

EVOLUTIONARY HISTORY OF TIGER MOSQUITO, *Aedes albopictus*, IN ITS NATIVE RANGE IN ASIA

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INTRODUCTION

The Asian Tiger Mosquito *Aedes albopictus*, is one of the 100 most invasive species in the world and a vector of human diseases. In the last 30 years, human activities, in particular the trade of used tires, have fostered the spread of this species from its native range in East Asia to Africa, Europe and the Americas. While the modern invasion of *Ae. albopictus* has been the focus of great interest by the scientific community, the population history of the species in its native range is still not understood. Here we analysed genetic variation at mitochondrial DNA markers with the aim to investigate phylogeographic structure and historical demography of the native populations of *Ae. Albopictus*. At this aim individuals from 18 localities across the native range of the species (Table 1) have been analysed for sequence variation at mitochondrial DNA genes encoding for the cytochrome oxidase I (COI) and nicotinamide adenine dinucleotide (ND5).

RESULTS

Seventy haplotypes were found, defined by 65 nucleotide substitutions. Overall haplotype and nucleotide diversity estimates were 0.954 (\pm 0.007) and 0.0030 (\pm 0.0017), respectively.

Phylogenetic analysis (Fig.1) showed the occurrence of two main clusters of haplotypes, which displayed a striking geographic pattern: all the individuals collected from mainland Asia (samples from 1 to 16, Table 1) belonged to the cluster I, while all the individuals from Indonesia belonged to the cluster II (samples 17-18, Table 1). SAMOVA also supported the occurrence of two main groups of populations corresponding respectively to mainland Asia and Indonesian populations (for $K = 2$ FCT = 0.63), while within mainland Asia no further phylogeographic structure was observed.

Code	Locality (Country)	Lat.	Long.	Sample size	h	π
1.	Choral uji (Japan).	34°21'N	133°29'E	14	0.472 \pm 0.136	0.0006 \pm 0.0005
2.	Tanaga Shima (Japan)	35°56'N	129°45'E	15	0.371 \pm 0.153	0.0006 \pm 0.0005
3.	Nagasaki (Japan)	33°02'N	129°49'E	4	-	-
4.	Okinawa (Japan)	26°51'N	127°43'E	11	0.509 \pm 0.100	0.0010 \pm 0.0008
5.	Hanguhou (China)	30°16'N	120°11'E	7	0.286 \pm 0.200	0.0006 \pm 0.0005
6.	Zhenyuan (China)	27°13'N	108°18'E	10	0.533 \pm 0.095	0.0004 \pm 0.0004
7.	Shanton (China)	23°22'N	116°40'E	8	0.893 \pm 0.111	0.0019 \pm 0.0013
8.	Taoyuan (Taiwan, China)	23°12'N	120°50'E	11	0.800 \pm 0.114	0.0012 \pm 0.0008
9.	Vinh Phur (Vietnam)	21°18'N	105°34'E	15	0.933 \pm 0.050	0.0023 \pm 0.0014
10.	Hanoi (Vietnam)	20°55'N	105°51'E	13	0.950 \pm 0.042	0.0014 \pm 0.0010
11.	Phuntsholing (Bhutan)	26°34'N	89°16'E	11	0.946 \pm 0.053	0.0021 \pm 0.0014
12.	Gelephu (Bhutan)	26°36'N	90°31'E	10	0.844 \pm 0.103	0.0018 \pm 0.0012
13.	Chiang Mai (Thailand)	18°51'N	98°36'E	13	0.923 \pm 0.050	0.0022 \pm 0.0013
14.	Lampang (Thailand)	18°41'N	99°47'E	10	0.933 \pm 0.077	0.0022 \pm 0.0014
15.	Ratchaburi (Thailand)	13°10'N	99°50'E	3	-	-
16.	Songkhla (Thailand)	6°48'N	100°35'E	13	0.539 \pm 0.161	0.0007 \pm 0.0005
17.	Toili (Indonesia)	1°06'S	122°49'E	10	0.822 \pm 0.097	0.0013 \pm 0.0009
18.	Semarang (Indonesia)	5°14'S	119°27'E	15	0.514 \pm 0.116	0.0007 \pm 0.0006

Table 1. Geographical origin, sample size, haplotype diversity, h (\pm SD), and nucleotide diversity, π (\pm SD) for the 18 sampled populations of *Aedes albopictus*.

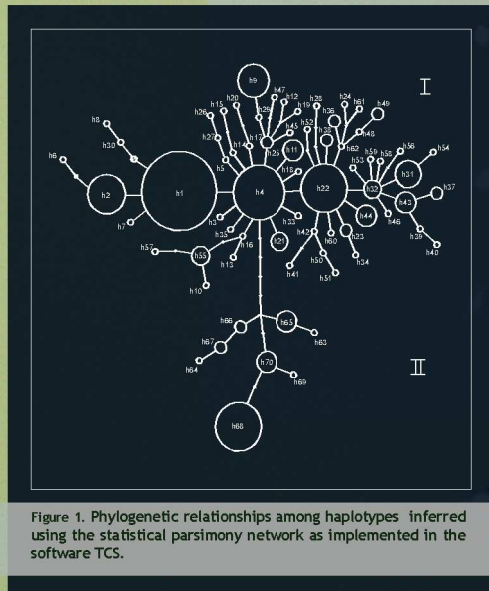


Figure 1. Phylogenetic relationships among haplotypes inferred using the statistical parsimony network as implemented in the software TCS.

Demographic analyses showed the signature of past demographic expansion for the populations on mainland Asia. Indeed, the mismatch distribution, computed as implemented in the software DnaSP 5.0, appeared smooth and bell-shaped (Fig. 2a) and the raggedness index ($r = 0.02$, $P = 0.04$) supported a fitting of the observed mismatch distribution to the expected distribution under the sudden expansion model. The time since expansion started for mainland Asian populations, was estimated from the parameter tau ($= 2\mu t$). Assuming $\mu = 1 \times 10^{-8}$ the expansion event would have taken place from roughly 35,000 years ago. No signature of past demographic growth was evidenced for Indonesian populations (mismatch distribution appeared multi-modal, Fig. 2b, and $r = 0.08$, $P = 0.48$).

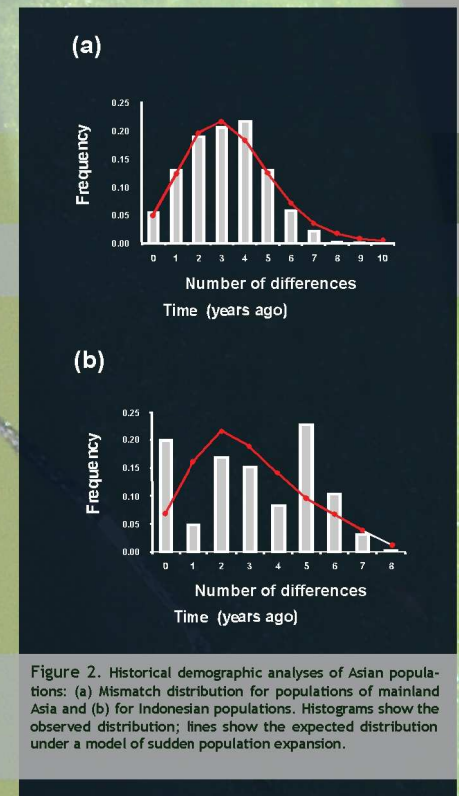


Figure 2. Historical demographic analyses of Asian populations: (a) Mismatch distribution for populations of mainland Asia and (b) for Indonesian populations. Histograms show the observed distribution; lines show the expected distribution under a model of sudden population expansion.

References

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DISCUSSION

Sequence analyses revealed non-negligible genetic diversity levels, changing the view that native populations of *Ae. albopictus* harbour little or no mitochondrial genetic diversity (Birungi & Munstermann, 2002 and reference therein). The pattern of geographic variation of mitochondrial genetic diversity observed (i.e. significant differentiation between Asian and Indonesian populations and no phylogeographic structure across all mainland Asian) is also consistent to the patterns observed at nuclear markers in previous studies conducted using allozymes (Kambhampati *et al.*, 1991; Urbanelli *et al.*, 2000). Further sampling throughout Indonesia is required to investigate the possible existence of further lineages in Indonesian islands, as well as their relationships with the Asian lineage. On the other hand, the lack of phylogeographic structure observed in mainland Asia could be explained by the past population expansion inferred. On the basis of the geographic distribution of genetic diversity and of the genealogical relationships between haplotypes (Table 1, Fig. 1), it can be hypothesized that, from a source area located approximately in the Indochinese Peninsula, the species would have experienced both demographic and spatial expansion, colonizing the temperate areas (China and Japan).

The time span in which the expansion was inferred, encompasses part of the last glacial phase (including the Last Glacial Maximum, 18,000-14,000 years ago) and the post glacial phase (since 14,000 years ago). In tropical regions, during glacial phase, the glacio-eustatic depression of the sea level fully exposed the Sunda shelf, which increased the habitat potentially available for the species. In temperate regions genetic and archaeological data suggest the occurrence of a south-north expansion into the East Asia mainland of the human populations after the LGM (Soares *et al.*, 2008). Because of the adaptability of *Ae. albopictus* to exploit human made breeding sites, the human expansion could have played a major role in the expansion of the tiger mosquito in the temperate areas.

Following this scenario the current geographic distribution of the tiger mosquito in Asia would be the result of the response of the species to the environmental changes due to Pleistocene climatic oscillations, and of its shared history with human populations since the post-glacial phase.