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Short Communication

ANALYSIS OF AFRICANIZED HONEY BEE MITOCHONDRIAL DNA REVEALS FURTHER DIVERSITY OF ORIGIN

Walter S. Sheppard[1], Thomas E. Rinderer[2], Lionel Garnery[3] and Hachiro Shimanuki[4]

ABSRTACT

Within the past 40 years, Africanized honey bees spread from Brazil and now occupy most areas habitable by the species *Apis mellifera*, from Argentina to the southwestern United States. The primary genetic source for Africanized honey bees is believed to be the sub-Saharan honey bee subspecies *A. m. scutellata*. Mitochondrial markers common in *A. m. scutellata* have been used to classify Africanized honey bees in population genetic and physiological studies. Assessment of composite mitochondrial haplotypes from Africanized honey bees, using 4 base recognizing restriction enzymes and COI-COII intergenic spacer length polymorphism, provided evidence for a more diverse mitochondrial heritage. Over 25% of the "African" mtDNA found in Africanized populations in Argentina are derived from non-*A. m. scutellata* sources.

INTRODUCTION

Honey bees of the Americas descended from various Old World subspecies introduced over the past several hundred years. Within the endemic Old World range of this insect, more than 24 morphologically distinct subspecies are recognized (Ruttner, 1992; Sheppard *et al.*, 1997). The subspecies vary extensively in behavior, including dance language (v. Frisch, 1951; Boch, 1957) and reproduction (Seeley, 1985; Ruttner 1988), reflecting adaptations to myriad climatic and ecological conditions. Within the last several hundred years at least eight European, Middle Eastern and north African subspecies were introduced into North America (Sheppard, 1989), although the overall population is generally considered "European".

A sub-Saharan subspecies, *Apis mellifera scutellata*, was imported into Brazil in the mid - 1950's to improve honey production in neotropical conditions (Kerr, 1957). Populations of "Africanized" honey bees, expressing *scutellata*-like reproductive, foraging and defensive behavior (Stort, 1974; Collins *et al.*, 1982; McNally and Schneider, 1992; Schneider and McNally, 1992), spread from this area and at present exist from Argentina to the southwestern United States. *A. m. scutellata* is a subspecies highly adapted to tropical ecological conditions, a fact reflected in an estimated 25-67-fold increase in the number of honey bee colonies in some neotropical areas, following Africanization (Taylor Jr., 1985). Temperate climatic limitations are known in Argentina (Kerr *et al.*, 1982) and expected to occur in North America (Taylor Jr., 1985).

Controversy over the relative contribution by *A. m. scutellata* and other subspecies to the genetic makeup of Africanized honey bees has been engendered by apparent discordance of data from mitochondrial DNA (Smith *et al.*, 1989; Hall and Muralidharan, 1989; Sheppard *et al.*, 1991a,b) and allozymes (Lobo *et al.*, 1989; Del Lama *et al.*, 1990; Sheppard *et al.* 1991b; Lobo and Krieger, 1992) or morphology (Lobo *et al.* 1989; Sheppard *et al.*, 1991a; Rinderer *et al.*, 1993). Allozymic and morphological character analyses suggest that about 20 to 30% of the genes of established populations of Africanized honey bees are of European ancestry (Lobo *et al.*, 1989; Rinderer *et al.*, 1993), whereas mitochondrial DNA haplotypes from such populations have been assigned almost exclusively to *A. m. scutellata* (Smith *et al.*, 1989; Hall and Muralidharan, 1989; Sheppard *et al.*, 1991a,b). Hypotheses to explain the paucity of European mitochondrial DNA found in Africanized populations include subspecific differences in reproductive rates and other fitness parameters in the tropics, large differences in colony densities and asymmetrical fitnesses of hybrids with European or African matrilines.

MATERIAL AND METHODS

We analyzed samples from 120 colonies of honey bees collected from Argentina with mtDNA previously classified as originating from introduced *A. m. scutellata* based on methods widely used in population studies (Smith *et al.*, 1989; Hall and Muralidharan, 1989; Sheppard *et al.*, 1991a,b; Crozier *et al.*, 1991; Hall and Smith, 1991; Harrison and Hall, 1993). Samples of colonies from Morocco (34), Kenya (10), South Africa (45) and Spain (16) were analyzed for mtDNA comparison. All samples were taken from colonies located in feral homesites or apiaries initiated with captured swarms and unmanaged for queen replacement. Mitochondrial determinations for this study

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[1] *Department of Entomology, Washington State University, Pullman, WA 99164, USA. Send correspondence to W.S.S. Fax: 509-335-1009. E-mail: shepp@mail.wsu.edu*

[2] *USDA-ARS Honey Bee Breeding, Genetics and Physiology Research Laboratory, 1157 Ben Hur Rd., Baton Rouge, LA 79820, USA.*

[3] *Laboratoire Populations Genetique et Evolution, Bat. 13 Avenue de la Terrasse, 91198 Gif-Sur-Yvette, France.*

[4] *USDA-ARS Bee Research Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA.*

reflect composite haplotype profiles based on three restriction enzymes and a size polymorphism in the COI-COII intergenic spacer (Garnery *et al.*, 1993; Sheppard *et al.*, 1996). Published methods of mtDNA analysis and details of naming the composite haplotypes are given in Table I.

RESULTS AND DISCUSSION

Individually, none of the markers were discriminatory when applied to the set of honey bee colonies from Argentinean, Iberian, North African or sub-Sahara African sources.

However, based on the composite haplotype approach, mtDNA from the 120 colonies of honey bees collected in Argentina was composed of a mixture of haplotypes most likely derived from both sub-Saharan and North African honey bee subspecies (Table I). Over 25% of the Africanized colonies from Argentina expressed a composite haplotype (ALBA), that was found in north African honey bees, but not in sub-Saharan *A. m. scutellata.* This raises the possibility that the proportion of haplotypes reported to originate from *A. m. scutellata* may have been overestimated in other New World populations, as well. If true, this could partially explain discrepancies among studies based on allozyme, morphological and mtDNA data. In our limited sampling of Old World populations, we found the haplotype (ALBA) only in colonies of the subspecies *A. m. intermissa* from Morocco. However, given that several studies report clinal variation or evidence of hybridization between the bees of North Africa and Spain (Smith *et al.*, 1991; Garnery *et al.*, 1995; Sheppard *et al.*, 1996), the possibility exists that the ALBA pattern arrived in Argentina with early Spanish settlers.

Table I
Composite mtDNA haplotypes* shared among Old World, African and Iberian honeybees.

mtDNA Haplotype	Argentina	Spain	Morocco	Kenya	S. *Africa*
AACD	2			6	
ABBA	7			2	2
ABCD	6			1	11
AHBA		1	5		
AICD	70				21
AIBA			2		1
ALBA	32		4		
Unshared†	3	15	23	1	10
Total colonies	120	16	34	10	45

*Four-letter composite mitochondrial DNA haplotype (e.g. ALBA) derived as: A = "African" *Eco*R1 haplotype (Smith *et al.*, 1989; Hall and Muraliharan, 1989; Sheppard *et al.*, 1991a,b), L = *Hin*F1 pattern "L" (Sheppard *et al.*, 1996). Other second letter designations denote arbitrarily named recognizable *Hin*F1 patterns (Sheppard *et al.*, 1996), B = COI-COII intergenic spacer size polymorphism PQ or PoQ (Garnery *et al.*, 1993). Other third letter designations from Garnery *et al.* (1993), A = Q, C = PQQ or PoQQ and D = PQQQ or PoQQQ and final letter A = pattern A_1 from *Dra*1 digestion of amplified intergenic spacer (other fourth letter designations denote arbitrarily named recognizable *Dra*1 digestion patterns of amplified COI-COII intergenic spacer (Garnery *et al.*, 1993), B = A_2, C = A_3, D = A_4 or $A_{4'}$, E = A_7 or $A_{7'}$, F = A_8, G = A_9, H = A_{16}, I = A_{17}, J = A_{22}).

† Unshared haplotypes and number of colonies were distributed as follows: Argentina, ABCG (1), AUDC (2); Spain, AHBI (1), AHCB (9), AHCD (1), AICB (1), AIDC (3); Morocco, AHBF (5), AHCG (12), AMBF (5), ANBA (1); Kenya, AACJ (1); South Africa, AABD (1), ABDE (1), ABDD (3), AKBD (1), AKCD (1), ALBD (1), ALCD (1), AWBA (1).

Reduced flight capacity or metabolism in hybrids has been suggested as an explanation for the predominance of African mtDNA in neotropical honey bee populations (Harrison and Hall, 1993). Mitochondrial classification of honey bee populations in this and another metabolic study (Harrison *et al.*, 1996) assumed an *A. m. scutellata* origin for the African mitochondrial haplotype detected. Similarly, numerous population studies on Africanized honey bees (Smith *et al.*, 1989; Hall and Muralidharan, 1989; Sheppard *et al.*, 1991a,b; Rinderer *et al.* 1991; Burgett *et al.*, 1995) and published methods for molecular identification of Africanized honey bees (Crozier *et al.*, 1991; Hall and Smith, 1991) regard African mtDNA detected in New World populations to be *A. m. scutellata* in origin. Further studies of Africanized honey bee mtDNA should be cognizant of the potential for erroneous assignment of subspecific parental origins, unless a combination of appropriate tests are used.

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RESUMO

Nos últimos 40 anos, abelhas africanizadas se espalharam a partir do Brasil e agora ocupam a maioria das áreas habitáveis pela espécie *Apis mellifera*, da Argentina ao sudoeste dos Estados Unidos. Acredita-se que a fonte genética primária das abelhas africanizadas seja a subespécie subsaariana de abelha *A. m. scutellata.* Marcadores mitocondriais comuns em *A. m. scutellata* têm sido usados para classificar abelhas africanizadas em estudos de fisiologia e genética de população. A avaliação de haplótipos mitocondriais compostos em abelhas africanizadas, usando 3 enzimas de restrição e um polimorfismo de comprimento no espaçador intergênico "COI-COII", evidenciou uma herança mitocondrial mais diversa. Mais de 25% do mtDNA "africano" encontrado em populações africanizadas na Argentina são derivados de fontes não relacionadas a *A. m. scutellata.*

REFERENCES

Boch, R. (1957). Rassenmässige Unterschiede in den Tänzen der Honigbiene (*Apis mellifica* L.). *Z. vgl. Physiol. 39*: 289-320.

Burgett, M., Shorney, S., Cordara, J., Gardiol, G. and **Sheppard, W.S.** (1995). The present status of Africanized honey bees in Uruguay. *Am. Bee J. 135*: 328-330.

Collins, A.M., Rinderer, T.E., Harbo, J.R. and **Bolton, A.B.** (1982). Colony defense by Africanized and European honey bees. *Science 218*: 72-74.

Crozier, Y.C., Koulianos, S. and **Crozier, R.H.** (1991). An improved test for Africanized honeybee mitochondrial DNA. *Experientia 47*: 968-969.

Del Lama, M.A., Lobo, J.A., Soares, A.E.E. and **Del Lama, S.N.** (1990). Genetic differentiation estimated by isozymic analysis of Africanized honey bee populations from Brazil and from Central America. *Apidologie 21*: 271-280.

Frisch, K. von (1951). Orientierungsvermögen und Sprache der Bienen. *Naturwissenschaften 38*: 105-112.

Garnery, L., Solignac, M., Celebrano, G. and **Cornuet, J.-M.** (1993). A simple test using restricted PCR-amplified mitochondrial DNA to study the genetic structure *of Apis mellifera* L. *Experientia 49*: 1016-1021.

Garnery, L., Mosshine, E.H., Oldroyd, B.P. and **Cornuet, J.-M.** (1995). Mitochondrial DNA variation in Moroccan and Spanish honey bee populations. *Mol. Ecol. 4*: 465-471.

Hall, G.H. and **Muralidharan, K.** (1989). Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. *Nature 339*: 213-215.

Hall, H.G. and **Smith, D.R.** (1991). Distinguishing African and European honey bee matrilines using amplified mitochondrial DNA. *Proc. Natl. Acad. Sci. USA 88*: 4548-4552.

Harrison, J.F. and **Hall, H.G.** (1993). African-European honeybee hybrids have low nonintermediate metabolic capacities. *Nature 363*: 258-259.

Harrison, J.F., Fewell, J.H., Roberts, S.P. and **Hall, H.G.** (1996). Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science 274*: 88-90.

Kerr, W.E. (1957). Introdução de abelhas africanas no Brasil. *Bras. Apícola 3*: 211-213.

Kerr, W.E., Del Rio, S. de L. and **Barrionuevo, M.D.** (1982). The southern limits of the distribution of the Africanized honey bee in South America. *Am. Bee. J. 122*: 196-198.

Lobo, J.A. (1995). Morphometric, allozymic and mitochondrial variability of Africanized honeybees in Costa Rica. *Heredity 75*: 133-141.

Lobo, J.A. and **Krieger, H.** (1992). Maximum likelihood estimates of gene frequencies and racial admixture in *Apis mellifera* L. (Africanized honeybees). *Heredity 68*: 441-448.

Lobo, J.A., Del Lama, M.A. and **Mestriner, M.A.** (1989). Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L.). *Evolution 43*: 794-802.

McNally, L.C. and **Schneider, S.S.** (1992). Seasonal cycles of growth, development and movement of the African honey bee, *Apis mellifera scutellata*, in Africa. *Insectes Soc. 39*: 167-179.

Rinderer, T.E., Stelzer, J.A., Oldroyd, B.P., Buco, S.M. and **Rubink, W.L.** (1991). Hybridization between European and Africanized honey bees in the neotropical Yucatan peninsula. *Science 253*: 309-311.

Rinderer, T.E., Oldroyd, B.P. and **Sheppard, W.S.** (1993). Africanized bees in the U.S. *Sci. Am. 269*: 52-58.

Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees.* Springer-Verlag, Berlin.

Ruttner, F. (1992). *Naturgeschichte der Honigbienen.* Ehrenwirth, München.

Schneider, S.S. and **McNally, L.C.** (1992). Seasonal patterns of foraging activity in colonies of the honey bee, *Apis mellifera scutellata*, in Africa. *Insectes Soc. 39*: 181-193.

Seeley, T.D. (1985). *Honeybee Ecology.* Princeton Univ. Press, Princeton.

Sheppard, W.S. (1989). A history of the introduction of honey bee races into the United States. *Am. Bee J. 129*: 617-619 & 664-667.

Sheppard, W.S., Rinderer, T.E., Mazzoli, J., Stelzer, J.A., and **Shimanuki, H.** (1991a). Gene flow between African- and European-derived honey bee populations in Argentina. *Nature 349*: 782-784.

Sheppard, W.S., Soares, A.E.E. and **DeJong, D.** (1991b). Hybrid status of honey bee populations near the historic origin of Africanization in Brazil. *Apidologie 22*: 643-652.

Sheppard, W.S., Rinderer, T.E., Meixner, M.D., Yoo, H.R., Stelzer, J.A., Schiff, N.M., Kamel, S.M. and **Krell, R.** (1996). *Hin*F1 variation in mitochondrial DNA of Old World honey bee races. *J. Hered. 87*: 35-40.

Sheppard, W.S., Arias, M.C., Grech, A. and **Meixner, M.D.** (1997). *Apis mellifera ruttneri*, a new honey bee subspecies from Malta. *Apidologie 28*: 287-293.

Smith, D.R., Brown, W.M. and **Taylor, O.R.** (1989). Neotropical Africanized bees have African mitochondrial DNA. *Nature 339*: 213-215.

Smith, D.R., Palopoli, M.F., Taylor, B.R., Garnery, L., Cornuet, J.-M., Solignac M. and **Brown, W.M.** (1991). Geographical overlap of two mitochondrial genomes in Spanish honeybees (*Apis mellifera iberica*). *J. Hered. 82*: 96-100.

Stort, A.C. (1974). Genetic study of aggressiveness of two subspecies of *Apis mellifera* in Brazil. 2. Time at which the first sting reached the leather ball. *J. Apic. Res. 14*: 171-175.

Taylor Jr., O.R. (1985). African bees: potential impact in the United States. *Bull. Entomol. Soc. Am. 31*: 14-24.

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