

ORIGINAL RESEARCH ARTICLE



Assessment of the mitochondrial origin of honey bees from Argentina

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Summary

The molecular diversity of the honey bee *Apis mellifera* in the province of Buenos Aires (Argentina) has been analysed through the study of the mitochondrial DNA. The mitochondrial haplotype corresponding to the intergenic region tRNA^{leu}-COII has been determined in samples from 300 colonies from 150 apiaries distributed in 71 localities of the province of Buenos Aires. Eight different haplotypes have been found, four of the African, three of the West European and one of the East European evolutionary lineages. The frequency of these haplotypes corroborates that the European yellow honey bee *Apis mellifera ligustica* is predominant in the Buenos Aires province whereas the black European bee *A. m. mellifera* is rare. The presence of the African haplotypes A1 and A4 common in Africanized honey bees, confirms a border of the africanization process located around the 30°–35° SL parallels. The other two African haplotypes (A8 and A11) are indicative of an Iberian or North African origin of some colonies. The influence of the transhumance and beekeeping practices is discussed in relation to the genetic variability detected.

Valoración del origen mitocondrial de las abejas de Argentina

La diversidad molecular de la abeja melífera *Apis mellifera* de la provincia de Buenos Aires (Argentina) ha sido analizada a partir del estudio del ADN mitocondrial. El haplotipo mitocondrial correspondiente a la región intergénica tARN^{leu}-COII ha sido determinado en muestras de 300 colonias de 150 apiarios distribuidos en 71 localidades de la provincia de Buenos Aires. Se han encontrado ocho haplotipos diferentes, cuatro del linaje evolutivo africano, tres del europeo occidental y uno del oriental. La frecuencia de estos haplotipos corrobora que la abeja amarilla europea *Apis mellifera ligustica*, es predominante en la provincia de Buenos Aires, mientras que la abeja negra europea *A. m. mellifera*, es escasa. La presencia de los haplotipos africanos A1 y A4 comunes en abejas africanizadas, confirma la presencia de una barrera al proceso de africanización localizada alrededor de los paralelos 30°–35° SL. La presencia de otros dos haplotipos africanos A8 y A11, indica que algunas colonias tienen origen ibérico o norteafricano. Se discute la influencia de la trashumancia y de las prácticas apícolas en relación con la variabilidad genética detectada.

Keywords: *Apis mellifera*, Argentina, Buenos Aires, mitochondrial DNA, haplotype, genetic variability, Africanization.

Introduction

Beekeeping activities have increased in Argentina since the beginning of the last century and nowadays this country is one of the main honey producers and exporters of the world (Hervias & Moggi, 2005). In this sense the province of Buenos Aires holds 65 % of the total number of hives for honey production (SAGPyA, 2006). The origin of the Argentinian honey bee populations is mainly

European, although the africanization process has reached the Northern side of the country. Africanized honey bees spread from Brazil and now occupy most of the areas suitable for the species *Apis mellifera*, from Argentina to the south-western United States.

Argentinian beekeepers traditionally chose the better honey producers looking at the external appearance of the honey bee, i.e., the colour of the abdomen, but a proper morphometrical analysis for the identification of the *A. mellifera* subspecies is lacking despite the

preliminary work of De Santis *et al.* (1983). At present, the data generated by molecular analysis of the mitochondrial DNA molecule (Garnery *et al.* 1993) are being widely used to identify *A. mellifera* subspecies (De la Rúa *et al.* 2005 and references therein) and therefore to clarify the origin of those subspecies introduced in the New World (Sheppard *et al.* 1999; Clarke *et al.* 2001; Pinto *et al.* 2004). 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, and provided evidence of a more diverse mitochondrial heritage. In fact, Sheppard *et al.* (1999) in their study of 120 honey bee colonies from Argentina concluded that over 25% of the 'African' mtDNA found in Africanized populations in Argentina are derived from non-*A. m. scutellata* sources.

In this study we have determined the genetic diversity using the mitochondrial polymorphism at the intergenic tRNA^{leu}-COII region, of honey bee populations in Buenos Aires, the largest and most important (in terms of beekeeping activities) province of Argentina. The characterization of the mitochondrial haplotype of these honey bee colonies follows several objectives: first, to test the origin of the honey bee populations occurring in this area, second, to determine the level and extent of Africanization of the Argentinian honey bees and third, to characterize the honey bees used by the beekeepers that sell queens to other localities and provinces in Argentina.

Materials and Methods

Sampling

A total of 300 colonies were sampled from May 2005 to February 2006. Two randomly colonies were chosen from 150 apiaries in 71 localities in the Buenos Aires province (Figure 1). Bees were immediately killed by immersion in absolute ethanol and kept at -20°C until they were processed in the laboratory. One individual worker colony bee has been analysed as representative of its source colony.

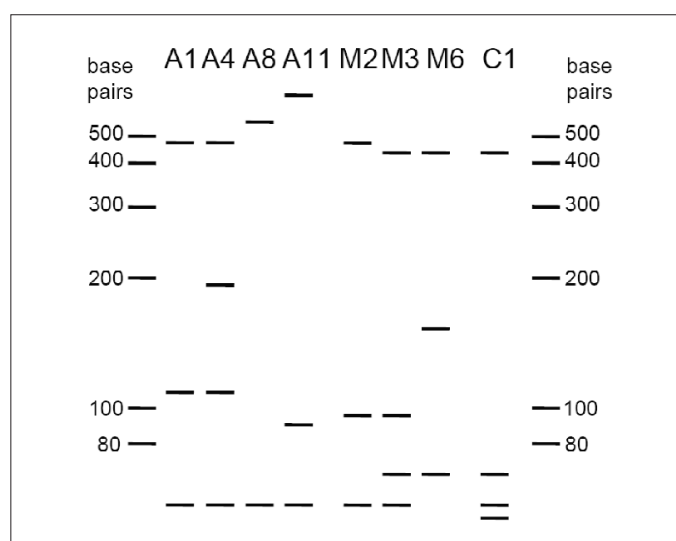


Fig 1. Illustration of restriction site fragments generated by *Dral* digests of the mitochondrial intergenic region (following Garnery *et al.* 1993) in the Argentinian honey bee populations.

DNA extraction

DNA isolation was performed following the Chelex method (Walsh *et al.* 1991) with slight modifications. One or two worker legs (one worker bee per colony) were dried for 30 min at 37°C and then homogenized with 100 µL of 5 % Chelex solution and proteinase K (10 mg/ml). This mix was incubated for 1 h at 55°C, 15 min at 99°C, 1 min at 37°C, and finally 15 min at 99°C in a thermocycler. Two µL of this solution were used for the PCR amplification.

Analysis of the mitochondrial polymorphism

The intergenic tRNA^{leu}-COII region was amplified and digested following Garnery *et al.* (1993), with the primers E2 located at the 5'-end of the tRNA^{leu} gene, and H2 located at the 5'-end of the COII gene. The size of the amplified products was determined after electrophoretic separation on 1.5% agarose gels. Ten µL aliquots of the PCR products were digested with five units of the *Dral* enzyme at 37°C for 4–12 h. The resulting fragments were visualized in 5% agarose (Nusieve) stained with ethidium bromide.

Results

Argentinian honey bee populations belong to three evolutionary lineages, as they bear A, M and C haplotypes. In total eight different haplotypes have been detected (Figure 1 and Table 1). Four of them correspond to the African evolutionary lineage (A1, A4, A8 and A11) and are present at a low frequency (5.02 %). Three belong to the West European lineage (M2, M3 and M6, 2.68 % of the total), and one to the East European lineage (C1); the C1 haplotype is the most frequent and widespread (92.31 %). The African haplotypes A1 and A4, present in the 3.34 % of the samples, have been observed at a high frequency in African subspecies including *A. m. scutellata* and also in Africanized honey bee populations from Mexico (Franck *et al.* 2001; Clarke *et al.* 2001). The haplotype A8 (0.33 %) is present in *A. m. iberiensis* and also in *A. m. intermissa*, whereas the haplotype A11 (1.34 %) is exclusive of the Atlantic populations of *A. m. iberiensis* (De la Rúa *et al.* 2006), reflecting that the possible origin of these honey bees could be the Iberian Peninsula. Among the M haplotypes, M2 and M6, present in 1 and 0.67 % of the sampled colonies respectively, have been observed in *A. m. mellifera* populations from France, but M3 present in 1 % of the Argentinian colonies, has been found only in *A. m. iberiensis* (Garnery *et al.* 1998, Cánovas *et al.* submitted). The C1 haplotype corresponds to the East-European evolutionary lineage that includes the Italian honey bee *A. m. ligustica* and also the Carniolan honey bee (*A. m. carnica*) and the Buckfast strain (Table 1).

Table 1. Haplotype frequency and presence of each haplotype in the *A. mellifera* evolutionary lineages and subspecies.

Mitochondrial haplotype	Frequency	Evolutionary lineage and subspecies
A1	2.68	African lineage (including Africanized honey bees)
A4	0.67	African lineage (including Africanized honey bees)
A8	0.33	African lineage (<i>A. m. iberiensis</i> , <i>A. m. intermissa</i>)
A11	1.34	African lineage (<i>A. m. iberiensis</i>)
M2	1.00	West European lineage (<i>A. m. mellifera</i>)
M3	1.00	West European lineage (<i>A. m. iberiensis</i>)
M6	0.67	West European lineage (<i>A. m. mellifera</i>)
C1	92.31	East European lineage (<i>A. m. carnica</i> , <i>A. m. ligustica</i> , Buckfast strain)

Discussion

The analysis of the mitochondrial DNA of the Argentinian honey bee population from Buenos Aires indicates that the matrilineal origin of these honey bees is European, mainly from subspecies belonging to the East European evolutionary lineage. Among these subspecies are *A. m. ligustica* or Italian honey bee, *A. m. carnica* or carniolan honey bee, and also the hybrid Buckfast strain. Although the molecular analysis performed in the present work, does not discriminate among these subspecies, according to the information provided by the beekeepers, these honey bee populations come mainly from Italy.

The high frequency of the haplotype characteristic of these honey bees (C1) is not surprising as Argentinian beekeepers have imported queens from Europe since the beginning of beekeeping activities in the country, coincident with the extensive human immigrations from Europe from 1900 onwards.

A selection based on the yellowish colour of the abdomen of the Italian queens is performed by local beekeepers with the aim of maintaining the high productivity and gentle character of these bees.

The honey bee colonies bearing African haplotypes are located mainly at the North and East sides of the province but at a very low frequency. These results fully agree with those from Diniz *et al.* (2003) about the genetic structure of honey bee populations from southern Brazil and Uruguay. In that investigation they concluded that the distribution limit of the Africanized honey bee colonies was located in northern Uruguay and that a transition area existed farther south in Uruguay. In concrete the natural limit to the Africanization process seems to be around parallels 35° to 40° SL, as proposed by Sheppard *et al.* (1999) in a previous analysis of samples from Argentina, therefore the province of Buenos Aires is situated exactly within this limit. Recently other nuclear markers as the SNPs (single nucleotide polymorphism) have confirmed the existence of two parallel transects in northern Argentina that revealed transitions from predominantly African genotypes at the north to predominantly C group genotypes at the south (Whitfield *et al.* 2006).

On the other hand the honey bees characterized by the African haplotypes could have an Iberian or even a North-African

origin, as they have been also observed in *A. m. iberiensis* and *A. m. intermissa* populations. This hypothesis fits in the case of the haplotypes A8 and A11 as these two haplotypes has been exclusively observed in honey bees from the southern Iberian Peninsula and northern Africa. Whether these introductions date back to 18th century introductions by Spanish settlers or have been recently imported, remains unresolved and needs to be clarified by further investigations.

In forty-one out of the 150 sampled apiaries, beekeepers perform queen rearing to sell honey bee queens and also honey bee brood to other professional beekeepers. All the colonies belonging to these apiaries did show the Italian haplotype except in two cases, in which one of the two colonies analysed per apiary had an African haplotype (A1). Therefore in these two apiaries Italian and Africanized (or Iberian derived) honey bees coexist.

The colonies characterized with the M haplotypes could have two possible European origins: either these colonies belong to the *A. m. mellifera* subspecies that is present in the Old World from France to Scandinavia and also in the United States, or come from the Iberian Peninsula where the northern *A. m. iberiensis* bear these haplotypes.

Transhumance practices are frequently performed by professional beekeepers that move their colonies to the surrounding provinces and also within the Buenos Aires province. 41% of the total professional beekeepers that contributed with samples to the present study moved their colonies during the last beekeeping season. These movements could influence the genetic composition of the transhumant colonies especially if they are transported to areas with a higher level of Africanization where the queen of the transhumant colonies could be replaced by Africanized queens.

Our results confirmed the homogeneous genetic profile of the Argentinian honey bee population from the province of Buenos Aires and that the Africanization process could have reached their limit as more extreme climatic conditions seem to act as natural barriers against the expansion of Africanized bees. The saturation of Italian queens performed by the beekeepers through selection and trade off may also act as an artificial barrier to this process.

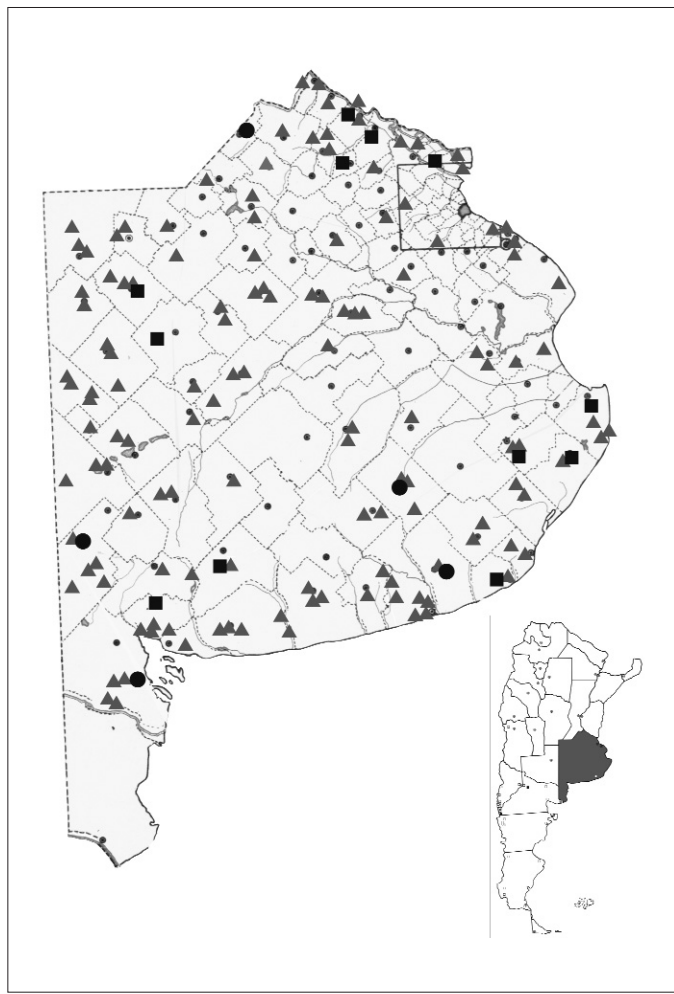


Fig 2. Distribution of the sampled apiaries through the Buenos Aires province (Argentina). A triangle indicates honey bee colonies belonging to the C evolutionary lineage, dots those corresponding to the M evolutionary lineage and squares to the African evolutionary lineage.

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