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Evidence of African honey bee mitotypes in the southern United States prior to Africanization as revealed by mtDNA sequence data

Maria Alice Pinto, apinto@ipb.pt, Centro de Investigação de Montanha, Campus de Santa Aplónia, Apartado 172, Bragança, Portugal, Walter S. Sheppard, shepp@mail.wsu.edu, Washington State University, Department of Entomology, 166 FSHN Bldg, Pullman, WA, John S. Johnston, spencerj@tamu.edu, Texas A&M University, Entomology, Heep Center, College Station, TX, William L. Rubink, wrubink@rubink.com, USDA, Entomology, Weslaco, TX, Robert N. Coulson, r-coulson@tamu.edu, Texas A&M University, Knowledge Engineering Laboratory, Entomology Department, Heep Center, Texas A&M University, College Station, TX, and John C. Patton, jcpatton@wfscgate.tamu.edu, Texas A&M University, Department of Wildlife and Fisheries, College Station, TX.

The standard protocol for assessing honey bees with a mitotype derived from an African origin is the amplification of a segment of cytochrome b and subsequent digestion of the amplified fragment with the restriction enzyme Bgl II. A previous survey of 451pre-Africanization honey bees from the southern U. S. revealed three bees with restriction pattern consistent with an African mitotype. To confirm these bees truly represented mitotypes of African origin we developed a new primer pair for amplification of cytochrome b, which utilizes internal sequencing primers to allow high quality direct sequence products. Using this system we amplified a mtDNA fragment of ~1,200 base-pairs (bp), taht included most of cytochrome b, serine (UCA) tRNA, and a small portion (s) of the ND-1 gene (s). Honey bees from ten morphometrically identified Apis mellifera subspecies (42 honey bee workers, each representing a different colony) from Old World and three honey bee workers (each representing a different colony) from the southern United States exhibiting an African phenotype as revealed by BglII restriction enzyme analysis were sequenced. The analysis showed that two of the three honey bees were of eastern European ancestry. These bees had lost the BglII cut site by a first position (C->A) transversion mutation. The third honey bee was found to have a sequence of African clade bees. This defining substitution for the African clade was found to be a third position (T->C) transiton mutation.

Species 1: Hymenoptera Apidae *Apis mellifera* (honey bee)
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