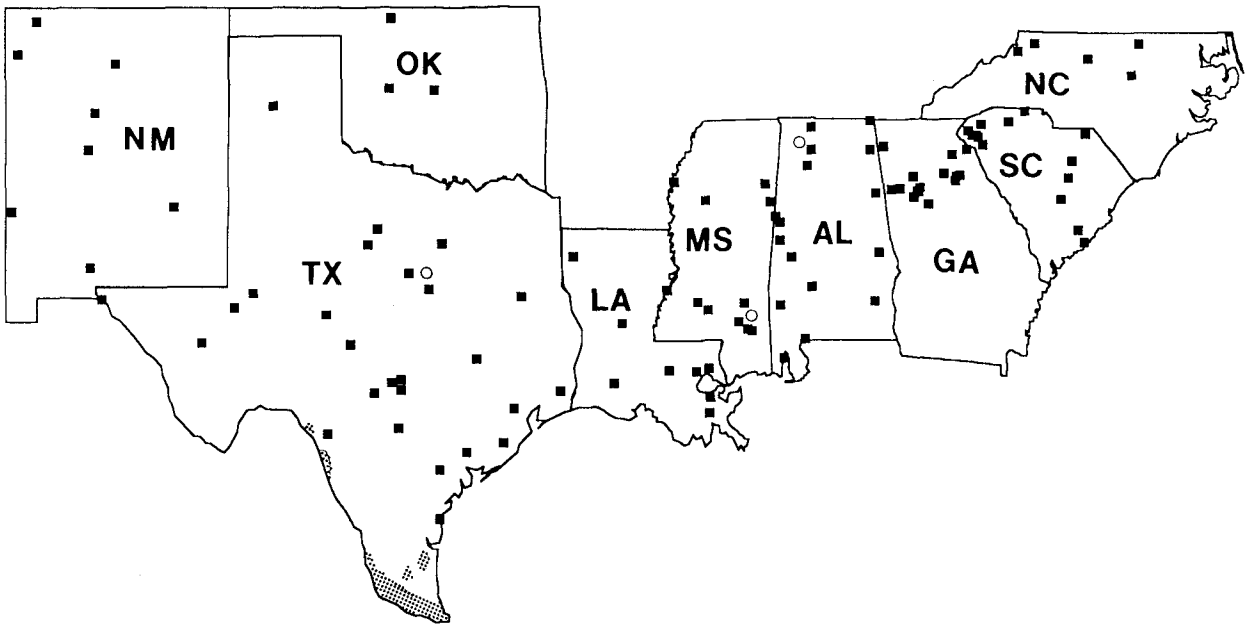

Figure 1. Collecting localities of feral colonies used in this study. The distribution of Africanized honey bees in the US at the time of the collection of the Texas samples is shown by the shaded area. Feral honey bee samples from Louisiana, New Mexico, Oklahoma and Texas were collected from August to October of 1991 and those from Alabama, Georgia, Mississippi, North Carolina and South Carolina were collected from July to August of 1992. Sites where samples with the *A. m. lamarckii* mtDNA pattern were collected are represented by open circles (Glen Rose, TX; Moselle, MS; Moulton, AL). All other sites are designated by closed squares.

transferred to nylon membrane and the blot hybridized with 32P nick translated probe made from honey bee mtDNA twice purified on a CsCl gradient. The mitochondrial bands were visualized by autoradiography. Morphometric analysis of selected colonies was performed using 2 discriminant analysis programs used to detect Africanization of New World populations[7,25].

Results and discussion

The majority of colonies exhibited *Eco*R1 fragment patterns typical of the Old World races *A. m. carnica* and *A. m. ligustica*, although approximately 21% exhibited the pattern indicative of *A. m. mellifera* and *A. m. iberica* (table). The large percentage of *carnica/ligustica* haplotypes probably reflects the influence of commercial colonies that have escaped since the mid and late 19th century introductions of these races. In addition, as descendents of imported *A. m. ligustica* constitute the majority of current US commercial honey bees, they probably continue to provide genetic input to feral populations. However, the *iberica/mellifera* markers found in 92 of the 422 feral colonies remain from 16th and 17th century importations.

Table. Mitochondrial DNA analysis of feral honey bee colonies from the southern United States

State	Distribution of haplotypes			total
	afr	mel/ibr	car/lig	
AL	1	14	24	39
GA	-	4	28	32
LA	-	10	41	51
MS	2	14	31	47
NC	-	3	27	30
NM	-	7	54	61
OK	-	-	3	3
SC-	-	21	59	80
TX	1	19	59	79
	4 (0.9)	92 (21.8)	326 (77.3)	422

Differentiation among African (afr), *A. m. mellifera/iberica* (mel/ibr), and *A. m. carnica/ligustica* (car/lig) haplotypes was based on EcoR1 restriction fragment length polymorphism. Percentages are given in parentheses.

Four colonies collected from Texas, Mississippi and Alabama exhibited an African mtDNA *Eco*R1 fragment pattern. Further analysis of these samples using the restriction enzyme *Hin*f1, which had been reported to differentiate *A. m. scutellata* (progenitor of the AHB) from *A. m. intermissa*[26], revealed that these samples were not derived from *A. m. scutellata* (fig. 2). The *Hin*f1 mitochondrial restriction pattern found in these four samples is previously unreported although, in a *Hin*f1 screening of 50 colonies of 5 African races, it was unique to *A. m. lamarckii* (n = 7; Sheppard et al. unpublished data), one of the North African subspecies known to have been introduced into the US in the 19th century[21]. Morphometric analyses of the 4 samples having African mitochondrial DNA determined them to be European. The combination of African mitochondrial DNA and European nuclear DNA (as indicated by European morphology) suggests an extensive history of backcrossing. The non-*scutellata* African mtDNA found in these 4 colonies indicates that the mitochondrial haplotype originated from at least one 19th century introduction.

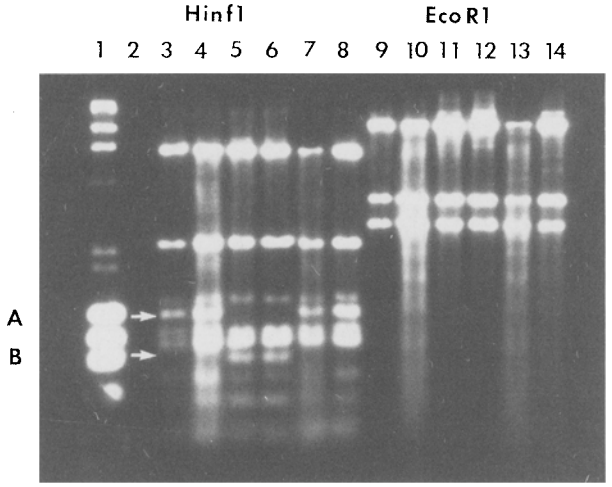

Figure 2. Autoradiograph (negative print) of 1.5% agarose gel showing honey bee mtDNA digested with *Hin*f1 (lanes 3–8) and *Eco*R1 (lanes 9–14). Lane 1 contains lambda/*Hin*dIII and PhiX 174/*Hae*III standards, lane 2 is blank, lanes 3–4 and 9–10 are Africanized bees from Brazil, lanes 5 and 11 are *A. m. lamarckii* from Egypt, lanes 6 and 12 are the sample from Glen Rose, Texas, lanes 7 and 13 are *A. m. intermissa* from Morocco, and lanes 8 and 14 are African *A. m. scutellata* from Kenya. All samples digested with *Eco*R1 share the same "African" haplotype. Arrows point to 2 *Hin*f1 fragments, A (1.3 Kb) and B (0.9 Kb), which distinguish *A. m. lamarckii* from *A. m. scutellata* and *A. m. intermissa* haplotypes. Fragment A is present in *A. m. scutellata* bees from Kenya, *A. m. scutellata*-derived Africanized bees from Brazil and *A. m. intermissa* from Morocco. Fragment B is present only in *A. m. lamarckii* and the US feral colonies exhibiting the African *Eco*R1 haplotype.

The probable racial source for these samples, the Egyptian honey bee, *A. m. lamarckii*, was first introduced into the eastern US in 1866[20]. Following this introduction, the African colonies swarmed and the new queens probably mated with European drones. After as few as four successive reproductive cycles, more than 90% of the African nuclear genes could be replaced by European genes. The non-recombining, maternally-inherited African mitochondrial DNA from the original queens remained present throughout these and subsequent generations. Similar scenarios probably explain the varied combination of mitochondrial and nuclear DNA markers of African and European origin found in transition zones between AHB and European-derived honey bees in Argentina and Uruguay, areas with high densities of European-derived populations in the Yucatan and some Africanized populations in neotropical Brazil[4,5,13,27,28]. Our findings demonstrate that future research on AHB population genetics in the United States must consider the diverse racial background of the introduced populations and the potential corresponding diversity of genetic markers. The assumption that a background of European genetic markers in North American populations is sufficient for comparisons with the expanding AHB population cannot be justified. Furthermore, the consequence of misidentifying the origin of 'African' genetic markers found in US populations, whether from the 1956 introduction of *A. m. scutellata* into Brazil or 19th century introductions of other African races into North America, is certainly significant for regulatory and monitoring efforts.

Acknowledgments. We thank the following for assistance in collecting bees: C. Adamek, L. Adair, J. Ambrose, J. Baker, W. Baker, M. Bassey, L. Beane, S. Blankenship, D. Boggs, J. Bradley, J. Brittingham, D. Burgess, T. Butler, T. Calhoun, W. Canada, L. Chapman, S. Dean, K. Delaplane, A. Dickson, A. Dietz, J. Elliot, M. Evans, T. Gaylord, R. Godwin, C. Guy, B. Halls, R. Hayes, W. Heard, M. Hood, S. Hummer, S. Hutto, J. Ingraham, P. Jackson, B. Jones, S. Kamel, J. Kelso, T. Kemp Jr., R. Krell, J. Lambert, T. Lane, R. Lenz, M. Lewis, R. Lovin, M. Marks, C. McCall, L. McEver, J. Mitchell, G. Moore, A. Morgan, M. Patrick, J. Polk, D. Pollet, P. Rawlings, W. Reddit, H. Reid, A. Rice, D. Richardson, L. Reynolds, C. Robinson, R. Rosson, D. Rush, K. Shacockis, F. Schoolcraft, J. Sharpland, H. Shelnutt Jr., S. Smith, E. Sugden, R. Taylor, J. Valkenburg, J. Villa, S. Waters, W. Watson, J. Williams, P. Williams, R. Williams, W. Wooten, the U.S. Army Corps of Engineers, the State Park Services of Alabama, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina and Texas and the National Park Service. We thank G. Gasparich, K. Hackett, B. McPheron and anonymous reviewers for commenting on earlier drafts of the manuscript.

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