Genet. Res., Camb. (2005), **86**, *pp.* 1–11. *With* 4 *figures.* © 2005 Cambridge University Press
doi:10.1017/S0016672305007627 Printed in the United Kingdom

Phylogeography of *Aedes* (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations

LAURENCE MOUSSON[1], CATHERINE DAUGA[2], THOMAS GARRIGUES[1], FRANCIS SCHAFFNER[3], MARIE VAZEILLE[1] AND ANNA-BELLA FAILLOUX[1]*‡

[1] *Insectes et Maladies Infectieuses* (*formerly Ecologie des Systèmes Vectoriels*), *Institut Pasteur, 25–28 rue du Dr Roux, 75724 Paris cedex 15, France*
[2] *Plate Forme 4 – Intégration et Analyse Génomiques, Institut Pasteur, 25–28 rue du Dr Roux, 75724 Paris cedex 15, France*
[3] *EID Méditerranée, 165 avenue Paul-Rimbaud, 34184 Montpellier cedex 4, France*

(*Received 28 January 2005 and in revised form 25 April 2005*)

Summary

Aedes (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* (Skuse) are the most important vectors of the dengue and yellow-fever viruses. Both took advantage of trade developments to spread throughout the tropics from their native area: *A. aegypti* originated from Africa and *A. albopictus* from South-East Asia. We investigated the relationships between *A. aegypti* and *A. albopictus* mosquitoes based on three mitochondrial-DNA genes (cytochrome *b*, cytochrome oxidase I and NADH dehydrogenase subunit 5). Little genetic variation was observed for *A. albopictus*, probably owing to the recent spreading of the species via human activities. For *A. aegypti*, most populations from South America were found to be genetically similar to populations from South-East Asia (Thailand and Vietnam), except for one sample from Boa Vista (northern Amazonia), which was more closely related to samples from Africa (Guinea and Ivory Coast). This suggests that African populations of *A. aegypti* introduced during the slave trade have persisted in Boa Vista, resisting eradication campaigns.

1. Introduction

The mosquitoes *Aedes* (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* (Skuse) are the most important vectors of the dengue and yellow-fever viruses. *A. aegypti* is found worldwide and has colonized most tropical countries. There are two forms of *A. aegypti* – *A. aegypti formosus* and *A. aegypti aegypti* – differing in ecology, behaviour, genetic variations and susceptibility to dengue viruses (MacClelland, 1974; Tabachnick & Powell, 1979; Failloux *et al.*, 2002). The taxonomic status of these two forms is debatable and there are no absolute diagnostic characteristics (Ravel *et al.*, 2002). However, gene flow between the two forms is restricted, probably because of differences in their spatial distributions (Failloux *et al.*, 2002). *A. aegypti aegypti* has been implicated in dengue epidemics worldwide, whereas *A. aegypti formosus* has been implicated in a dengue forest cycle in West Africa (Gubler, 1997). The pale domestic and anthropophilic form, *A. aegypti aegypti* breeds essentially in man-made sites, whereas the dark, peridomestic and less anthropophilic form *A. aegypti formosus* is found mostly in Africa, preferring to colonize natural breeding sites.

A. albopictus originated in the forests of South-East Asia (Smith, 1956) and is commonly found in peri-urban, rural and forested areas. *A. albopictus* displays no ecological specialization, has succeeded in colonizing temperate zones such as the USA (Sprenger & Wuithiranyagool, 1986) and Europe (Adhami & Murati, 1987; Sabatini *et al.*, 1990; Schaffner & Karch, 2000; Schaffner *et al.*, 2004), and is currently invading African countries (Fontenille & Toto, 2001; Toto *et al.*, 2003). *A. albopictus* is nowadays

* Present address: UP Génétique moléculaire des Bunyaviridés, Institut Pasteur, 25–28 rue du Dr Roux, 75724 Paris cedex 15, France.
‡ Corresponding author. Institut Pasteur, UP Génétique moléculaire des Bunyaviridés, 25 rue du Dr Roux, 75724 Paris cedex 15, France. Tel: +33 1 406 13617. Fax: +33 1 40613151. e-mail: afaillou@pasteur.fr

implicated only in sporadic dengue cases (e.g. in Mexico in 1997; Ibanez-Bernal *et al*., 1997).

Mosquitoes spread by means of active adult flight and passive transportation of immature stages (i.e. larvae and eggs) via international trade. From the 15th century onwards, successive waves of invasion of the vector mosquitoes *A. aegypti*, *Culex pipiens* and, more recently, *A. albopictus* have been facilitated by commercial routes. As a result, *A. aegypti* replaced *A. albopictus* in South-East Asian cities in the first half of the 20th century (Hawley, 1988). Conversely, in the Americas, the introduction of *A. albopictus* was associated with a decline in the abundance of *A. aegypti* in the 1980s (O'Meara *et al*., 1995). Current intensification of intercontinental traffic might result in an increase in invasive species affecting human health as vectors of various pathogens (Roderick, 1996). *A. aegypti* and *A. albopictus* have spread outside their natural distribution areas and the sources of introduced populations remain to be identified.

Mitochondrial DNA (mtDNA) is commonly used for molecular evolution studies in insects (Kambhampati, 1995; Tang *et al*., 1996). Mitochondrial genes have proved to be particularly useful for detecting genetic divergence in mosquitoes and reconstructing the dispersal history of these insects (Kambhampati & Rai, 1991). Because mtDNA has a smaller effective population than that does nuclear DNA, it is more sensitive to genetic drift, resulting in greater genetic differentiation between populations (Avise, 1994). We analysed the sequence diversity of three mitochondrial genes – encoding cytochrome *b* (*Cytb*), cytochrome oxidase I (*COI*) and NADH dehydrogenase subunit 5 (*ND5*) – in 30 specimens of *A. aegypti* and *A. albopictus* collected in 15 countries from Europe, Africa, Asia and North and South America. We found: (i) that mitochondrial genes evolved faster in *A. aegypti* than in *A. albopictus*; and (ii) that *A. aegypti* populations were polyphyletic, whereas *A. albopictus* populations from Asia and South America originated from two distinct lineages. The species *A. aegypti* from Boa Vista (Brazil) shared close relationships with *A. aegypti formosus* from Africa, suggesting that some Brazilian mosquitoes originate from African populations that have survived control programmes.

2. Materials and methods

(i) *Field collections*

Specimens were collected as larvae or pupae and were reared in secure laboratory in artificial conditions to the adult stage. They were then frozen and stored at −80 °C. The location and characteristics of each mosquito strain are shown in Table 1. The three African specimens of *A. aegypti* corresponded to the forest-dwelling form, *A. aegypti formosus*. The other specimens were collected outside Africa and corresponded to the pale domestic form, *A. aegypti aegypti*.

Table 1. *Geographic origin of the mosquitoes analysed*

City	Country	Year of collection
Aedes aegypti		
Libreville	Gabon	1997
Boa Vista	Brazil	2001
Bouaké	Ivory coast	2000
Boulbinet	Guinea	2001
Europa Is.	Europa Is.	1998
Foz Iguaçu	Brazil	2001
Guadeloupe	France	1985
Hanoi	Vietnam	2000
Ho Chi Minh City	Vietnam	2000
Mahaleja	Madagascar	1998
Rivière Salée	Martinique	2001
Nha Trang	Vietnam	2000
Paea	French Polynesia	1994
Phnom Penh	Cambodia	2001
Quixeramobim	Brazil	2001
Rio Branco	Brazil	2001
Chiang Mai	Thailand	2000
Aedes albopictus		
Represa do Cigano	Brazil	2001
Hanoi	Vietnam	2000
Jacksonville	USA	2001
Seam Reap	Cambodia	2001
Diego Suarez	Madagascar	1999
Montsecret	France	2000
Naintré	France	1999
Nha Trang	Vietnam	2000
Oahu	Hawaii	1971
La Possession	Réunion	2000
La Providence	Réunion	2000
Sao Luis	Brazil	2001
Chiang Mai	Thailand	2000

(ii) *DNA extraction*

Specimens from each location were ground in 250 μl 10% chelex (BioRad®) in 0·1% SDS, 1% Tween 20, 1% NP40 and the homogenate was incubated for 30 min at 56 °C, then 30 min at 95 °C. DNA was purified by precipitation in ethanol. DNA samples were used as templates for the amplification of specific fragments of mtDNA: a 307 bp fragment for *Cytb*, a 597 bp fragment for *COI* and a 450 bp fragment for *ND5*. Three sets of primers were used: for *Cytb*, L14841 (5′-AAAAAGCTTCCATCCAACATCTC-AGCATGATGAAA-3′) and H15149 (5′-AAACT-GCAGCCCCTCAGAATGATATTTGTCCTCA-3′) (Kocher *et al*., 1989); for *COI*, CI-J-1632 (5′-TGAT-CAAATTTATAAT-3′) and CI-N-2191 (5′-GGTA-AAATTAAAATATAAACTTC-3′) (Kambhampati

https://doi.org/10.1017/S0016672305007627 Published online by Cambridge University Press

& Smith, 1995); and, for *ND5*, ND5FOR (5′-TCCTTAGAATAAAATCCCGC-3′) and ND5REV (5′-GTTTCTGCTTTAGTTCATTCTTC-3′) (Birungi & Munstermann, 2002).

Each reaction was performed in a Perkin-Elmer thermal cycler 2400, in a final volume of 20 μl. For *Cytb* and *COI*, the PCR mixture contained 100 ng genomic DNA, 1× buffer, 2·5 mM $MgCl_2$, 250 μM each dNTP, 100 nM each primer and 1 unit Eurobio Taq polymerase. Amplification was achieved by heating at 95 °C for 5 min and then subjecting the mixture to 35 cycles of 97 °C for 30 s, annealing temperature (50 °C for *Cytb* and 40 °C for *COI*) for 45 s, and 72 °C for 1 min. The mixture was then subjected to a final extension step at 72 °C for 5 min. For *ND5*, the PCR mixture contained 200 μM each dNTP, 200 nM each primer and 0·5 units Eurobio Taq polymerase, and the amplification programme was as follows: 98 °C for 2 min followed by five cycles of 95 °C for 30 s, 45 °C for 30 s and 72 °C for 45 s, then 25 cycles of 95 °C for 30 s, 46 °C for 45 s and 72 °C for 45 s, and a final extension step at 72 °C for 5 min. PCR products were separated by agarose-gel electrophoresis and purified using the Qiaquick Gel extraction kit (Qiagen). Purified DNA fragments (100 ng) were directly sequenced in an automated DNA sequencer (ABI PRISM® 310), using the dideoxynucleotide-chain-termination method with ddNTPs labelled with a specific fluorochrome. Sequences were assembled using Mac Molly software (Soft Gene GmbH). Sequences were aligned using ClustalW, with default parameters, in Bioedit software (Hall, 1999).

Table 2. *Accession numbers of sequences deposited in EMBL Nucleotide Sequence Database*

	Accession numbers		
	Cytb	*COI*	*ND5*
Aedes aegypti			
Libreville	AJ970943	AJ970960	–
Boa Vista	AJ970944	AJ970961	AJ970977
Bouaké	AJ970945	AJ970962	AJ970978
Boulbinet	AJ970946	AJ970963	AJ970979
Europa Is.	AJ970947	AJ970964	AJ970980
Foz Iguaçu	AJ970948	AJ970965	AJ970981
Guadeloupe	AJ970949	AJ970966	–
Hanoi	AJ970950	AJ970967	AJ970982
Ho Chi Minh City	AJ970951	AJ970968	–
Mahaleja	AJ970952	AJ970969	–
Rivière Salée	AJ970953	AJ970970	AJ970983
Nha Trang	AJ970954	AJ970971	AJ970984
Paea	AJ970955	AJ970972	AJ970985
Phnom Penh	AJ970956	AJ970973	AJ970986
Quixeramobim	AJ970957	AJ970974	AJ970987
Rio Branco	AJ970958	AJ970975	AJ970988
Chiang Mai	AJ970959	AJ970976	AJ970989
Aedes albopictus			
Represa do Cigano	AJ970990	AJ971003	AJ971016
Hanoi	AJ970991	AJ971004	AJ971017
Jacksonville	AJ970992	AJ971005	AJ971018
Seam Reap	AJ970993	AJ971006	AJ971019
Diego Suarez	AJ970994	AJ971007	AJ971020
Montsecret	AJ970995	AJ971008	AJ971021
Naintré	AJ970996	AJ971009	AJ971022
Nha Trang	AJ970997	AJ971010	AJ971023
Oahu	AJ970998	AJ971011	AJ971024
La Possession	AJ970999	AJ971012	AJ971025
La Providence	AJ971000	AJ971013	AJ971026
Sao Luis	AJ971001	AJ971014	AJ971027
Chiang Mai	AJ971002	AJ971015	AJ971028

–, no sequence available.

(iii) *Phylogenetic analysis*

We carried out a combined analysis for the three genes to improve the reliability of phylogenetic information and the resolution of relationships between *A. aegypti* and *A. albopictus*. The three genes *Cytb*, *COI* and *ND5* were analysed together for 13 specimens of each species for which the three gene sequences were available. We carried out combined analysis for *Cytb* and *COI* on 17 specimens of *A. aegypti* (for which both gene sequences were available). The partition-homogeneity test function of PAUP 4.0b10 (software described by Swofford, 1998) (using a heuristic search and tree-bisection–reconnection (TBR) branch-swapping option) was used to determine whether data sets were incongruent (Farris *et al*., 1995).

Phylogenetic relationships between specimens were determined using the maximum-likelihood (ML) method implemented in PAUP. A test run with MODELTEST 3.0 software (Posada & Crandall, 1998, 2001) compared our sequence data set with various evolutionary matrix models and made it possible to identify the models that best fitted the data. Trees were then constructed with the full heuristic search option and TBR branch swapping. The significance of internal branches was evaluated using 100 bootstrap replications. Nodes represented in more than 90% of bootstrap replicates (btp) were considered to be strongly supported, btp values of 70% to 89%, were considered to indicate moderate support and values of 50% to 69% to indicate weak support. Nodes with bootstrap values lower than 50% are not shown because they were not considered to be supported by the test.

3. Results

(i) *Sequence variation*

Mitochondrial genes from 17 specimens of *A. aegypti* and 13 specimens of *A. albopictus* were sequenced (Table 2, Fig. 1). For the *Cytb* gene of *A. aegypti*, we

https://doi.org/10.1017/S0016672305007627 Published online by Cambridge University Press

(A)

	Cytb	*COI*	*ND5*
	1111111112222	11111122222222222333333344444555555	111133344
	6680114445780388	34455667799913457900113334589335679936779012444 5	2239247726800
	8907060562689067	56702072803420245909480362176252923406580969167 0	3652300368314
Libreville (Gabon)	TAAAAATTGAAAGAAG	AGA-A-AATAA-GCTAG-AAGATTCTTGAAT-AA-TCTAGGA--A-TT	
Boa Vista (Brazil)	...GGGCC.G..A...	.....G.....	CCGTGCGTCCCCG
Bouaké (Ivory Coast)	...GGG.C.G..A...	.....G.....A.....	.....T..A
Boulbinet (Guinea)	...GGGCC.G..A...	.....G.....C	.....T..A
Europa Is.	.....G.C....A...	CA.....GC....T....TG..C.....T.....CGA.....C.	TTAC.T.CTTTTA
Foz Iguaçu (Brazil)	.....G.C....A...	.....C.....	TTAC.T..TTT.A
Guadeloupe (France)	.G.GGG.C.G..A...	.....C.....T.R.....C.....C..	
Hanoi (Vietnam)	...GGG.C.G..A...	.....C.....	...C..A..T.TA
Ho Chi Minh City (Vietnam)	C..G.G.C....A..A	.....G.....TC.....	
Mahaleja (Madagascar)	..G.GG.CA.GGA.GA	.A..G.--.-..ATCGA...AG.CT..TGGC.G.T.....A...-...	
Riviere salée (Martinique)	...GGG.C.G..A...	.A.....GC....T.....G..C...C.....C.CGA.....C.	TTAC.T.CTTT.A
Nha Trang (Vietnam)	...GGG.C.G..A...	.....G.....G.....C.C.....	...C..AG.T.TA
Paea (French Polynesia)	...GGG.C.G..A...	...T.....C.....TT....	...C..A..T.TA
Phnom Penh (Cambodia)	.....G.C....AG..	.A.....GC....T.....G..C..CC.....C.CGA.....C.	TTAC.T..TTTTA
Quixeramobim (Brazil)	.....G.C....A...	.A.....GC....T.....G..C..CC.....C.CGA.....C.	TTAC.T..TTT.A
Rio Branco (Brazil)	...GGG.C.G..A...	NNN.....C.....	...C..A..T.TA
Chiang Mai (Thailand)	...RRG.C.R..A...	.....G.....C.....-.....	...CA.A..T.TA

(B)

	Cytb	*COI*	*ND5*
	1123	233	2
	784893	122634	2
	306864	912408	3
Represa do Cigano (Brazil)	TGGATA	ATACGC	C
Hanoi (Vietnam)	.A....	.....	T
Jacksonville (USA)	.....	....A.	T
Seam Reap (Cambodia)	..A.C.	...T..	T
Diego Suarez (Madagascar)	.....	....A.	T
MontSecret (France)	.....	....A.	T
Naintré (France)	.....	....A.	T
Nha Trang (Vietnam)	..A...	.....	T
Oahu (Hawaii)	...G.G	TAT.A.	T
La Possession (Réunion)	C.....	....AT	T
La Providence (Réunion)	.....	....A.	T
Sao Luis (Brazil)	.....	.....	.
Chiang Mai (Thailand)	..A.C.	.....	T

Fig. 1. (*a*) Variable nucleic acids in *Aedes aegypti*, showing 34 polymorphic sites for *COI*, 16 for *Cytb* and 13 for *ND5*. (*b*) Variable nucleic acids in *Aedes albopictus* showing six polymorphic sites for *COI* and *Cytb* and only one polymorphic site for *ND5*.

obtained fragments of 368 bp to 376 bp and a final alignment of 376 sites. 16 sites (4·25%) were polymorphic and four of these (1·06%) were phylogenetically informative. For *A. albopictus*, the alignment of *Cytb* gene fragments (375–376 bp) gave a total of 2·13% polymorphic sites and 0·53% informative sites.

For the *COI* gene, *A. aegypti* gave fragments of 507–599 bp. The alignment of 17 sequences showed that 5·67% of sites were polymorphic and 2·33% were informative. For *A. albopictus*, the alignment of fragments (407–552 bp) showed that 1·11% of sites were polymorphic and 0·18% were informative.

For the *ND5* gene, *A. aegypti* gave fragments of 410–432 bp for a global alignment of 420 bp. Polymorphic sites accounted for 3·09% of these sites and 2·38% of sites were informative. For *A. albopictus*, the alignment of *ND5* fragments (423–449 bp) showed that only one site (0·24%) was polymorphic and informative.

The *COI* gene therefore seems to be the most variable of these genes in *A. aegypti* and the least informative in *A. albopictus*, whereas the *ND5* gene was the most informative in *A. aegypti* and the least variable in *A. albopictus*. For *A. albopictus*, the *Cytb* gene was the most variable and informative of the three phylogenetic markers used. The degree of polymorphism of mitochondrial genes differed between species. The mutation rate of mitochondrial genes in *A. aegypti* was about six times higher than that in *A. albopictus*.

(ii) *Combined data and phylogenetic analysis of* A. aegypti

When we carried out a combined analysis for all three genes from 13 specimens of *A. aegypti*, we found 28 informative characters in 1380 bp (360 bp for the *Cytb* gene, 600 bp for the *COI* gene and 420 bp for the *ND5* gene). The partition-homogeneity test implemented in PAUP revealed that the three genes were significantly incongruent ($P = 0{\cdot}01$ for *Cytb* combined with *COI* and P value $= 0{\cdot}05$ for all three). Despite this result, we adopted the 'total evidence' approach (Huelsenbeck *et al.*, 1996) because phylogenetic trees based on individual genes showed poor resolution of nodes rather than clear discrepancies (data not shown). We combined genes evolving at different rates under the hypothesis that they might interact positively to resolve different levels of a phylogenetic tree, by maximizing the informative and explanatory power of sequences.

https://doi.org/10.1017/S0016672305007627 Published online by Cambridge University Press

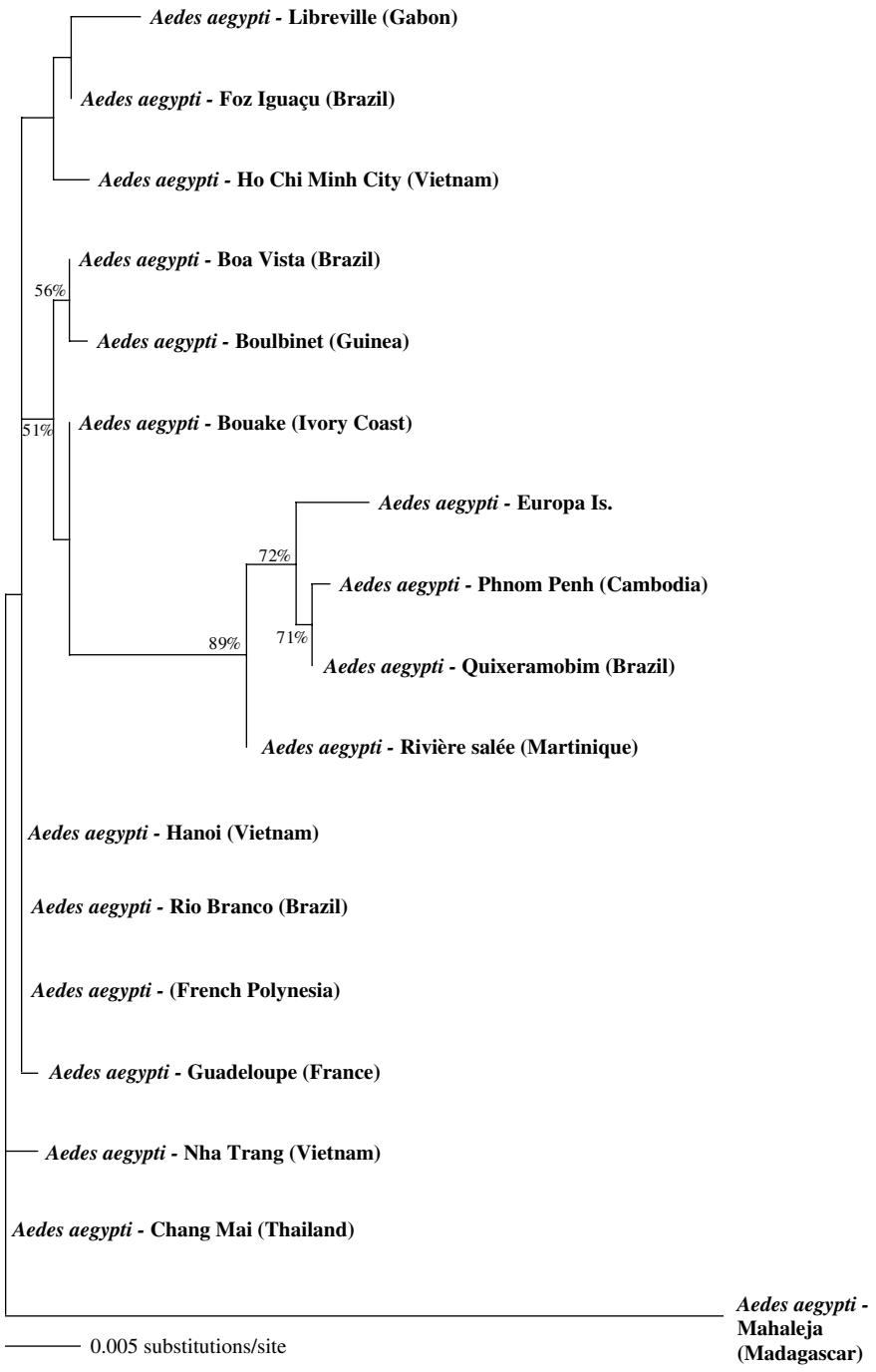

Fig. 2. Maximum-likelihood tree for 17 *Aedes aegypti* specimens based on *Cytb* and *COI* sequences. The best-fitting evolutionary model found for our data set by MODELTEST was the Hasegawa, Kishino and Yano model (HKY + I + G), with a proportion of invariable sites (I) equal to 0·79 and a γ-distribution shape parameter (G) of 0·615. Numbers indicate bootstrap values for nodes retained by more than 50% of bootstrap replicates. The designation of sequences corresponds to the geographical region of isolation.

The phylogenetic tree based on the combination of *Cytb* and *COI* sequences available for 17 samples identified a group (btp = 51%) including specimens from South America, the Caribbean, South-East Asia, the Indian-Ocean region and Africa (Fig. 2). Within this group, we identified a subgroup (btp = 56%) associating *A. aegypti* from northern Amazonia (Boa Vista) with *A. aegypti* from Guinea (Boulbinet). A second subgroup (btp = 89%) grouped *A. aegypti* from Cambodia (Phnom Penh), North-East Brazil (Quixeramobim), Martinique (Rivière salée) and a specimen from Europa Island. *A. aegypti* from Gabon (Libreville), South Brazil (Foz Iguaçu) and South Vietnam (Ho Chi Minh City) formed another group

https://doi.org/10.1017/S0016672305007627 Published online by Cambridge University Press

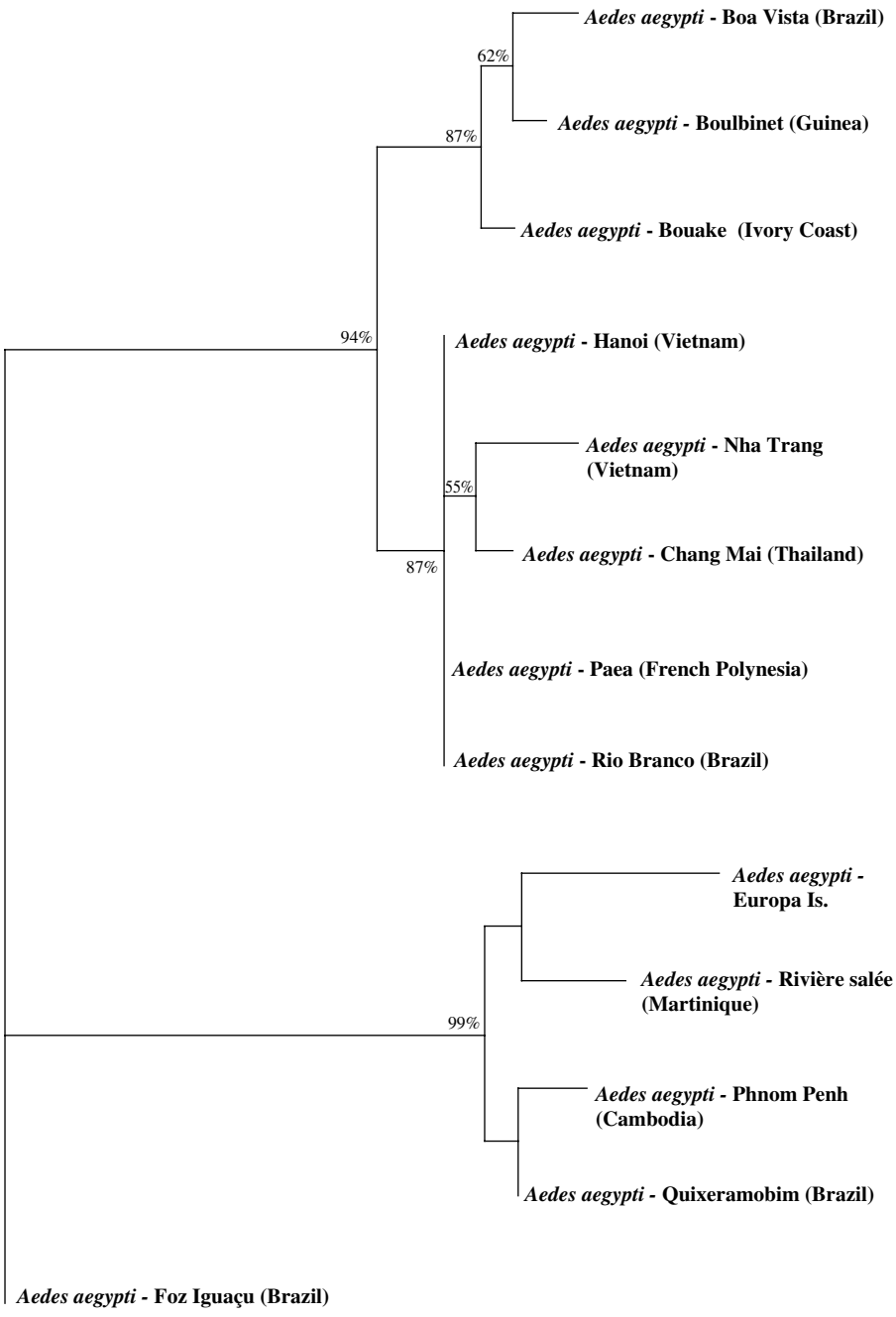

Fig. 3. Maximum-likelihood tree for 13 *Aedes aegypti* specimens based on *ND5*, *Cytb* and *COI* sequences. The best-fitting evolutionary model found for our data set by MODELTEST was the Hasegawa, Kishino and Yano model (HKY + I + G) with a proportion of invariable sites (I) equal to 0·77 and a γ-distribution shape parameter (G) of 0·0553. Numbers indicate bootstrap values for nodes retained by more than 50 % of bootstrap replicates. The designation of sequences corresponds to the geographical region of isolation.

that was not validated by a significant bootstrap value.

On the phylogenetic tree based on combined analysis of three genes (*Cytb*, *COI* and *ND5*) from 13 *A. aegypti* samples, we distinguished a strongly supported (btp = 94 %) group including South-East Asian, African and Brazilian mosquitoes (Fig. 3). This group showed two successive paraphyletic branching patterns: (1) one (btp = 87 %) showing the North Amazonian strain collected in Boa Vista to be closely related to strains collected in Africa, Guinea (Boulbinet) and Ivory Coast (Bouaké); and (2) another (btp = 87 %) showing *A. aegypti* from South-East Asia (Hanoi and Nha Trang from Vietnam, and

https://doi.org/10.1017/S0016672305007627 Published online by Cambridge University Press

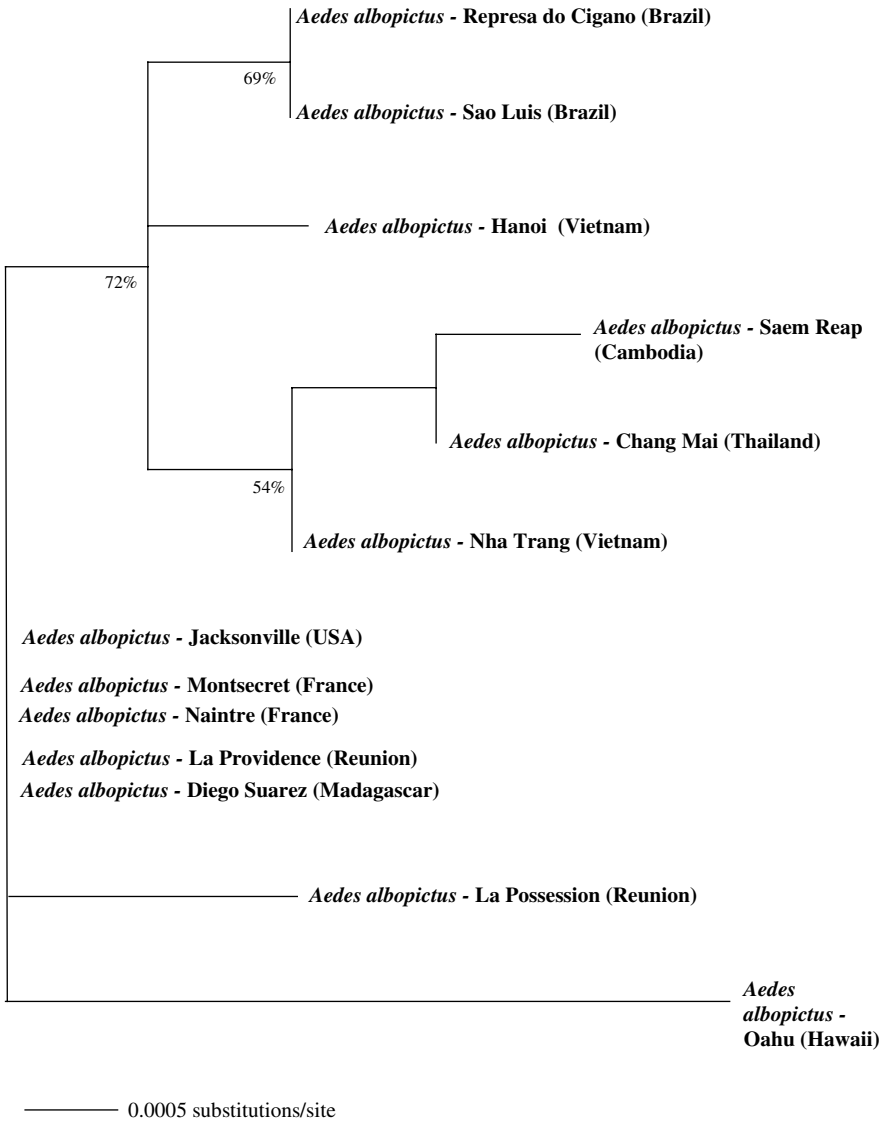

Fig. 4. Maximum-likelihood tree for 13 *Aedes albopictus* specimens based on *ND5*, *Cytb* and *COI* sequences. The best-fitting evolutionary model found for our data set by MODELTEST was the Hasegawa, Kishino and Yano model (HKY), with no invariable site and equal rates for all sites. Numbers indicate bootstrap values for nodes retained by more than 50% of bootstrap replicates. The designation of sequences corresponds to the geographical region of isolation.

Chiang Mai from Thailand) to be phylogenetically closely related to mosquitoes from French Polynesia (Paea) and Western Amazonia (Rio Branco, Brazil). Finally, we identified a group (btp = 99%) including two insular specimens, from Europa Island and Martinique (Rivière salée), and mosquitoes from Cambodia (Phnom Penh) and North-East Amazonia (Quixeramobim, Brazil).

(iii) *Combined data and phylogenetic analysis of* A. albopictus

For *A. albopictus*, we detected only four informative characters in 1320 bp (360 bp for the *Cytb* gene, 540 bp for the *COI* gene, and 420 bp for the *ND5* gene). The partition-homogeneity test implemented by PAUP revealed that the three genes were not significantly incongruent ($P = 1$). We therefore carried out a combined analysis to enhance phylogenetic information.

The phylogenetic tree obtained contained one main group (btp = 72%), including only specimens from South-East Asia and Brazil (Fig. 4). This group consisted of two subgroups: (1) one (btp = 69%) including mosquitoes collected in the South-East region (Represa do Cigano) and the North-East region (Sao Luis) of Brazil; and (2) another (btp = 54%) comprising *A. albopictus* from Cambodia (Seam Reap),

https://doi.org/10.1017/S0016672305007627 Published online by Cambridge University Press

Thailand (Chiang Mai) and Vietnam (Nha Trang). Interestingly, specimens from the USA (Jacksonville), France (Naintré and Montsecret), Madagascar (Diego Suarez) and Réunion (La Providence) were indistinguishable.

4. Discussion

Three mitochondrial loci were used in this study to describe the natural history of the sampled *A. aegypti* and *A. albopictus* specimens, and to provide insight into the evolutionary processes underlying the genetic diversity and geographical distribution of these species.

(i) *Evolution of* A. aegypti *and* A. albopictus

The three mitochondrial genes analysed showed only low levels of genetic variation in both species. The rate of molecular evolution in mosquitoes might be affected by decreases in population size, generating low levels of mtDNA variability (Davies *et al*., 1999). Repeated population bottlenecks lead to losses in genetic variation because of random genetic drift. In this case, intensive control activities reduced variability by dramatically decreasing population densities (Yan *et al*., 1998). Low levels of sequence variation might also be a consequence of the recent and rapid expansion of the range of a few mtDNA haplotypes via modern transport, a passive mode of dispersal, as previously described for *A. albopictus* populations in America (Birungi & Munstermann, 2002). Other explanations might account for the low level of genetic variation: such as more effective selection against mildly deleterious mutations, the consequence of a species-specific replication-dependent model in mtDNA (Weinreich, 2001).

Moreover, genes from some island populations, including *A. aegypti* from Madagascar (Mahaleja) and *A. albopictus* from Hawaii (Oahu) and La Réunion (La Possession), had the highest rates of sequence evolution in their respective samples. Before become an island, Madagascar was connected to East Africa, a source of colonizing mosquitoes. Isolation has affected species diversity and subsequently led to high levels of endemism, which might account for the level of genetic diversity observed (Gillespie & Roderick, 2002). Volcanic islands (e.g. Hawaii and La Réunion) have never been in direct contact with a source of colonizing insects. They therefore have abundant empty ecological niche space and species numbers depend on the degree of isolation.

A. aegypti and *A. albopictus* populations seem to have different evolutionary histories. The general structure of the phylogenetic trees based on mitochondrial genes showed that populations of *A. aegypti* were polyphyletic, whereas some populations of *A. albopictus* emerged from separate lineages. Mitochondrial genes were less informative for *A. albopictus* than for *A. aegypti*.

(ii) *Biogeography of* A. aegypti

Phylogenetic trees revealed close relationships between specimens of *A. aegypti* from South America, Asia and/or Africa in various lineages (Figs 2, 3). This probably reflects the mixing of genotypes owing to the recurrent spreading of mosquitoes over the various continents. The two forms, *A. aegypti aegypti* and *A. aegypti formosus*, occupied different areas with only limited gene flow between them. No diagnostic molecular characteristic is yet available to distinguish one form from the other. *A. aegypti aegypti* is much more susceptible to dengue infections in the laboratory than *A. aegypti formosus* (Failloux *et al*., 2002). In tropical Africa, the forest-dwelling form, *A. aegypti formosus*, might have progressively differentiated into *A. aegypti aegypti*, which is better adapted to domestic environments. *A. aegypti aegypti*, which is known to be a poor flyer, limiting its dispersal around breeding sites, used human trading activities to spread throughout the tropics. It first spread to the New World from West Africa via the African slave trade (from the 15th to the 19th centuries). It then spread into Asia via commercial exchanges in the 18th–19th centuries and finally, throughout the world after World War II (Failloux *et al*., 2002).

A. aegypti populations in South America seem to have been established from different founding sources, reflecting successive waves of colonization before and after eradication programmes. Intensive control was first implemented at the start of the 20th century, to control yellow-fever epidemics in South America. Such control was first carried out by the Rockefeller Foundation in 1916, followed by the Pan-American Health Organization in 1940–1960. Control programmes were halted in the 1970s before total eradication of the species was achieved. Thus, *A. aegypti* is still present in Suriname, the Guyanas, Venezuela, the Southern USA and some Caribbean Islands. The species reinvaded Brazil through the state of Bahia and Rio de Janeiro, in 1976 and 1977, respectively (Schatzmayr, 2000). Most Brazilian strains are genetically more closely related to South-East Asian strains (Thailand and Vietnam) and were probably reintroduced once control programmes had ended. This might explain why Brazil suffers so many urban dengue epidemics, because most Brazilian *A. aegypti* populations are highly susceptible to dengue infections (Lourenço-de-Oliveira *et al*., 2004). Conversely, the Boa Vista strain displays strong genetic similarity to African mosquitoes

(Guinea and Ivory Coast), suggesting that some mosquitoes introduced by the slave trade survived control programmes and persist in this region. In Brazil, the first dengue epidemic occurred in Boa Vista in the early 1980s (Vasconcelos *et al*., 1999) but did not spread elsewhere in the country. *A. aegypti* from Boa Vista has been compared with samples collected in the Venezuelan border city Maracay, where the species had not been eradicated, and infection rates for dengue-2 virus (Lourenço-de-Oliveira *et al*., 2004) and yellow-fever virus (Lourenço-de-Oliveira *et al*., 2002) in experimental infection situations (Vazeille-Falcoz *et al*., 1999) were found to be similar.

(iii) *Biogeography of* A. albopictus

For *A. albopictus*, populations collected in Represa do Cigano and Sao Luis on the East coast of Brazil formed a lineage paraphyletic to Asian lineages (Cambodia, Vietnam and Thailand) (Fig. 4). Brazilian populations showed no diapause, suggesting a tropical origin for their founders (Hawley *et al*., 1987). *A. albopictus* was identified in Brazil after its detection in North America (Forattini, 1986). The occurrence of a single widespread mtDNA in Brazil provides evidence for a single introduction into the country (Birungi & Munstermann, 2002). *A. albopictus* has not been implicated in dengue epidemics in Brazil even though dengue virus has been isolated from the species (Serufo *et al*., 1993). Because this species uses bromeliads as larval habitats, bringing it into close contact with enzootic arbovirus cycles, it is thought to be involved in the transmission of yellow-fever virus (Natal *et al*., 1997). *A. albopictus*, indigenous to South-East Asia, was the major vector of dengue viruses before the introduction of *A. aegypti*, probably around 1915 (Stanton, 1920).

A. albopictus has been established in southern Europe since the 1970s (Albania in 1979) (Adhami & Murati, 1987), in Italy since 1990 (Sabatini *et al*., 1990), in France since 1999 (Schaffner & Karch, 2000), in Belgium since 2000 (Schaffner *et al*., 2004), in Serbia and Montenegro since 2001 (Petric *et al*., unpublished), in Switzerland since 2003 (Flacio *et al*., 2004), and in Spain since 2004 (C. Aranda, personal communication). Populations from France (Montsecret and Naintré) have been found to be genetically related to populations from the southern USA (Jacksonville) and islands in the Indian Ocean (Madagascar and La Réunion). The low levels of sequence variation within *A. albopictus* might be caused by recent colonization through expansion, on shipments of used tyres (Hawley *et al*., 1987).

In conclusion, this analysis of mitochondrial genes provides insight into the origins of colonizing *A. aegypti* populations, particularly in Brazil, and highlights the role of control programmes and human trading activities in shaping the genetic composition of mosquito populations.

We thank G. Lecointre for advice on phylogenetic analysis and N. Ayad for rearing mosquitoes. We also thank the following for collecting mosquitoes: N. Elissa (CIRMF, Gabon), R. Lourenço-de-Oliveira (Instituto Oswaldo, Fiocruz, Rio de Janeiro), J.-P. Hervé ('Lutte contre les Insectes Nuisibles', IRD, Montpellier, France), A. Yébakima and C. Charles (Centre de Démoustication, Martinique), R. Girod (DRASS, La Réunion), V. S. Nam and N. T. Yen (National Institute of Hygiene and Epidemiology, Hanoi, Vietnam), T. H. Hoang and L. L. Loan (Pasteur Institute of Ho Chi Minh City, Vietnam), J.-M. Reynes and Y. Buisson (Pasteur Institute of Cambodia, Phnom Penh, Cambodia), L. V. Lo and T. N. V. An (Pasteur Institute of Nha Trang, Vietnam), and J.-B. Duchemin (Pasteur Institute of Madagascar). This work was supported by the Pasteur Institute in Paris (grant 62258) and by the 'Entente Inter-Départementale pour la démoustication Méditerranée' surveillance programme for *A. albopictus* supported by the French Labour and Solidarity Ministry, General Directorate for Health.

References

Adhami, J. & Murati, N. (1987). Presence of the mosquito *Aedes albopictus* in Albania. *Revista Mjekesöre* **1**, 13–16.

Avise, J. C. (1994). *Molecular Markers, Natural History and Evolution*. New York: Chapman & Hall.

Birungi, J. & Munstermann, L. E. (2002). Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial *ND5* sequences: evidence for an independent invasion into Brazil and United States. *Annals of the Entomological Society of America* **95**, 125–132.

Davies, N., Villablanca, F. X. & Roderick, G. K. (1999). Determining the source of newly founded populations: multilocus genotyping in nonequilibrium population genetics. *Trends in Ecology and Evolution* **14**, 17–21.

Failloux, A.-B., Vazeille, M. & Rodhain, F. (2002). Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. *Journal of Molecular Evolution* **55**, 653–663.

Farris, J. S., Källersjo, M., Kluge, A. G. & Bult, C. (1995). Testing significance of incongruence. *Cladistics* **10**, 315–319.

Flacio, E., Lüthy, P., Patocchi, N., Guidotti, F., Tonolla, M. & Peduzzi, R. (2004). Primo ritrovamento di *Aedes albopictus* in Svizzera. *Bollettino della Società Ticinese di Scienze Naturali* (*STSN*) **92**, 141–142.

Fontenille, D. & Toto, J. C. (2001). *Aedes* (*Stegomyia*) *albopictus* (Skuse), a potential new Dengue vector in southern Cameroon. *Emerging Infectious Diseases* **7**, 1066–1067.

Forattini, O. P. (1986). Identificação de *Aedes* (*Stegomyia*) *albopictus* (Skuse) no Brasil. *Revista de Saúde Pública* **20**, 244–245.

Gillespie, R. G. & Roderick, G. K. (2002). Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology* **47**, 595–632.

Gubler, D. J. (1997). Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In *Dengue and Dengue Hemorrhagic*

Fever (ed. Gubler, D. J. & Kuno, G.), pp. 1–22. Wallingford, UK: CAB International.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**, 95–98.

Hawley, W. A. (1988). The biology of *Aedes albopictus*. *Journal of the American Mosquito Control Association* **4**, 1–39.

Hawley, W. A., Reiter, P., Copeland, R. S., Pumpuni, C. B. & Craig, G. B., Jr (1987). *Aedes albopictus* in North America: probable introduction in used tires from Northern Asia. *Science* **236**, 1114–1116.

Huelsenbeck, J. P., Bull, J. J. & Cunningham, C. W. (1996). Combining data in phylogenetic analysis. *Trends in Ecology and Evolution* **11**, 152–158.

Ibanez-Bernal, S., Briseno, B., Mutebi, J. P., Argot, E., Rodriguez, G., Martinez-Campos, C., Paz, R., de la Fuente-San Roman, P., Tapia-Conyer, R. & Flisser, A. (1997). First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. *Medical and Veterinary Entomology* **11**, 305–309.

Kambhampati, S. (1995). A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proceedings of the National Academy of Sciences of the USA* **92**, 2017–2020.

Kambhampati, S. & Rai, K. S. (1991). Mitochondrial DNA variation within and among populations of the mosquito *Aedes albopictus*. *Genome* **34**, 288–292.

Kambhampati, S. & Smith, P. T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Molecular Biology* **4**, 233–236.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA* **86**, 6196–6200.

Lourenço-de-Oliveira, R., Vazeille, M., Filippis, A. M. B. & Failloux, A. B. (2002). Oral susceptibility to yellow fever virus of *Aedes aegypti* from Brazil. *Memórias do Instituto Oswaldo Cruz* **97**, 437–439.

Lourenço de Oliveira, R., Vazeille, M., Bispo de Filippis, A. M. & Failloux, A. B. (2004). *Aedes aegypti* in Brazil: genetically differentiated populations with high susceptibility to dengue and yellow fever viruses. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **98**, 43–54.

MacClelland, G. A. H. (1974). A worldwide survey of variation in scale pattern of the abdominal tergum of *Aedes aegypti* (L.) (Diptera: Culicidae). *Transactions of the Royal Entomological Society of London* **126**, 239–259.

Natal, D., Urbinatti, P. R., Taipe-Lagos, C., Ceret, W., Diederichsen, A., Souza, R. G. & Souza, R. P. (1997). Encontro de *Aedes* (*Stegomyia*) *albopictus* em Bromeliaceae na periferia de São Paulo, SP, Brasil. *Revista de Saúde Pública* **31**, 517–518.

O'Meara, G. F., Evans, L. F., Jr, Gettman, A. D. & Cuda, J. P. (1995). Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. *Journal of Medical Entomology* **32**, 554–562.

Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Posada, D. & Crandall, K. A. (2001). Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50**, 580–601.

Ravel, S., Hervé, J. P., Diarrassouba, S., Kone, A. & Cuny, G. (2002). Microsatellite markers for population genetic studies in *Aedes aegypti* (Diptera: Culicidae) from Côte d'Ivoire: evidence for a microgeographic genetic differentiation of mosquitoes from Bouaké. *Acta Tropica* **82**, 39–49.

Roderick, G. K. (1996). Geographic structure of insect populations: gene flow, phylogeography, and their uses. *Annual Review of Entomology* **41**, 325–352.

Sabatini, A., Raineri, A. V., Trovato, G. & Coluzzi, M. (1990). *Aedes albopictus* in Italia e possible diffusione della specie nell'area Mediterranea. *Parassitologia* **32**, 301–304.

Schaffner, F. & Karch, S. (2000). Première observation d'*Aedes albopictus* (Skuse, 1894) en France métropolitaine. *Comptes Rendus de l'Académie des Sciences* (*Série III*) – *Sciences de la vie* **323**, 373–375.

Schaffner, F., Van Bortel, W. & Coosemans, M. (2004). First record of *Aedes* (*Stegomyia*) *albopictus* in Belgium. *Journal of the American Mosquito Control Association* **20**, 201–203.

Schatzmayr, H. G. (2000). Dengue situation in Brazil by year 2000. *Memorias do Instituto Oswaldo Cruz* **95**, 179–181.

Serufo, J. C., de Oca, H. M., Tavares, V. A., Souza, A. M., Rosa, R. V., Jamal, M. C., Lemos, J. R., Oliveira, M. A., Nogueira, R. M. & Schatzmayr, H. G. (1993). Isolation of dengue virus type 1 from larvae of *Aedes albopictus* in Campos Altos city, State of Minas Gerais, Brazil. *Memorias do Instituto Oswaldo Cruz* **88**, 503–504.

Smith, C. E. G. (1956). The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *Journal of Tropical Medicine and Hygiene* **59**, 243–251.

Sprenger, D. & Wuithiranyagool, T. (1986). The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *Journal of the American Mosquito Control Association* **2**, 217–219.

Stanton, A. T. (1920). Mosquitoes of far eastern ports with special reference to the prevalence of *Stegomyia fasciata*. *Bulletin of Entomological Research* **10**, 333–334.

Swofford, D. L. (1998). PAUP. Phylogenetic analysis using parsimony, version 3.1.1. Computer program distributed by the Illinois National History Survey. Champaign, IL, USA.

Tabachnick, W. J. & Powell, J. R. (1979). A world-wide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti*. *Genetical Research* **34**, 215–229.

Tang, J., Pruess, K. & Unnasch, T. R. (1996). Genotyping North American black flies by means of mitochondrial ribosomal RNA sequences. *Canadian Journal of Zoology* **74**, 39–46.

Toto, J. C., Abaga, S., Carnevale, P. & Simard, F. (2003). First report of the oriental mosquito *Aedes albopictus* on the West African Island of Bioko, Equatorial Guinea. *Medical and Veterinary Entomology* **17**, 343–346.

Vasconcelos, P. F. C., Travassos-da-Rosa, A. P. A., Pinheiro, F. P., Rodrigues, S. G., Travassos-da-Rosa, E. S., Cruz, A. C. R. & Travassos-da-Rosa, J. F. S. (1999). *Aedes aegypti*, dengue and re-urbanization of yellow fever in Brazil and other South American countries – past and present situation and future perspectives. *Dengue Bulletin* **23**, 55–66.

Vazeille-Falcoz, M., Mousson, L., Rodhain, F., Chungue, E. & Failloux, A.-B. (1999). Variation in oral susceptibility to dengue type 2 virus of populations of *Aedes aegypti* from the islands of Tahiti and Moorea, French Polynesia. *American Journal of Tropical Medicine and Hygiene* **60**, 292–299.

Weinreich, D. M. (2001). The rates of molecular evolution in rodent and primate mitochondrial DNA. *Journal of Molecular Evolution* **52**, 40–50.

Yan, G., Chadee, D. D. & Severson, D. W. (1998). Evidence of genetic hitchhiking effect associated with insecticide resistance in *Aedes aegypti*. *Genetics* **148**, 793–800.