Medical and Veterinary Entomology

Medical and Veterinary Entomology (2010), doi: 10.1111/j.1365-2915.2010.00869.x

Geographic and ecological distribution of the dengue and chikungunya virus vectors *Aedes aegypti* and *Aedes albopictus* in three major Cameroonian towns

B. KAMGANG[1,2], J. Y. HAPPI[3], P. BOISIER[4], F. NJIOKOU[5], J.-P. HERVÉ[1], F. SIMARD[1,6] and C. PAUPY[1,7]

[1]Unité de Recherche Caractérisation et Contrôle des populations de Vecteurs (CCPV), Institut de Recherche pour le Développement (IRD), Montpellier, France, [2]Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon, [3]Département de Géographie, Faculté des Lettres et Sciences Humaines, Université de Yaoundé I, Yaoundé, Cameroon, [4]Unité d'épidémiologie, Centre Pasteur du Cameroun, Yaoundé, Cameroon, [5]Laboratoire de Biologie Général, Faculté des Sciences, Université de Yaoundé I, Yaoundé, Cameroon, [6]Laboratoire d'entomologie médicale, Institut de Recherches en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina-Faso and [7]Unité de Recherche en Ecologie de la Santé (URES) Département de Xxx, Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon

Abstract. *Aedes albopictus* (Diptera: Culicidae) was first reported in Central Africa in 2000, together with the indigenous mosquito species *Aedes aegypti* (Diptera: Culicidae). Because *Ae. albopictus* can also transmit arboviruses, its introduction is a public health concern. We undertook a comparative study in three Cameroonian towns (Sahelian domain: Garoua; equatorial domain: Douala and Yaoundé) in order to document infestation by the two species and their ecological preferences. High and variable levels of pre-imaginal *Ae. aegypti* and *Ae. albopictus* infestation were detected. Only *Ae. aegypti* was encountered in Garoua, whereas both species were found in Douala and Yaoundé, albeit with significant differences in their relative prevalence. Peridomestic water containers were the most strongly colonized and productive larval habitats for both species. No major differences in types of larval habitat were found, but *Ae. albopictus* preferentially bred in containers containing plant debris or surrounded by vegetation, whereas *Ae. aegypti* tended to breed in containers located in environments with a high density of buildings. These findings may have important implications for vector control strategies.

Key words. *Aedes aegypti*, *Aedes albopictus*, larval ecology, typology, urban environment, Cameroon.

Introduction

Aedes aegypti (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894), two mosquitoes belonging to the *Stegomyia* subgenus, are major vectors of human arboviruses. *Aedes aegypti* is the main worldwide vector of dengue virus (DENV), chikungunya virus (CHIKV) and yellow fever virus (YFV) (Kow *et al*., 2001; Gubler, 2002). Although *Ae. albopictus* has long been considered a secondary vector (Gratz, 2004), its vector status was recently revisited after the emergence of CHIKV epidemics involving this species in countries bordering the Indian Ocean (Vazeille *et al*., 2007; Delatte *et al*., 2008a, 2008b), as well as in Central Africa (Leroy *et al*., 2009; Paupy *et al*., 2009) and Europe (Charrel *et al*., 2008).

Correspondence: Christophe Paupy, Centre International de Recherches Médicales de Franceville (CIRMF), BP 769, Franceville, Gabon. Tel.: +241 67 70 96; fax: +241 67 72 95; E-mail: paupy@ird.fr

In sub-Saharan Africa, a region considered to represent its native geographic range (Mattingly, 1957), *Ae. aegypti* infests all countries and occurs in a broad range of environments, from sylvan to urban. In West Africa, this species has been responsible for historic epidemics of YFV (Monath, 1991; Barrett & Higgs, 2007) and CHIKV (Thonnon *et al*., 1999). Unlike *Ae. aegypti*, *Ae. albopictus*, a species native to Southeast Asia (Smith, 1956), has a very recent history in Africa, where it was first reported in 1990 in South Africa (Cornel & Hunt, 1991) and in 1991 in Nigeria, where it rapidly pullulated (Savage *et al*., 1992). During the current decade, *Ae. albopictus* has spread to several Central African countries (Paupy *et al*., 2009), such as Cameroon, where it occurs in most towns up to a latitude of 6° N (Simard *et al*., 2005). In urban environments previously colonized by *Ae. aegypti*, the density of *Ae. albopictus* has increased to levels compatible with arbovirus transmission. *Aedes albopictus* is suspected to play a major role in the transmission of DENV and CHIKV in Cameroon (Peyrefitte *et al*., 2007) and in Gabon, where it is now abundant, particularly in suburban areas of towns such as Libreville (Paupy *et al*., in press).

Spatial and ecological coexistence of *Ae. aegypti* and *Ae. albopictus* has been documented in several parts of the world and the larvae sometimes share common developmental sites (Braks *et al*., 2003; Simard *et al*., 2005; Chen *et al*., 2006). In South American and Southeast Asian areas where the two species are sympatric, they segregate in different habitats under the influence of environmental factors (Braks *et al*., 2003; Rey *et al*., 2006; Tsuda *et al*., 2006). *Aedes aegypti* generally predominates in densely crowded urban areas, whereas *Ae. albopictus* predominates in suburban and rural areas. Nevertheless, *Ae. albopictus* is also able to colonize urban habitats, especially when *Ae. aegypti* is absent (Delatte *et al*., 2008a). Spatial overlap of the two species is thought to result in competitive interaction. Displacement of *Ae. aegypti* after *Ae. albopictus* invasion has been documented in the southeastern U.S.A. and Brazil (Lounibos, 2002; Juliano *et al*., 2005), and is suspected in La Réunion and Mayotte (Bagny *et al*., 2009a, 2009b). Conversely, in Asia, *Ae. aegypti* has an overall competitive advantage over *Ae. albopictus*, especially in urban areas (Rudnick, 1965; Gilotra *et al*., 1967). The outcome of competitive interactions between these species has not yet been studied in an African context.

Invasion of Central Africa by genetically DENV/CHIKV competent *Ae. albopictus* (Paupy *et al*., in press) and subsequent changes in *Aedes* populations may affect arbovirus epidemiology and have the potential to trigger major outbreaks of DENV/CHIKV. The control of such diseases is based on vector surveillance and control and requires extensive background information on the biology of the mosquitoes involved. We therefore assessed the degree of infestation of *Ae. aegypti* and *Ae. albopictus* in three large Cameroonian towns, focusing on the micro-environments (larval habitats) and macro-environments exploited by the two species.

Materials and methods

Study sites

Garoua (09° 18′ N, 13° 25′ E) is the main town in northern Cameroon, with a population close to 400 000 inhabitants. The town is typically Sahelian, with recognizable modern residential blocks developed by local authorities in central areas and traditional architectural structures mostly found in the peripheral indigenous zones (Bopda & Simeu-Kamdem, 2007). Inhabitants traditionally store water inside and around their dwellings in terracotta jars (canaris), even in areas where piped water is available. The climate of Garoua is tropical-Sudanian (mean annual rainfall and temperature of 950 mm and 28°C, respectively) and the rainy season extends from April to October. Douala (04° 00′ N, 09° 43′ E) is located on the Atlantic coast. It possesses the main harbour and is the most populated city in Cameroon, with 2 million inhabitants. Two-thirds of the city is concentrated on the southern bank of the Wouri River and the town centre (including administrative and commercial structures) is located around the harbour. The spread of Douala city has resulted from the building of both privately and publicly owned residential estates and from unplanned residential development, resulting in a huge housing belt over 6 km deep (Bopda & Simeu-Kamdem, 2007). The climate of Douala is equatorial-monsoon, with heavy summer rains (mean annual rainfall and temperature of 4000 mm and 26.7°C, respectively) and a rainy season extending from March to November. Yaoundé (03° 51′ N, 11° 30′ E) is the political capital of Cameroon. It is located 250 km inland, east of Douala, and is the second largest city, with a population close to 1.5 million. Yaoundé is laid out concentrically; the centre contains most administrative and commercial structures and is surrounded by a patchwork of old native districts which still have a strong social and cultural identity. Peripheral quarters are mostly populated by waves of recent arrivals (Bopda & Simeu-Kamdem, 2007). The climate is sub-equatorial Guinean (mean annual rainfall and temperature of 1600 mm and 25°C, respectively) and there are two distinct rainy seasons, the first extending from March to June and the second from September to November.

Entomological surveys

We conducted entomological surveys in September 2006, November–December 2006 and November–December 2007 (during or at the end of the rainy seasons) in Garoua, Douala and Yaoundé, respectively. The surveys were carried out in clusters of homes selected by using a randomized sampling plan weighted by human population density (Cameroon National Census, 2005, unpublished data). The clusters consisted of three to six houses located around GPS points randomly selected prior to field investigations. During the field surveys, the team rallied at each GPS location to define the first houses of each cluster. If there were no houses at the GPS coordinates, the first house located to the north was selected. Each cluster was prospected to record all natural and artificial water containers (defined as ‘potential vector containers’), and immature stages (larvae and pupae) of *Ae. aegypti* and/or *Ae. albopictus* ('positive containers'). When present, immature stages were collected for counting and identification. Larval development sites were geo-referenced using GPS and data were noted on: the type of container; its total volume; the volume, source, use and quality (clear, tinted, containing organic matter) of the water; the presence of plant debris inside the container; the presence of vegetation around the container, and sun exposure. Based on the nature, source and use of the water, positive containers were classified into three main categories: domestic, peridomestic and natural. Domestic containers were defined as human-filled receptacles, whereas peridomestic containers (e.g. discarded containers) and natural containers (rock and tree holes, leaf axils, empty shells and nuts, etc.) were those filled by rain. Larvae and pupae were returned to insectaries to count L3 and L4 larvae and pupae of *Aedes* (*Stegomyia*) species, which were then isolated from other *Culicinae* species and reared to adult stage for taxonomic identification. The number of immature stages of each *Stegomyian* species was estimated from the proportion of emerging adults of each species, assuming equal mortality rates for the two species at the larval and pupal stages.

Entomological indices

The levels of *Ae. aegypti* and *Ae. albopictus* infestation were expressed with standard indices based on immature stages (Pan American Health Organization, 1994), including the house index (H_I, percentage of houses positive for larvae and/or pupae) and the Breteau index (B_I, number of positive containers per 100 houses). Additional indices based on the presence/absence and number of larvae or pupae were also used, including the larval index (L_I, number of larvae per 100 houses), the pupal index (P_I, number of pupae per 100 houses) and the larval-pupal index [$(L + P)_I$, total number of immature stages per 100 houses]. The productivity of a given container type is defined as the number of L3 and L4 larvae or pupae in each container type divided by the total number of L3 and L4 larvae or pupae in all container types (Hammond *et al.*, 2007).

Environmental characterization

The urban environment was characterized mainly on the basis of building density, estimated from the analysis of topographical maps (1 : 15 000 and 1 : 25 000), Google Earth images (2006, 2007 and 2009) and additional Aster images (2004). Three classes were defined: high building density corresponded to areas with an average of 50–60 houses per ha and 60–80% of built-up surface cover; medium building density corresponded to areas with 30–50 houses per ha and 40–60% of built-up surface cover, and low building density was defined as areas with <30 houses per ha and <30% of built-up surface cover. Sampled clusters and positive larval habitats were projected onto maps using MapInfo Professional 8.0 software (MapInfo Corp., Troy, NY, U.S.A.).

Statistical analysis

Type of container (six classes), water quality (three classes), presence of plant debris inside the container (two classes), presence of vegetation around the container (two classes), sun exposure (three classes), building density (three classes) and presence of any immature stage of *Ae. albopictus* (two classes) or *Ae. aegypti* (two classes) were defined as categorical variables, and percentages were compared using either Pearson's chi-squared test or Fisher's exact test. Container volumes, water volume in the containers, and the numbers of L3 and L4 larvae and pupae were defined as numerical variables. They were expressed as means and standard deviations and were compared using non-parametric Kruskal–Wallis ANOVA. The relationships between the presence of L3 and L4 larvae and pupae (immature stages) of *Aedes* spp. (*Ae. aegypti* or *Ae. albopictus*) and potential explanatory variables were first tested with univariate analysis. P-values <0.05 were considered significant. Then the presence/absence of immature stages was analysed using binary logistic regression with a conditional backward stepwise procedure. The potential predictors corresponded to the main larval habitat characteristics, as described above. All variables significantly associated with the dependent variable in univariate analysis, and variables with P-values <0.25, were introduced into the model. The goodness of fit of the final model was assessed using the Hosmer and Lemeshow statistic. All statistical analyses were performed using SPSS Version 12.0 (SPSS, Inc., Chicago, IL, U.S.A.).

Results

Pre-imaginal infestation

Respectively, 267 houses (67 clusters), 302 houses (73 clusters) and 287 houses (69 clusters) were investigated in Garoua, Douala and Yaoundé.

In Garoua, all adult specimens that emerged from field-collected larvae/pupae were identified as *Ae. aegypti*. Out of 646 potential larval breeding places recorded in Garoua, 131 (20.3%) contained immature stages of *Aedes* spp. and all were identified as *Ae. aegypti*. The H_I and B_I values were 36.0 and 49.1, respectively (Table 1). The L_I, P_I and $(L + P)_I$ values were 860.7, 218.0 and 1078.7, respectively (Table 1). All indices were significantly ($P < 0.05$, χ^2 test, data not shown) higher in areas of low building density than in medium- and high-density areas.

In Douala, out of 432 potential larval breeding places, 97 (22.5%) contained immature stages of *Aedes* spp.; larval infestation indices were 16.9 (H_I) and 32.1 (B_I). The L_I, P_I and $(L + P)_I$ values were 748.0, 129.5 and 877.5, respectively (Table 1). Both *Ae. aegypti* and *Ae. albopictus* were recorded, and species-specific indices (calculated separately for each species) were higher for *Ae. aegypti* than for *Ae. albopictus* (Table 1). When these specific indices were compared, the probability of homogeneity across species was rejected only for L_I and P_I ($P < 10^{-6}$, χ^2 test). Except for L_I and $(L + P)_I$ in the medium-density building class, where the values were higher for *Ae. albopictus* than for *Ae. aegypti*, all the indices were

Table 1. Infestation indices of immature stages of *Aedes aegypti* and *Aedes albopictus* according to building density in Garoua, Douala and Yaoundé.

		House index	Breteau index	Larval index	Pupal index	(Larval + pupal) index
Building density						
Garoua						
High $n = 85$	*Ae. aegypti*	35.3	51.4	726.6	239.9	1.492.5
Medium $n = 64$	*Ae. aegypti*	34.8	41.6	1.012.4	133.7	1.146.1
Low $n = 5$	*Ae. aegypti*	100.0	100.0	2.800.0	960.0	3.760.0
All $\mathbf{n = 131}$	***Ae. aegypti***	**36.0**	**49.1**	**860.7**	**218.0**	**1.078.7**
Douala						
High	*Ae. aegypti*	20.5	28.6	561.6	129.5	691.1
$n = 36$	*Ae. albopictus*	13.4	17.9	190.2	58.0	248.2
Medium	*Ae. aegypti*	20.0	21.6	452.8	60.8	513.6
$n = 50$	*Ae. albopictus*	19.2	27.2	560.8	56.0	616.8
Low	*Ae. aegypti*	13.8	13.8	180.0	53.8	233.8
$n = 11$	*Ae. albopictus*	6.2	6.2	53.8	6.2	60.0
All	***Ae. aegypti***	**17.9**	**22.5**	**436.4**	**86.4**	**522.8**
$\mathbf{n = 97}$	***Ae. albopictus***	**15.2**	**19.2**	**317.2**	**47.7**	**364.9**
	Ae. aegypti and/or Ae. albopictus	**16.9**	**32.1**	**748.0**	**129.5**	**877.5**
Yaoundé						
High	*Ae. aegypti*	10.2	10.2	30.7	10.2	40.9
$n = 17$	*Ae. albopictus*	17.0	17.0	168.2	40.9	209.1
Medium	*Ae. aegypti*	11.5	13.9	112.3	63.9	176.2
$n = 27$	*Ae. albopictus*	17.2	19.7	164.8	56.6	221.3
Low	*Ae. aegypti*	3.9	3.9	18.2	2.6	20.8
$n = 12$	*Ae. albopictus*	11.7	13.0	150.6	49.4	200.0
All	***Ae. aegypti***	**9.0**	**10.1**	**47.5**	**29.3**	**76.7**
$\mathbf{n = 56}$	***Ae. albopictus***	**15.0**	**17.8**	**160.5**	**52.6**	**213.3**
	Ae. aegypti and/or Ae. albopictus	**13.2**	**19.5**	**208.2**	**81.9**	**290.0**

n, number of positive containers infested with at least one L3–L4 larva or pupa of *Ae. aegypti* or *Ae. albopictus*.

higher for *Ae. aegypti* in all the urban environments studied. The differences were significant only for B_I in high-density areas ($P < 0.05$, χ^2 test) and for L_I and P_I in all three building density classes ($P < 10^{-6}$, χ^2 test).

In Yaoundé, out of 216 potential larval breeding sites surveyed, 56 (25.9%) contained immature stages of *Aedes* spp. Both species were found. The global larval infestation indices were 13.2 (H_I) and 19.5 (B_I), and the L_I, P_I and $(L + P)_I$ values were 208.2, 81.9 and 290.0, respectively (Table 2). Examined by building density class, all indices in Yaoundé were higher for *Ae. albopictus* than for *Ae. aegypti*, except for P_I in the medium-density class (Table 2). The differences were significant for L_I in all classes (P-values ranging from 10^{-5} to 10^{-6}, data not shown) and for P_I in the low- and high-density classes ($P < 10^{-6}$, data not shown). The differences in H_I and B_I were significant only in areas of low building density ($P = 0.03$ and $P = 0.02$, respectively, data not shown) (Table 2). In Douala and Yaoundé, the proportions of containers infested by *Ae. aegypti* only, *Ae. albopictus* only and by both species (Fig. 1) differed significantly ($P < 10^{-5}$, χ^2 test). The proportion of containers infested by *Ae. albopictus* (with or without *Ae. aegypti*) was significantly higher in Yaoundé than in Douala ($P < 10^{-5}$, χ^2 test), whereas the proportion of containers infested by *Ae. aegypti* (with or without *Ae. albopictus*) did not differ ($P > 0.05$, χ^2 test) between the two towns. Thus, *Ae. albopictus* was significantly more prevalent in Yaoundé than in Douala and the prevalence of *Ae. aegypti* was similar in the two towns.

Typology of larval habitats

In Garoua, most of the positive containers (76.4%) were peridomestic (Table 2). In addition, peridomestic containers harboured the largest number of larvae/pupae and were therefore the most productive (Table 3). Most of the positive containers in Douala and Yaoundé were also peridomestic (87.6% and 78.6%, respectively). In Douala, the most productive containers were used tyres and miscellaneous containers for *Ae. aegypti*, and discarded tanks for *Ae. albopictus* (Table 3). In Yaoundé, the most productive containers were discarded tanks for *Ae. aegypti* and used tyres for *Ae. albopictus* (Table 3).

We used a binary logistic regression model to test the association between container characteristics and the presence of immature stages of *Aedes* spp. The analyses showed that

Table 2. Infestation and larval habitat typology of *Aedes aegypti* and *Aedes albopictus* in three major towns in Cameroon.

	Garoua			Douala					Yaoundé				
	n inspected	% positive	% *Ae. aegypti* only	*n* inspected	% positive	% *Ae. aegypti* only	% *Ae. albopictus* only	% mixed	*n* inspected	% positive	% *Ae. aegypti* only	% *Ae. albopictus* only	% mixed
	$n = 646$	$n = 131$	$n = 131$	$n = 432$	$n = 97$	$n = 39$	$n = 29$	$n = 29$	$n = 216$	$n = 56$	$n = 6$	$n = 27$	$n = 23$
Type of container													
Domestic	**207**	**22.9**	**22.9**	**76**	**12.4**	**15.4**	**10.4**	**10.3**	**56**	**19.6**	**33.4**	**18.5**	**17.4**
Water storage	205	22.1	22.1	62	8.3	7.7	6.9	10.3	39	10.7	16.7	3.7	17.4
Flower pots	2	0.8	0.8	14	4.1	7.7	3.5	0.0	17	8.9	16.7	14.8	0.0
Peri-domestic	**437**	**76.4**	**76.4**	**352**	**87.6**	**84.6**	**89.6**	**89.7**	**158**	**78.6**	**66.6**	**77.8**	**82.6**
Used tyres	89	17.6	17.6	121	23.7	35.9	10.3	20.7	45	30.4	0.0	29.6	39.1
Discarded tanks	304	44.3	44.3	132	37.1	17.9	41.4	41.4	77	30.4	33.3	29.6	30.4
Miscellaneous	44	14.5	14.5	99	26.8	30.8	37.9	27.6	36	17.9	33.3	18.6	13.1
Natural	**2**	**0.7**	**0.7**	**4**	**0.0**	**0.0**	**0.0**	**0.0**	**2**	**1.8**	**0.0**	**3.7**	**0.0**

n inspected, number of potential containers inspected; % positive, percentage of containers infested with at least one larva or pupa of one species; *Ae. aegypti* only, containers containing only *Ae. aegypti*; *Ae. albopictus* only, containers containing only *Ae. albopictus*; mixed, containers infested with 'at least one larva/pupa of each species'.

in Garoua the presence of immature stages of *Ae. aegypti* was significantly and positively [relative risk (RR) > 1] associated with the presence of vegetation in the immediate vicinity of the potential container [RR = 16.42, 95% confidence interval (CI) 3.84–70.3] and with the presence of plant debris inside the container (RR = 5.22, 95% CI 1.14–23.92). In Douala and Yaoundé, we found no significant association between the presence of immature stages of *Ae. aegypti* and any of the variables. The presence of immature stages of *Ae. albopictus* was positively and significantly associated with the presence of plant debris inside the container (Douala: RR = 2.8, 95% CI 1.12–6.78) and with vegetation around the container (Yaoundé: RR = 9.33, 95% CI 2.84–30.70). The number of *Ae. aegypti* immature stages inside the container (Douala: RR = 0.97, 95% CI 0.94–0.99) was a significant predictor for both the presence and abundance of *Ae. albopictus*.

Environmental distribution

Finally, we investigated the relationship between building density and the spatial distribution of larval habitats of *Ae. aegypti* and *Ae. albopictus* (Fig. 2). Except in Douala, the number of containers colonized by immature stages of *Ae. aegypti* was significantly higher in areas of high building density than in medium- or low-density areas ($P < 0.05$, Kruskal–Wallis test). In Douala and Yaoundé, the spatial distribution of *Ae. albopictus* was not related to building density.

Discussion

We assessed the pre-imaginal infestation rates of *Ae. aegypti* and *Ae. albopictus* in three major Cameroonian towns, along with the typology of containers colonized by the two species. Our findings confirmed that *Ae. aegypti* was present in all three towns, whereas *Ae. albopictus* was limited to the southern towns (Douala and Yaoundé). In Douala and Yaoundé, the two species co-localized, but there were significant differences in their relative proportions and spatial distribution, resulting from differences in environmental characteristics such as building density and vegetation.

We found significant differences in larval infestation rates of the *Aedes* spp. according to the town. Indeed, the number of immature stages of *Aedes* spp. and the larval/pupal indices (H_I and B_I) were higher in Garoua than in Douala or Yaoundé, suggesting higher potential human exposure to *Aedes* spp. bites in Garoua. This situation was probably related to the larger number of potential containers (2.41 per house) in Garoua than in Douala and Yaoundé (0.32 and 0.19 per house, respectively). Infestation of terracotta jars and other tanks traditionally used in Garoua and throughout northern Cameroon to store water or cereals was low, contrary to the situation observed in other parts of the world, particularly Asia (Strickman & Kittayapong, 1993; Hammond *et al*., 2007). Previous observations made in Garoua during the dry season suggested that *Ae. aegypti* rarely colonizes earthen

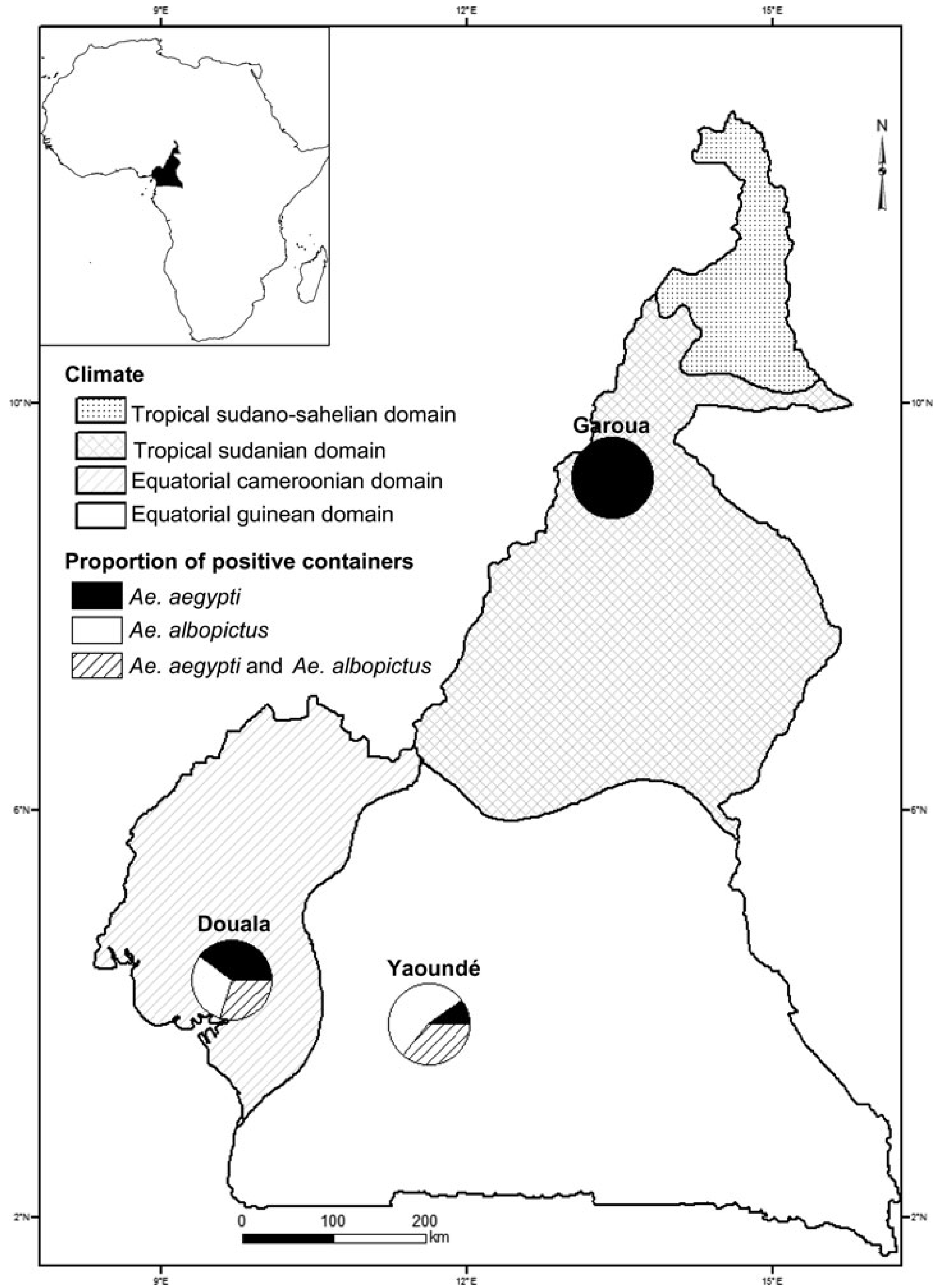

Fig. 1. Location of study sites in Cameroon (2006–2007) and proportions of containers colonized by *Aedes aegypti*, *Aedes albopictus* or both species.

Table 3. Number of larvae (L3–L4) and pupae for *Aedes aegypti* and *Aedes albopictus* in each container type according to investigated town.

	Garoua		Douala				Yaoundé			
	Ae. aegypti		*Ae. aegypti*		*Ae. albopictus*		*Ae. aegypti*		*Ae. albopictus*	
	L3–L4, *n* (mean ± SD)*	Pupae, *n* (mean ± SD)	L3–L4, *n* (mean ± SD)	Pupae, *n* (mean ± SD)	L3–L4, *n* (mean ± SD)	Pupae, *n* (mean ± SD)	L3–L4, *n* (mean ± SD)	Pupae, *n* (mean ± SD)	L3–L4, *n* (mean ± SD)	Pupae, *n* (mean ± SD)
Type of container										
Domestic	**430 (15.9 ± 17.8)**	**125 (8.3 ± 12.1)**	**244 (24.4 ± 31.8)**	**39 (4.9 ± 9.0)**	**109 (12.1 ± 17.2)**	**7 (1.2 ± 1.3)**	**40 (4.0 ± 5.3)**	**5 (1.3 ± 0.5)**	**113 (12.6 ± 10.8)**	**15 (2.1 ± 2.1)**
Water storage	420 (16.2 ± 18.1)	125 (8.3 ± 12.0)	211 (35.2 ± 37.3)	38 (9.5 ± 2.5)	89 (17.8 ± 20.6)	5 (2.5 ± 0.7)	30 (6.0 ± 5.7)	4 (1.3 ± 5.6)	78 (15.6 ± 12.6)	11 (3.7 ± 2.3)
Flower pots	10 (10.0 ± 0.0)	0	33 (11.0 ± 12.5)	1 (1.0 ± 0.0)	20 (20.0 ± 0.0)	2 (2.0 ± 0.0)	10 (10.0 ± 0.0)	1 (1.0 ± 0.0)	35 (11.7 ± 7.0)	4 (2.0 ± 0.0)
Peridomestic	**1878 (21.3 ± 25.9)**	**457 (7.4 ± 8.7)**	**1080 (18.6 ± 19.8)**	**222 (6.3 ± 9.6)**	**851 (17.0 ± 18.4)**	**138 (4.9 ± 5.1)**	**102 (4.9 ± 9.1)**	**84 (7.0 ± 18.0)**	**354 (10.1 ± 7.4)**	**137 (5.5 ± 4.5)**
Discarded tanks	1184 (23.2 ± 29.7)	237 (6.6 ± 8.7)	266 (14.0 ± 27.9)	42 (3.2 ± 2.6)	408 (18.5 ± 21.9)	68 (6.2 ± 6.7)	66 (7.3 ± 13.6)	68 (17 ± 31.3)	86 (6.1 ± 3.7)	31 (3.4 ± 4.1)
Used tyres	329 (19.2 ± 22.2)	86 (9.6 ± 9.3)	491 (17 ± 9.4)	80 (12.5 ± 15.8)	118 (17.1 ± 17.3)	13 (5.2 ± 4.4)	28 (2.7 ± 2.0)	15 (2.1 ± 2.2)	217 (10.2 ± 5.9)	94 (7.2 ± 4.4)
Miscellaneous	365 (18.3 ± 8.5)	134 (7.2 ± 8.5)	323 (24.5 ± 17.1)	100 (5.7 ± 8.3)	325 (13.1 ± 11.1)	57 (2.2 ± 1.6)	8 (3.1 ± 2.7)	1 (1.0 ± 0.0)	51 (13.6 ± 8.7)	12 (4.0 ± 3.6)
Natural	7 (7.0 ± 0.0)	—	—	—	—	—	—	—	3 (3.0 ± 0.0)	—
All	**2315 (20.0 ± 24.2)**	**582 (7.5 ± 9.4)**	**1324 (19.5 ± 21.7)**	**261 (6.1 ± 9.4)**	**960 (16.3 ± 18.2)**	**145 (4.3 ± 4.9)**	**142 (24.0 ± 24.0)**	**89 (15.0 ± 27.0)**	**467 (93.0 ± 72.0)**	**152 (25.0 ± 35.0)**

*Only *Ae. aegypti* was found in Garoua.
n, number; SD, standard deviation.

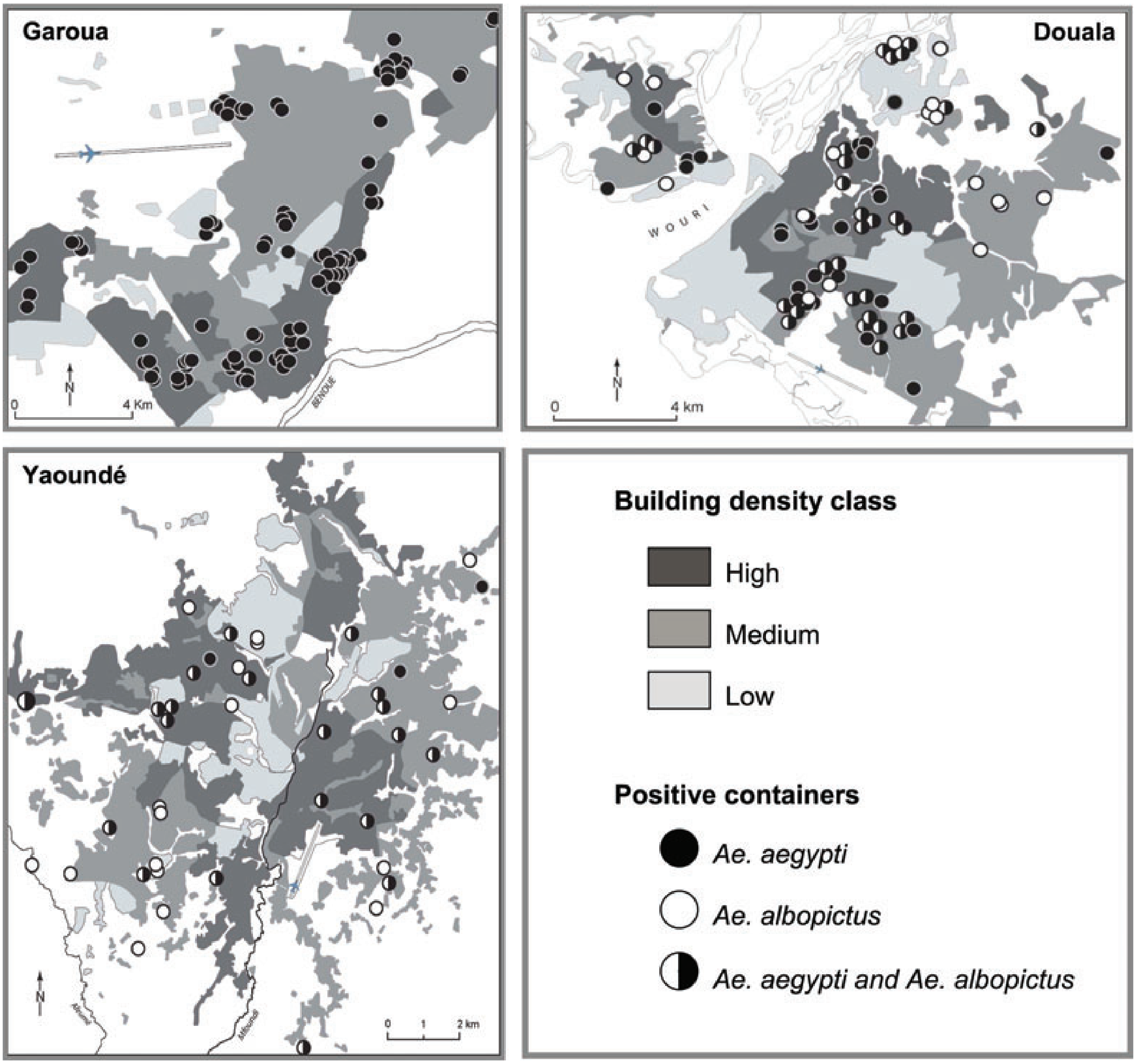

Fig. 2. Distribution of *Aedes aegypti* and *Aedes albopictus* larval habitats according to building density in three major Cameroonian towns.

jars, even when rain-filled containers are absent (C. Paupy, unpublished data, 2007). One possible reason for the lack of *Ae. aegypti* larvae in drinking water storage tanks is regular cleaning, although socioanthropological studies of the uses and management of these containers are needed to confirm this. By contrast, when abandoned in the peridomestic environment and filled with rainwater, terracotta jars represented the main sites for *Ae. aegypti* development.

Aedes albopictus was detected in the two main towns in southern Cameroon (Douala and Yaoundé), but not in Garoua, which is located at a latitude of about 09° N. This is consistent with a previous study suggesting that the northern limit of *Ae. albopictus* in Cameroon is around 6° N (Simard *et al*., 2005). The absence of *Ae. albopictus* north of this limit may result from unfavourable climatic conditions. In Garoua, the climate is tropical Sudanian, with a short rainy season that extends from April to October, an average annual rainfall of <950 mm and mean monthly temperatures of 26–32°C. Models predicting areas favourable for *Ae. albopictus* (Benedict *et al*., 2007) suggest that such climatic conditions are probably not compatible with the propagation of the species. In addition, *Ae. albopictus* eggs suffer high mortality rates in conditions of extreme humidity and temperature (Juliano *et al*., 2002). *Aedes aegypti* is better adapted to arid conditions such as those occurring in Garoua and its eggs resist low hygrometry and high temperatures better (Braks *et al*., 2003). Conversely, climatic conditions in southern Cameroon (equatorial climate, mean annual temperature <26.5°C) (Table 1) are more favourable for *Ae. albopictus*, which is widespread in this area (Fontenille & Toto, 2001; Simard *et al*., 2005).

In all three towns the larvae of both species preferentially colonized peridomestic containers, except for terracotta jars, which were present only in Garoua. In keeping with previous observations made in Cameroon (Fontenille & Toto, 2001; Simard *et al*., 2005), peridomestic containers represented the bulk of the containers infested by *Ae. aegypti* or *Ae. albopictus*. In many sub-Saharan towns, unplanned urbanization and lack of waste management lead to a multiplication of water collections, thus favouring the proliferation of *Aedes* species. Although the two species studied here were found to breed in similar container types, *Ae. albopictus* preferred those containing plant debris or surrounded by vegetation. This suggests that micro-environmental factors influence the presence of the two species, above and beyond the container type itself.

Aedes aegypti and *Ae. albopictus* co-existed in Douala and Yaoundé although their prevalences differed markedly. *Aedes aegypti* was the most abundant species in Douala, as was *Ae. albopictus* in Yaoundé. This probably reflects differences in environmental factors (biotic and abiotic). Intercontinental trade, especially the shipment of used tyres, is the main source of dissemination of *Ae. albopictus* worldwide (Reiter, 1998; Gratz, 2004). Because Douala possesses the only functional commercial harbour in Cameroon, as well as an international airport, *Ae. albopictus* was probably first introduced into Douala and secondarily into Yaoundé via the road network. If time of colonization was the main factor explaining the spatial distribution of *Ae. albopictus*, a higher density would have been expected in Douala than in Yaoundé. Alternatively, environmental factors (climate, vegetation, building density, etc.) may be responsible for the different prevalences of *Ae. aegypti* and *Ae. albopictus* observed here. Previous studies have suggested that *Ae. albopictus* preferentially colonizes environments with vegetation and mainly breeds in natural containers such as tree holes and leaf axils, whereas *Ae. aegypti* prefers to breed in artificial containers located in environments with higher building density (Chan *et al*., 1971; Braks *et al*., 2003; Cox *et al*., 2007). Because Yaoundé is more wooded than Douala, it may provide a more favourable environment for *Ae. albopictus*. In addition, we found that *Ae. albopictus* tended to breed in containers surrounded by vegetation in Yaoundé. By contrast with *Ae. albopictus*, and in keeping with previous studies, *Ae. aegypti* also seems to prefer built-up areas (Garoua) or places with a high building density (Douala) in Cameroon. *Aedes albopictus* and *Ae. aegypti* are competing species because they use the same blood sources and larval habitats, and competitive displacement has been documented outside Africa (Chan *et al*., 1971; Lounibos, 2002; Juliano *et al*., 2005). Competition probably also occurs in Cameroon, where the two species share the same resources.

In conclusion, this survey shows high infestation rates of *Ae. aegypti* and *Ae. albopictus* in three major Cameroonian towns, implying a strong potential for human arbovirus transmission. Additional investigations of larger numbers of houses at different dates are needed. Our data on the typology and productivity of larval development sites should be useful for vector control programmes.

Acknowledgements

We thank the inhabitants of Garoua, Douala and Yaoundé for their full participation in the study. We are also grateful to Elysée Nchoutpouen for significant help during field work and mosquito rearing. This work was supported by the French government through Agence Nationale pour la Recherche, which funded the Epidengue Project (ANR 05 SEST 010-01), and the Institut de Recherche pour le Développement (IRD).

References

Bagny, L., Delatte, H., Elissa, N., Quilici, S. & Fontenille, D. (2009a) *Aedes* (Diptera: Culicidae) vectors of arboviruses in Mayotte (Indian Ocean): distribution area and larval habitats. *Journal of Medical Entomology*, **46**, 198–207.

Bagny, L., Delatte, H., Quilici, S. & Fontenille, D. (2009b) Progressive decrease in *Ae. aegypti* distribution in Reunion Island since the 1900s. *Journal of Medical Entomology*, **46**, 1541–1545.

Barrett, A.D.T. & Higgs, S. (2007) Yellow fever: a disease that has yet to be conquered. *Annual Review of Entomology*, **52**, 209–229.

Benedict, M.Q., Levine, R.S., Hawley, W. & Lounibos, L.P. (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne and Zoonotic Diseases*, **7**, 76–85.

Bopda, A. & Simeu-Kamdem, M. (2007) Urban development. *Atlas of Cameroon* (ed. by D. Ben Yahmed, N. Houstin & C. Seignobos), pp. 105–119. Les Editions JA-Edition du Jaguar, Paris.

Braks, M.A., Honorio, N.A., Lourenco-De-Oliveira, R., Juliano, S.A. & Lounibos, L.P. (2003) Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. *Journal of Medical Entomology*, **40**, 785–794.

Chan, K.L., Chan, Y.C. & Ho, B.C. (1971) *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 4. Competition between species. *Bulletin of World Health Organization*, **44**, 643–649.

Charrel, R.N., De Lamballerie, X. & Raoult, D. (2008) Seasonality of mosquitoes and chikungunya in Italy. *Lancet Infectious Diseases*, **8**, 5–6.

Chen, C.D., Nazni, W.A., Lee, H.L., Seleena, B., Mohd Masri, S., Chiang, Y.F. & Sofian-Azirun, M. (2006) Mixed breeding of *Aedes aegypti* (L.) and *Aedes albopictus* Skuse in four dengue endemic areas in Kuala Lumpur and Selangor. *Tropical Biomedicine*, **23**, 224–227.

Cornel, A.J. & Hunt, R.H. (1991) *Aedes albopictus* in Africa? First records of live specimens in imported tyres in Cape Town. *Journal of the American Mosquito Control Association*, **7**, 107–108.

Cox, J., Grillet, M.E., Ramos, O.M., Amador, M. & Barrera, R. (2007) Habitat segregation of dengue vectors along an urban environmental gradient. *American Journal of Tropical Medicine and Hygiene*, **76**, 820–826.

Delatte, H., Dehecq, J.S., Thiria, J., Domerg, C., Paupy, C. & Fontenille, D. (2008a) Geographic distribution and developmental sites of *Aedes albopictus* (Diptera: Culicidae) during a chikungunya epidemic event. *Vector Borne and Zoonotic Diseases*, **8**, 25–34.

Delatte, H., Paupy, C., Dehecq, J.S., Thiria, J., Failloux, A.B. & Fontenille, D. (2008b) *Aedes albopictus*, vector of chikungunya and dengue viruses in Reunion Island: biology and control. *Parasite*, **15**, 3–13.

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

Gilotra, S.K., Rozeboom, L.E. & Bhattacharya, N.C. (1967) Observation on possible competitive displacement between populations of *Aedes aegypti* and *Aedes albopictus* Skuse in Calcutta. *Bulletin of World Health Organization*, **37**, 437–446.

Gratz, N.G. (2004) Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology*, **18**, 215–227.

Gubler, D.J. (2002) Epidemic dengue/dengue haemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends in Microbiology*, **10**, 100–103.

Hammond, S.N., Gordon, A.L., Lugo Edel, C. *et al*. (2007) Characterization of *Aedes aegypti* (Diptera: Culicidae) production sites in urban Nicaragua. *Journal of Medical Entomology*, **44**, 851–860.

Juliano, S.A. & Lounibos, L.P. (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology Letters*, **8**, 558–574.

Juliano, S.A., O'Meara, G.F., Morrill, J.R. & Cutwa, M.M. (2002) Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia*, **130**, 458–469.

Kow, C.Y., Koon, L.L. & Pang, F.Y. (2001) Detection of dengue viruses in field-caught male *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Singapore by type-specific PCR. *Journal of Medical Entomology*, **38**, 475–479.

Leroy, E.M., Nkoghe, D., Ollomo, B. *et al*. (2009) Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon. *Emerging Infectious Diseases*, **15**, 591–593.

Lounibos, L.P. (2002) Invasions by insect vectors of human diseases. *Annual Review of Entomology*, **47**, 233–266.

Mattingly, P.F. (1957) Genetical aspects of the *Aedes aegypti* problem. I. Taxonomy and bionomics. *Annals of Tropical Medicine and Parasitology*, **51**, 392–408.

Monath, T.P. (1991) Yellow fever: Victor, Victoria? Conqueror, conquest? Epidemics and research in the last forty years and prospects for the future. *American Journal of Tropical Medicine and Hygiene*, **45**, 1–43.

Pan American Health Organization (PAHO) (1994) *Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control*. PAHO, Washington, DC.

Paupy, C., Delatte, H., Bagny, L., Corbel, V. & Fontenille D. (2009) *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes and Infections*, **11**, 1177–1185.

Paupy, C., Ollomo, B., Kamgang, B. *et al*. (2010b) Role of *Ae. albopictus* versus *Ae. aegypti* in dengue and chikungunya emergences in Central Africa: laboratory and field evidence. *Vector Borne and Zoonotic Diseases*, in press.

Peyrefitte, C.N., Rousset, D., Pastorino, B.A. *et al*. (2007) Chikungunya virus, Cameroon, 2006. *Emerging Infectious Diseases*, **13**, 768–771.

Reiter, P. (1998) *Aedes albopictus* and the world trade in used tyres, 1988–1995: the shape of things to come? *Journal of American Mosquito Control Association*, **14**, 83–94.

Rey, J.R., Nishiruma, B., Wagner, M., Braks, M.A., O'Connell, S.M. & Lounibos, L.P. (2006) Habitat segregation of mosquito arbovirus vectors in South Florida. *Journal of Medical Entomology*, **43**, 1134–1141.

Rudnick, A. (1965) Studies on the ecology of dengue in Malaysia: a preliminary report. *Journal of Medical Entomology*, **2**, 203–208.

Savage, H.M., Ezike, V.I., Nwankwo, A.C.N., Spiegel, R. & Miller, B.R. (1992) First record of breeding populations of *Aedes albopictus* in continental Africa: implications for arboviral transmission. *Journal of the American Mosquito Control Association*, **8**, 101–103.

Simard, F., Nchoutpouen, E., Toto, J.C. & Fontenille, D. (2005) Geographic distribution and breeding site preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) in Cameroon, Central Africa. *Journal of Medical Entomology*, **42**, 726–731.

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**, 3–11.

Strickman, D. & Kittayapong, P. (1993) Laboratory demonstration of oviposition by *Aedes aegypti* (Diptera: Culicidae) in covered water jars. *Journal of Medical Entomology*, **30**, 947–949.

Thonnon, J., Spiegel, A., Diallo, M., Diallo, A. & Fontenille, D. (1999) Chikungunya virus outbreak in Senegal in 1996 and 1997. *Bulletin de la Société de Pathologie Exotique*, **92**, 79–82.

Tsuda, Y., Suwonkerd, W., Chawprom, S., Prajakwong, S. & Takagi, M. (2006) Different spatial distribution of *Aedes aegypti* and *Aedes albopictus* along an urban–rural gradient and the relating environmental factors examined in three villages in northern Thailand. *Journal of the American Mosquito Control Association*, **22**, 222–228.

Vazeille, M., Moutailler, S., Coudrier, D. *et al*. (2007) Two chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus*. *PLoS ONE*, **11**, e1168.

Accepted 25 February 2010

