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SCIENTIFIC NOTE

INTRODUCTION OF DIFFERENT LINEAGES OF *AEDES AEGYPTI* IN ARGENTINA

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ABSTRACT. Based on sequence analysis of the mitochondrial gene ND4, we determined the presence in Argentina of 3 haplotypes representing different *Aedes* (*Stegomyia*) *aegypti* lineages previously identified in other countries of the Americas, Africa, and Asia. Haplotype 17, the most frequent in Argentina, was previously detected in Brazil. Haplotype 7, restricted in our study to the northwest of Argentina and Bolivia, was formerly found in low frequency in the USA, Brazil, Mexico, and Senegal. Also haplotype 11, belonging to a different haplogroup than the other two, was observed in the present study; it had been reported before in Africa and Asia, but not in the Americas. The coexistence of haplotypes belonging to divergent haplogroups supports the hypothesis of multiple introductions of the species in Argentina.

KEY WORDS *Aedes aegypti*, mtDNA lineages, dengue

The tropical and subtropical mosquito *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) is the most important vector of several arboviruses, including yellow fever and dengue, which cause between 50 and 100 million new infections in the world every year (Gubler 2004). From January to June 2009, a dengue outbreak spread widely throughout Argentina, causing 5 deaths and 27,752 infections in 13 provinces (National Ministry of Health 2009). *Aedes aegypti* was considered eradicated from Argentina in 1964, as the result of a continental control campaign organized by the Pan-American Health Organization (Ousset et al. 1967); however, reinfestation through the northeastern region was reported in 1986 (Boffi 1998). High population densities of *Ae. aegypti* in most of the northern and central provinces of Argentina, the constant introduction of different dengue serotypes from neighboring countries, and the low immunity levels in a large part of the human population are alarming factors that could cause future epidemics in the region. In the present study we examined the distribution of mitochondrial lineages of *Ae. aegypti* in Argentina, using the ND4 gene fragment previously chosen for similar studies performed in other countries from America, Asia, and Africa (Da Costa-da-Silva et al. 2005, Bracco et al. 2007).

The larvae of *Ae. aegypti* were collected during summer 2007 through summer 2008 in 9 cities from Argentina, 1 from Bolivia, and 1 from Uruguay, as listed in Table 1. Samples were taken from artificial water containers, transported to the laboratory in 90% ethanol, and finally stored at −20°C. Homogenization and DNA extraction were performed as described in Rondan Dueñas et al. (2009). A 336-base-pair (bp) fragment of the mitochondrial gene ND4 was amplified by polymerase chain reaction (PCR), using primers ND4F (5′-ATT GCC TAA GGC TCA TGT AG-3′) and ND4R (5′-TCG GCTTCC TAG TCG TTC AT-3′) as described in Bracco et al. (2007). Amplifications were carried out in 50-µl reactions with 2 mM Mg^{++}, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.5 mM of each primer, 0.2 mM of each dNTP, 2 units of DNA *Taq* polymerase (GE Healthcare/Amersham, Little Chalfont, Buckinghamshire, United Kingdom), and 1 µl of template DNA. The PCR temperature profiles consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min, and final extension at 72°C for 7 min.

Analyses were carried out on 336-bp of the mtDNA ND4 sequences from 60 *Ae. aegypti* individuals collected in 11 localities of Argentina, Bolivia, and Uruguay. Nucleotide sequences were checked in chromatograms using Chromas (McCarthy 1996) and then were aligned using the Clustal algorithm in MEGA 4.0 (Tamura et al. 2007). A BLASTn search was performed in National Center for Biotechnology Information GenBank database to confirm the identity of our sequences in relation to others corresponding to the *Ae. aegypti* ND4 gene. The DNA polymorphism was screened using DnaSP software (Rozas et al. 2003). The characterization of 3 haplotypes gave an estimated nucleotide diversity of $\pi = 0.00936$; the observed haplotipic diversity (*Hd*) was 0.434. This value was similar to the one obtained in previous analyses performed in Thailand and Perú ($\pi = 0.008$) (Bosio et al.

Table 1. Distribution and frequency of the mitochondrial ND4 haplotypes in *Aedes aegypti* populations from Argentina, Bolivia (Yacuiba), and Uruguay (Fray Bentos).

Sampling locality	Geographic coordinates	Haplotype (*n*)
Córdoba	31°23′S, 64°10′W	11 (1), 17 (4)
Buenos Aires	34°36′S, 58°22′W	17 (5)
Avellaneda	29°06′S, 59°39′W	11 (1), 17 (5)
Rafaela	31°15′S, 61°29′W	11 (1), 17 (5)
Clorinda	25°17′S, 57°43′W	17 (5)
Puerto Iguazú	25°35′S, 54°34′W	17 (5)
Tartagal	22°31′S, 63°47′W	7 (3), 17 (2)
Santiago del Estero	27°47′S, 64°16′W	17 (6)
Tucumán	26°56′S, 65°16′W	17 (6)
Yacuiba (Bolivia)	22°02′S, 63°40′W	11 (1), 7 (5)
Fray Bentos (Uruguay)	33°07′S, 58°18′W	11 (4), 17 (2)

2005, Da Costa-da-Silva et al. 2005). Haplotype frequencies and geographic distribution are shown in Fig. 1. The sequences were analyzed together with those reported by Bracco et al. (2007) for several American, Asian, and African localities, and were included in the haplotype network published by the authors, following the nomenclature used in their study. Two of the 3 haplotypes detected in our study (11 and 17) were placed in the same haplogroup in the network. Haplotype 17 showed the highest frequency (83.33%), and it was represented in almost all the localities except Yacuiba (Bolivia). In previous studies, this haplotype was detected with a broad distribution in Brazil (Bracco et al. 2007), but it was absent from Mexico (Gorrochotegui-Escalante et al. 2002) and Peru (Da Costa-da-Silva et al. 2005). The fact that this haplotype was observed in high frequency in Argentina, and in the previous study in Brazil, reinforces the idea of important levels of genetic exchange between and within both countries, proposed by Rondan Dueñas et al. (2009). Haplotype 7 was found only in Yacuiba and Tartagal; in former studies, it was detected in low frequency in the USA, Mexico, Brazil, and Senegal (Gorrochotegui-Escalante et al. 2002, Bracco et al. 2007, Lima Júnior and Scarpassa 2009). Bracco et al. (2007) suggested that the group containing this haplotype might have been introduced from the USA and/or Venezuela throughout other American countries as a consequence of a long-distance colonization of populations that persisted after the continental control campaign. Haplotype 11 was found in low frequency in the Argentinean localities of Córdoba, Avellaneda, Rafaela, and also in Yacuiba (Bolivia) and Fray Bentos (Uruguay). This haplotype had not been reported in the Americas before, but it was detected in Africa (Guinea and Uganda) and Asia (Singapore) (Bracco et al. 2007). Haplotypes representing introductions from different geographical origins have also been reported by other authors using the same molecular marker in populations from Peru (Da Costa-da-Silva et al. 2005) and Venezuela (Herrera et al. 2006).

On the basis of sequence analyses of different mitochondrial genes, Mousson et al. (2005) dealt with a multiple introductions hypothesis of *Ae. aegypti* in America. The authors proposed that South American populations were established from different founding lineages, represented by successive colonization waves before and after the massive eradication programs. In the Americas, control operations were interrupted during the 1970s, most likely before achieving the total eradication of the species. Consequently, the genetic diversity originally imported through early invasions might have persisted, at least partially, depending on the rate of local success of the control campaigns.

Although the haplotype diversity obtained for the ND4 gene in our study was relatively low, haplotype 7 is separated from haplotypes 11 and 17 by 11 and 14 mutational steps, respectively. In Argentina, the coexistence of haplotypes belonging to divergent haplogroups, shared with populations from the ancestral geographic distribution range, is in agreement with a hypothesis of multiple introductions of the species and persistence of relictual populations after the continental control campaign, rather than a rapid genetic local differentiation scenario.

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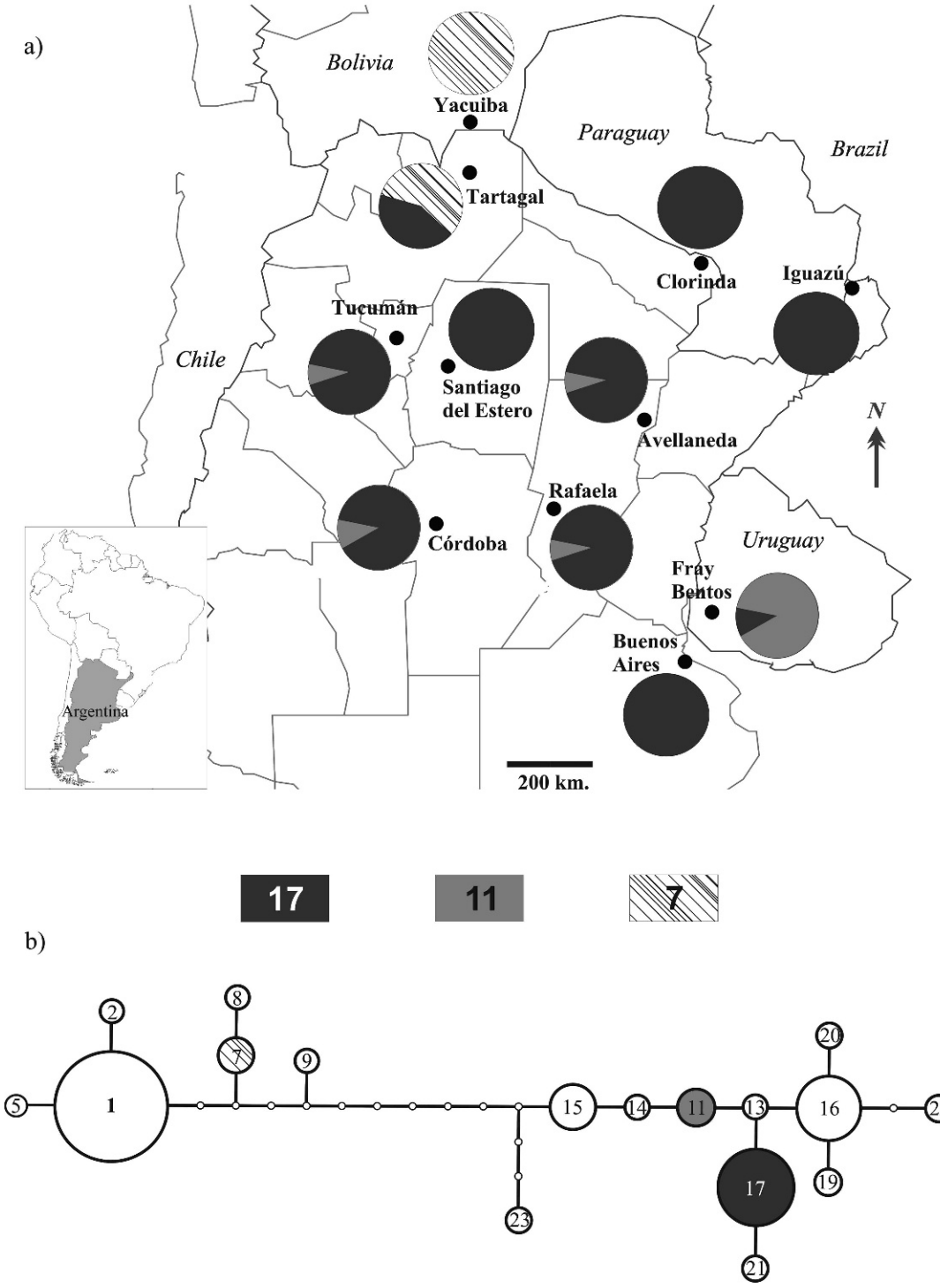

Fig. 1. (a) Partial map of Argentina showing the distribution of ND4 haplotypes in the populations analyzed in our study; each haplotype is identified by a number (7, 11, 17) and its frequency is proportional to the assigned area within each circle. (b) Position of the observed haplotypes (7, 11, 17) in the network was reported by Bracco et al. (2007). Each circle corresponds to a haplotype (1 to 22), and the size is proportional to its frequency. Each line represents a mutational step, and white dots correspond to hypothetical haplotypes not found in the sampled individuals.

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