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Short Communication

Molecular characterization of *Aedes aegypti* (L.) (Diptera: Culicidae) of Easter Island based on analysis of the mitochondrial ND4 gene



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ABSTRACT

*Aedes aegypti* mosquitoes are the main vector of viruses Dengue, Zika and Chikungunya. Shortly after the first report of the dengue vector *Ae. aegypti* in Easter Island (Rapa Nui) in late 2000, the first disease outbreak dengue occurred. Viral serotyping during the 2002 outbreak revealed a close relationship with Pacific DENV-1 genotype IV viruses, supporting the idea that the virus most likely originated in Tahiti. Mitochondrial NADH dehydrogenase subunit 4 (ND4) DNA sequences generated from 68 specimens of *Ae. aegypti* from Easter Island reporting a unique finding of a single maternal lineage of *Ae. aegypti* on Easter Island.

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Dengue is by far the most prevalent mosquito-borne arboviral disease, with millions of cases throughout the tropical world each year (Carrington and Simmons, 2014), the antropophilic mosquito *Aedes aegypti* is the principal vector. The *Ae. aegypti*, originally an Afrotropical forest mosquito, adapts readily to artificial breeding containers in domestic environments. As a result, the species has been introduced into many tropical and subtropical countries of the world through human commerce and movement (Brown et al., 2013). From the 16th century onwards, the species invaded the Americas (Lounibos, 2002). The species was quick to colonize the New World, and this was accompanied by repeated and devastating yellow fever epidemics in seaports (Powell and Tabachnick, 2013). From the mid-19th century onwards, it became widespread and common in much of India and southeast Asia. Dispersal of *Ae. aegypti* in the Polynesian islands, between 1924 and 1986 has been attributed in large part to the construction of new airports and consequential movement of goods and people between the islands.

Easter Island (Rapa Nui) is a relatively small, Polynesian island in the southeastern Pacific Ocean, at the southeastern most point of the Polynesian Triangle. Easter Island covers an area of 163.6 km<sup>2</sup> with a population of 5761 persons. The *Ae. aegypti* was first reported from Easter Island in 2000 (Perret et al., 2003). This discovery was

shortly followed by the Islands' first outbreak of Dengue fever in 2002. Between January and May 2002, some 636 cases of Dengue fever (affecting 11% of the total population) were positively verified through epidemiological nexus and serological testing (Olea, 2003; Perret et al., 2003). Molecular characterization and phylogenetic analysis of the Easter Island infections concluded that this outbreak was due to one serotype, most closely related to the Pacific DENV-1 genotype IV viruses (Cáceres et al., 2008).

Herein, we undertake the first genetic analysis of *Ae. aegypti* on Easter Island using of the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene. The ND4 gene has proven useful for genetic analysis of population structure and colonization events for species of *Aedes aegypti* (Urdaneta-Marquez et al., 2008).

In this study, we analyzed 68 specimens of *Ae. aegypti* (larvae or adults) were collected in two years (2007 and 2011), randomly on Easter Island, mainly in and around the town of Hanga Roa (27°09'S, 109°25'W) in artificial containers near homes.

Genomic DNA was extracted from 68 specimens of *Ae. aegypti* using the commercially available DNeasy Blood & Tissue Kit (QIAgen®, USA). Amplification of the NADH dehydrogenase subunit 4 mitochondrial DNA gene (ND4) was carried out using the ND4F & ND4R primers and amplification protocol of Bracco et al. (2007). PCR products were cleaned, diluted and sequenced in both directions in the Laboratory of Molecular Genetics of the Public Health Institute of Chile. Chromatograms were edited and aligned in the BioEdit Software version 7.0 (Hall, 1999). Verification

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of the correct target sequence was carried out using the individual consensus sequences using the online NCBI Basic Local Alignment Search Tool (BLAST)). Unique haplotypes were identified using DNA Sequence Polymorphism (DnaSP) software v.5.0 (Librado and Rozas, 2009). Haplotype network was then generated using the algorithm Median Joining Network Software v.4.6.0.0 (Bandelt et al., 1999), to establish the relationships of ND4 haplotypes of *Ae. aegypti* from Easter Island with other populations of the species.

The ND4 sequences obtained in this study were novel and were deposited in NCBI GenBank (GenBank Accession Number: KR052993 to KR053060) comprised one single maternal haplotype, indicating extremely low genetic variability, most probably due to a single point source introduction event. To better understand the possible origin of the introduction of *Ae. aegypti* to Easter Island, were selected 40 sequences from GenBank representing samples collected in America, South East Asia and Polynesia, and compared this with Chilean sample. Easter Island samples show only 1 of the 28 haplotype obtained, and it was shared with the haplotype found in Thailand, America and some countries of South East Asia. The Chilean samples shared haplotypes with samples collected in Brazil, Mexico, Thailand, North America and global sequence corresponding to the Tahiti/Cambodia/Singapore/Brazil samples.

This work reports a unique finding of a single maternal lineage of *Ae. aegypti*. To try to better understand the possible origin of the samples tested from Easter Island, there is not funded genetic variability, which could be explained by the geographical isolation of the island. The presence of single haplotype founded suggests that has been only one introduction event of *Ae. aegypti* on Easter Island.

Although the mitochondrial ND4 gene is widely used for analyzing intraspecific genetic study, recent studies have been reported NUMT (nuclear mitochondrial DNA) in *Ae. aegypti* that can alter the conclusions drawn if they are either analyzed as mitochondrial copies (Hlaing et al., 2009). Therefore, it would be useful in future studies using nuclear markers, allowing a better understanding of the genetic structure of populations of *Ae. aegypti* of Easter Island campaigns help to optimize the monitoring and eradication of this species, which is relevant to prevent further outbreaks of Dengue on the Easter Island.

## Conflicts of interest

The authors declare no conflicts of interest.

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