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ORIGINAL RESEARCH ARTICLE

An update of Africanization in honey bee (*Apis mellifera*) populations in Buenos Aires, Argentina

María L. Genchi García^{a,b,c,*}, Francisco José Reynaldi^{b,d}  and Claudio Marcelo Bravi^{a,d} 

^aInstituto Multidisciplinario de Biología Celular (CCT-La Plata CONICET, CIC-PBA, UNLP), La Plata, Argentina; ^bCátedra de Virología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina; ^cComisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA), La Plata, Argentina; ^dCCT-CONICET La Plata, La Plata, Argentina

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European and African honey bee populations have been separated and influenced by different environments. Furthermore, *Apis mellifera* is the only species of honey bees that evolved in Europe, the Middle East, and Africa, where several subspecies are recognized. *A. m. scutellata* was introduced in Brazil in 1956, resulting in the spread of African bees throughout South and Central America. The aim of the present study was to determine the prevalence and geographical distribution of Africanized bees in Buenos Aires province (Argentina) employing a mtDNA cytochrome b polymorphism. A total of 430 colonies were sampled between 2013 and 2014; eighteen out of them (4,2%) belonged to African lineage. Our results confirm that European haplotypes are the most prevalent in Buenos Aires Province, and that the process of African gene introgression remains stable since 2005.

Actualización del estado de africanización en poblaciones de abejas melíferas (*Apis mellifera*) en Buenos Aires, Argentina

Las poblaciones de abejas melíferas europeas y africanas han estado separadas e influenciadas por distintos ambientes. Por ello, *Apis mellifera* es la única especie de abejas que evolucionó en Europa, Medio Oriente y África, donde pueden reconocerse varias subespecies. La introducción de *A. m. scutellata*, en Brasil en 1956 resultó en la expansión de la abeja africana a lo largo de Centroamérica y Sudamérica. El objetivo del presente estudio fue determinar la prevalencia y distribución geográfica de las abejas africanizadas en la provincia de Buenos Aires (Argentina), utilizando un polimorfismo mitocondrial de la región del citocromo b. Fueron muestreadas 430 colonias entre 2013 y 2014, de las cuales 18 (4,2%) resultaron positivas para linajes africanos. Nuestros resultados confirman que los haplotipos europeos son los más prevalentes en la provincia de Buenos Aires. El proceso de introgresión de genes africanos en esta región se ha mantenido estable desde 2005.

Keywords: Africanization; honey bees; *Apis mellifera*; mitochondrial DNA; Buenos Aires; Argentina

Introduction

European and African honey bee populations have been separated and influenced by different environments. Mutations and natural selection favored adaptations to different conditions, which shaped them and generated variation in their morphological, physiological, and behavioral characteristics. This isolation and adaptations to different environments resulted in different honey bee subspecies or ecotypes adapted to a particular ecological region. *Apis mellifera* is the only species of honey bee that evolved in Europe, the Middle East, and Africa, where several subspecies are recognized (Ruttner, 1988).

In 1956, in an attempt to study whether African honey bees (*A. m. scutellata*) and their hybrids would be better adapted to tropical climates and improve honey harvest, queens were imported from South Africa to Brazil (Kerr, 1967). But this introduction resulted in the spread of African bees throughout South, Central, and North America. The hybridization process is complex and became frequent between African and European honey bees resulting in asymmetrical gene introgression

(Clarke, Rinderer, Franck, Quezada-Euán, & Oldroyd, 2002; Kraus, Franck, & Vandame, 2007; Lobo Segura, 2000; Moritz, Härtel, & Neumann, 2005; Pinto et al., 2005; Schneider, Degrandi-Hoffman, & Smith, 2004). According to that, Africanized bees have retained a predominantly African genotype due to the fact that existed a major flow of African genes towards European bees, that in inverse sense (Kraus et al., 2007).

Africanized bees are one of the most biologically successful invasive species (Caron, 2001) because they have colonized and prevailed in more than 20 American countries (Guzmán-Novoa, Correa Benítez, Espinosa Montaña, & Guzmán Novoa, 2011). Africanization gradually replaced European honey bee populations by populations with African characteristics (Caron, 2001; Collet, Ferreira, Arias, Soares, & Del Lama, 2006; Dávila, Ortiz Menéndro, & De Huiza, 1980; Guzmán-Novoa et al., 2011; Lobo Segura, 2000; Salamanca Grosso, 2009).

The genetic background of the Argentinian honey bee populations is mainly European, although the

*Corresponding author. Email: ml.genchigarca@gmail.com

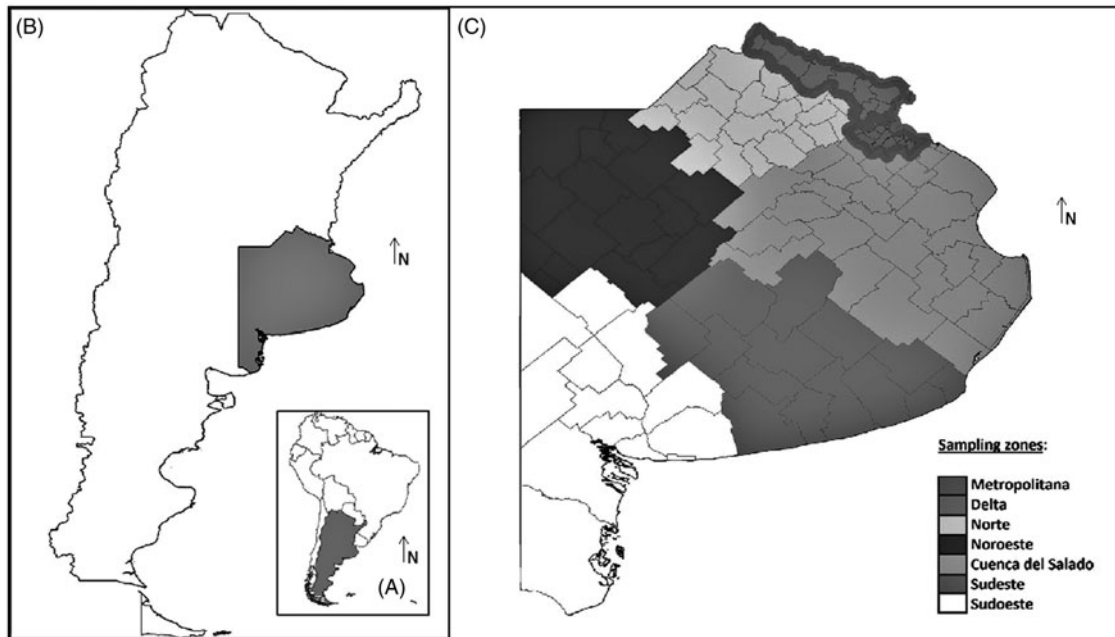


Figure 1. (A) Argentina, (B) Buenos Aires province, (C) distribution of sampling zones in Buenos Aires Province. Africanized areas are highlight.

Africanization process has reached the country. Particularly, the southern limit of Africanization is around 35°S latitude (Abrahamovich, Atela, De La Rúa, & Galián, 2007) beyond which only European honey bees exist, with temporary migrations of Africanized swarms during summer.

Honey bees are invaluable to commercial agriculture, propagation of plants through pollination, and human society by producing various products (Allen-Wardell et al., 1998). Beekeeping activities have increased in Argentina and nowadays is one of the main honey producers and exporters of the world. In this sense, the province of Buenos Aires holds 65% of the total number of hives for honey production.

Several methods of molecular and morphological analyses have been used to study Africanization, such as microsatellites (Pinto et al., 2005; Rangel et al., 2016), restriction analyses of mitochondrial DNA (Branchiccela et al., 2014; Dominguez-Ayala et al., 2016; Kono & Kohn, 2015; Quezada-Euán, Pérez-Castro, & May-Itzá, 2003; Rangel et al., 2016), sequencing (Branchiccela et al., 2014; Kono & Kohn, 2015), and morphometry (Branchiccela et al., 2014; Dominguez-Ayala et al., 2016; Kono & Kohn, 2015; Quezada-Euán et al., 2003). However, analyses of the non-recombining mitochondrial DNA (mtDNA) has been widely employed (Cornuet & Garnery, 1991; Pinto et al., 2003), building upon the fact that mtDNA studies uncovered that *A. mellifera* evolved in three major geographical branches. Secondary contacts between branches resulted into introgression areas (Smith, 1991).

The aim of the present study was to determine the prevalence and geographical distribution of Africanized bees in Buenos Aires province employing mtDNA

cytochrome b polymorphism. This information allows us to update the southern limit of the process of Africanization in Buenos Aires, Argentina, determined by maternal lineage

Materials and methods

Buenos Aires province was divided into seven sampling zones: Metropolitana, Delta, Sudeste, Sudoeste, Cuenca del Salado, Norte, and Noroeste (Figure 1). A total of 430 colonies were sampled during autumn, winter, and spring of 2013 and 2014. Samples were stored at -20°C in 96% ethanol before DNA extraction. *Varroa destructor* levels were quantified according to the World Organization for Animal Health validated method (World Organization for Animal Health [OIE], 2008). Total DNA was isolated from the thorax of two bees per colony with DNAzol[®] Reagent (Invitrogen[™], Massachusetts, USA), according to the manufacturer protocol.

Mitochondrial DNA analysis was carried out according to Pinto et al. (2003). Briefly, a 485 bp fragment of the *cytochrome b* gene was amplified with primers 5'-TAT GTA CTA CCA TGA GGA CAA ATA TC-3' and 5'-ATT ACA CCT CCT AAT TTA TTA GGA AT-3' (Crozier, Koulianos, & Crozier, 1991). PCR was performed in 50 μl total volume containing 5 μl of DNA with a temperature profile modified from Pinto et al. (2003)—initial denaturalization of 94°C for 45 s, 35 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 2 min. The product was digested with *Bgl*III (Promega, Wisconsin, USA) using the conditions recommended by the supplier. Total digestion volumes were then electrophoresed on

a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. African and European bees were distinguished by means of *Bgl*II RFLP patterns: while amplicons derived from non-African mtDNAs lineages carry a *Bgl*II recognition site that results in two fragments of 194 and 291 bp after digestion, those derived from bees carrying *A. m. scutellata* mtDNA remained undigested.

Results

In line with previous results, 412 out of 430 (95.8%) Buenos Aires colonies analyzed belonged to non-African bees. The colonies derived from African bees were found to coexist with the European ones in just two of the seven areas, Metropolitana and Delta. In relation to *V. destructor* levels, 56% of the samples (241/430) were free of *V. destructor*, 20% (86/430) had less than 1%; 21% (90/430) had 1–4%, and 3% of the samples (13/430) had between 4% and 20%. Particularly, Sudeste and Sudoeste Regions presented 86–90% of hives with levels below 1%, while, Metropolitana, Delta, and Noroeste had 54–56% with more than 1% of *V. destructor* (García et al., 2014).

Discussion

Previous mtDNA surveys of 300 Buenos Aires feral colonies sampled in 2005–2006 reported 5.02% of African maternal lineage (Abrahamovich et al., 2007). We have found a similar percentage of the 4.2% of colonies carrying African mitotype eleven years later. Moreover, we both observed that Africanized populations were found mainly in the north-east of Buenos Aires province. This scenario is in stark contrast with what has been reported in other Latin America countries such as Colombia (Salamanca Grosso, 2009), Brazil and Uruguay (Branchiccela et al., 2014; Collet et al., 2006; Nelson, Wallberg, Simões, Lawson, & Webster, 2017), Costa Rica (Lobo Segura, 2000), Venezuela (Clarke, Oldroyd, Javier, Quezada-Euán, & Rinderer, 2001), Peru (Dávila et al., 1980; Quezada-Euán et al., 2003), and Mexico (Dominguez-Ayala et al., 2016; Guzmán-Novoa et al., 2011), where the African haplotypes were found to be more prevalent.

According to Büchler et al. (2014), the interaction of genotype and environment is an indicator for local adaptation and fitness. Therefore, the southern Africanization limit could be related to the temperature and precipitation indexes in the zone, and confirm a border of the process located around 35°S latitude (Abrahamovich et al., 2007). Likewise, Kono and Kohn (2015) pointed a similar limit around 35.5°N latitude for the USA. In line with Randi (2008), the conservation of honey bees with successful adaptations to a particular ecological region should be a priority. Conservation of genetic diversity of honey bees is important to preserve

a high genetic adaptability of populations in different zones (Büchler et al., 2014).

Our results confirm that European haplotypes are the most prevalent in Buenos Aires Province. The process of African gene introgression remains stable since 2005. Although the southern expansion of Africanized bees may be restricted by environmental factors, it should be noted that cultural factors are also at play since local beekeepers tend to select negatively for aggressive behavior, and purchase of European queens for replacements are restricted to certified breeders.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Francisco José Reynaldi  <http://orcid.org/0000-0002-1531-4905>

Claudio Marcelo Bravi  <http://orcid.org/0000-0002-2499-4471>

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