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Variation over space and time of *Aedes aegypti* in Phnom Penh (Cambodia): genetic structure and oral susceptibility to a dengue virus

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Summary

We studied spatial and temporal variation in 20–23 *Aedes aegypti* samples collected in Phnom Penh and its suburbs to estimate the population genetic structure using allozymes and the susceptibility to a dengue-2 virus. Based on seven allozyme systems, we detected low levels of genetic exchanges (i.e. high, significant F_{ST} values) between populations collected in the city centre, and different patterns of genetic structure for samples collected in the suburbs, depending on the type of environment and the date of collection. In the southern suburbs and the Chroy Chang Var Peninsula, differentiation became highly significant at the end of the dry season, whereas the opposite situation was observed for collections from the northern suburbs. Vector competence assessed by oral infections with a dengue-2 virus was lower for samples collected in the city centre than in the suburbs. A significant decrease of dengue susceptibility was observed in populations during the dry season. This study allows a model of *Ae. aegypti* population functioning in Phnom Penh to be suggested. Dynamics of dengue virus diffusion depend on the population genetic structure of the vector and its evolution over space and time.

1. Introduction

Since the emergence of dengue hemorrhagic fever (DHF) in the mid-1950s in South-East Asia, DHF occurs mostly throughout Asia, the Americas and the Pacific region, and its incidence is increasing rapidly worldwide (Gubler, 2002). The lack of effective vaccines and treatment, the rapid urbanization, the emergence of insecticide-resistant mosquitoes and the increasing spread of viruses and vectors throughout the world require the development of alternative methods to control dengue transmission. Among them, the release of genetically engineered, dengue-resistant mosquitoes could disrupt virus transmission to human populations. These transgenic vectors would contain heritable anti-dengue genes or sequences that could irreversibly alter the vector competence. Thus, replication of dengue viruses in the midgut or salivary glands could be prevented (Olson *et al.*, 2002). However, whether these genetically modified mosquito populations could really become established is still the subject of much debate. In particular, the dynamics of colonization and impact of population size on genetic structure should be addressed.

At least 1 million of the 12 millions inhabitants of Cambodia lives in the Phnom Penh province and 570 000 are settled in the urban area. The multiple breeding sites created in the urban centre favour vector proliferation (C. Paupy *et al.*, unpublished). Recurrent introductions of new serotypes of dengue virus into naïve or partially immune populations have resulted in an increasing frequency in dengue outbreaks (N. Chantha, personal communication).

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Table 1. *Sampling sites of* Aedes aegypti *collected in Phnom Penh in February and July 2001*

Sample	Area[a]	District	Location	Type of breeding site	Sample	Area[a]	District	Location	Type of breeding site
February[b]					July[c]				
PP1	Centre	Chamkar Mon	Boeung Trabek	Big jar	PP1′	Centre	Chamkar Mon	Boeung Trabek	Tyre
PP2	Centre	Chamkar Mon	Tonle Bassac	Big jar	PP2′	Centre	Chamkar Mon	Tonle Bassac	Tyre
PP3	Centre	Meanchey	Boeung Tom Ponn	Small jar	PP3′	Centre	Meanchey	Boeung Tom Ponn	Big jar
PP4	Centre	Meanchey	Stueng Meanchey	Big jar	PP4(a)′	Centre	Meanchey	Stueng Meanchey	Watering place
					PP4(b)′	Centre	Meanchey	Stueng Meanchey	Big jar
PP5	Centre	Toul Kok	Toeuk Laok 3	Small jar	PP5′	Centre	Toul Kok	Toeuk Laok 3	Big jar
PP6	Centre	Toul Kok	Boeung kok 2	Big jar	PP6′	Centre	Toul Kok	Boeung kok 2	Big jar
PP7	Centre	Daun Penh	Srash Chak	Big jar	PP7′	Centre	Daun Penh	Srash Chak	Big jar
PP8	Centre	Daun Penh	Srash Chak	Big jar	PP8′	Centre	Daun Penh	Srash Chak	Small jar
PP9	Centre	Russei Keo	Toul Sangke	Big jar	PP9′	Centre	Russei Keo	Russei Keo	Big jar
PP10	Peninsula	Russei Keo	Chroy Chang Var	Metal drum	PP10′	Peninsula	Russei Keo	Chroy Chang Var	Big jar
PP11	North	Russei Keo	Russei Keo	Big jar	PP11′	North	Russei Keo	Russei Keo	Small jar
PP12	North	Russei Keo	Kilometre 6	Big jar	–				
PP13	North	Russei Keo	Chraing Cham Res 1	Small jar	PP13′	North	Russei Keo	Chraing Cham Res 1	Small jar
PP14	Peninsula	Russei Keo	Prek Leab	Small jar	PP14(a)′	Peninsula	Russei Keo	Prek Leab	Small jar
					PP14(b)′	Peninsula	Russei Keo	Prek Leab	Small jar
PP15	West	Russei Keo	Phnom Penh Thmey	Big jar	PP15′	West	Russei Keo	Phnom Penh Thmey	Big jar
PP16	West	Russei Keo	Toek Thla	Broken jar	PP16′	West	Russei Keo	Toek Thla	Small jar
PP17	West	Russei Keo	Toek Thla	Big jar	–				
PP18	West	Dankor	Chorm Chao	Big jar	PP18′	West	Dankor	Chorm Chao	Big jar
PP19	South	Meanchey	Stueng Meanchey	Big jar	–				
PP20	South	Meanchey	Chak Angrae kraom	Plastic drum	PP20′	South	Meanchey	Chak Angrae kraom	Small jar
PP21	South	Meanchey	Chak Angrae Kraom	Small jar	PP21′	South	Meanchey	Chak Angrae Kraom	Flower vase
PP22	South	Meanchey	Chak Angrae Kraom	Small jar	–				
PP23	South	Meanchey	Prek Pra	Small jar	PP23′	South	Meanchey	Prek Pra	Small jar

[a] Center, city centre; north, northern suburbs; peninsula, Chroy Chang Var peninsula; south, southern suburbs; west, western suburbs; –, no sample.
[b] Beginning of the dry season.
[c] End of the dry season.

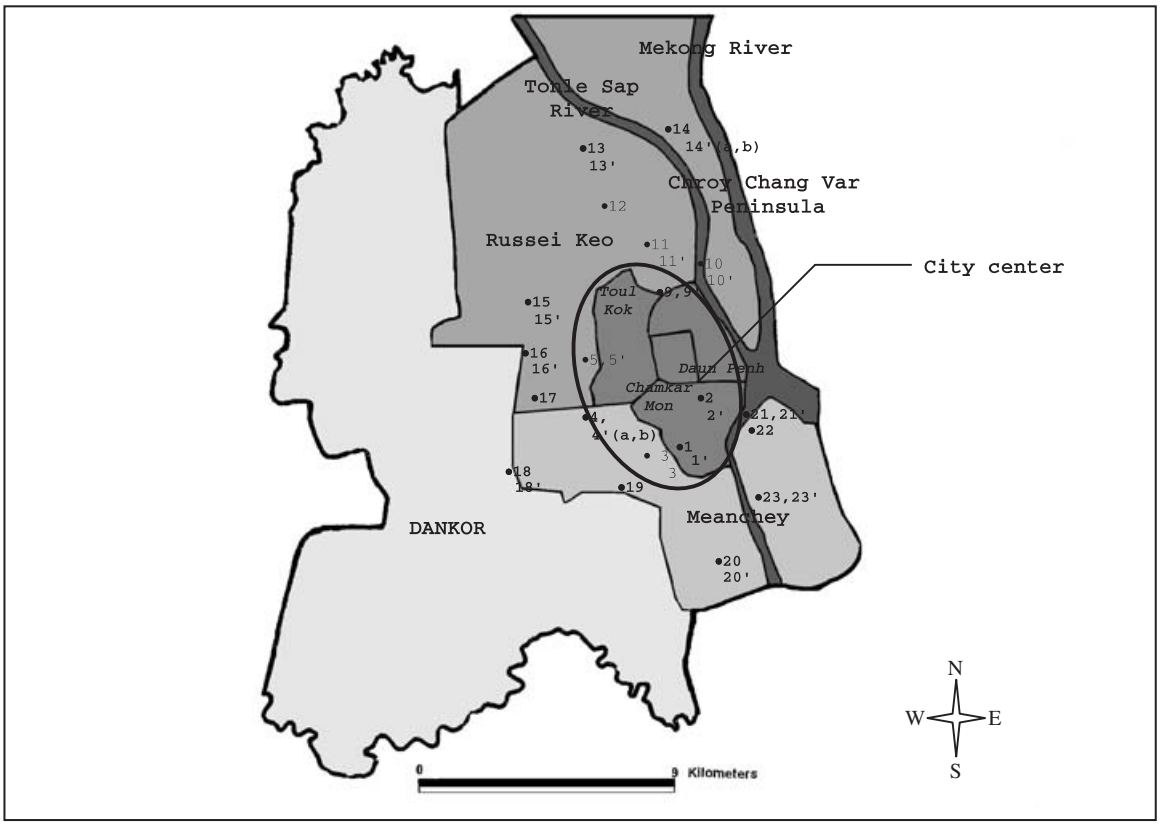

Fig. 1. Map of Phnom Penh showing the location of *Aedes aegypti* samples collected in 2001.

The area of dengue transmission matches the distribution of the main vector, *Aedes aegypti*. In Asia, where the species was introduced at the end of the 19th century, *Ae. aegypti* is a domestic, anthropophilic mosquito, breeding around houses in artificial containers (Strickman & Kittayapong, 1993), has a short flight range (Tsuda *et al.*, 2001) and gets multiple blood-meals before oviposition (Scott *et al.*, 1997). In Cambodia, where this mosquito has recently been introduced (C. Paupy *et al.*, unpublished), water storage containers (particularly concrete jars with a capacity of 100 litres and more) are the most common larval habitat, exhibiting a high infestation rate throughout the year (N. Chantha, personal communication). In Phnom Penh, more than 40% of indoor resting mosquitoes are *Ae. aegypti* (Kohn, 1990).

Insect populations are often fall, leading to a loss in genetic diversity (Huber *et al.*, 2002a,b). These changes in genetic structure can be induced by natural (e.g. availability of habitats) or human-mediated (e.g. insecticide control) environmental changes. Investigating the genetic structure of populations at different geographic scales and different temporal periods is useful for predicting the spread of some genes of interest (Failloux *et al.*, 2002). In this study, we examined genetic changes at seven allozyme systems and variation in vector competence to a dengue-2 virus of 20–22 *Ae. aegypti* samples collected in Phnom Penh and suburbs. We quantified geographic variation among *Ae. aegypti* populations distributed throughout the city of Phnom Penh and suburbs, and temporal variation of populations sampled twice over a 6-month period, at the beginning and the end of the dry season.

2. Materials and methods

(i) *Mosquito sampling*

Ae. aegypti larvae and pupae were collected in breeding sites sampled in Phnom Penh City in 2001; 23 samples were taken in February and 20 in July (Table 1, Fig. 1). The first collection corresponded to the beginning of the dry season and the second to the end of the dry season.

Sampling was carried out in different areas: (1) in the city centre, where the habitat was dense with brick houses supplied with running water, and fields and vegetation were almost absent; (2) in the southern suburbs, where the human density was high and the houses were not provided with piped water, a habitat of the slum type; (3) in the western suburbs, where concrete houses were separated by fields and vegetation, an area that seemed to be more residential; and (4) in the northern suburbs and in the Chroy Chang Var peninsula, where wooden houses

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Table 2. F_{IS} *and deviations from Hardy–Weinberg expectations observed at six polymorphic loci in 42* Aedes aegypti *samples collected in Cambodia in 2001*

		Got-2		G-3-pdh		Hk-2		Mdh		Pgi		Pgm		All loci
	N	N_{all}	F_{IS}	N_{all}	F_{IS}	N_{all}	F_{IS}	N_{all}	F_{IS}	N_{all}	F_{IS}	N_{all}	F_{IS}	*P*
February														
City centre														
PP1	48	1	–	1	–	2	0·117	4	0·122	2	–0·014	5	0·141	0·153
PP2	48	1	–	1	–	1	–	3	–0·159	3	**0·850**	4	0·062	**0·000**
PP3	27	1	–	2	–0·020	2	–	2	0·098	2	–	4	0·168	0·890
PP4	48	1	–	2	–0·175	3	–0·098	2	–0·372	2	0·086	4	0·356	0·017
PP5	48	2	0·486	1	–	2	–0·033	3	–0·144	3	0·161	4	0·133	0·241
PP6	48	1	–	2	–0·080	2	–0·022	3	–0·067	3	–0·051	3	0·167	0·955
PP7	48	1	–	2	–	2	–	3	–0·250	3	0·305	3	0·116	0·116
PP8	48	1	–	1	–	2	–0·011	3	–0·123	2	0·793	4	–0·041	**0·005**
PP9	48	2	–0·022	1	–	2	**0·793**	3	0·090	3	–0·035	3	–0·014	0·189
Suburbs (Peninsula)														
PP10	48	1	–	1	–	1	–	3	–0·165	2	–0·011	3	0·231	0·321
PP14	48	2	–0·011	2	–	2	–0·011	3	–0·078	2	–0·011	4	0·045	0·132
Suburbs (North)														
PP11	48	2	–0·011	2	–0·136	1	–	3	–0·108	3	0·171	2	0·174	0·728
PP12	38	1	–	2	–0·072	1	–	3	–0·159	2	–0·014	2	0·062	0·9554
PP13	48	2	0·662	1	–	3	0·468	2	–0·027	2	–0·022	2	–0·106	0·1523
Suburbs (West)														
PP15	48	2	–0·011	1	–	2	–	3	0·013	2	–0·022	4	–0·014	0·3650
PP16	48	2	–	2	0·029	2	–0·044	3	–0·035	3	–0·107	4	–0·169	1
PP17	48	1	–	1	–	1	–	3	0·099	2	0·651	4	0·121	**0·002**
PP18	48	2	–0·011	2	–0·044	1	–	3	0·120	3	–0·022	4	–0·088	0·977
Suburbs (South)														
PP19	48	1	–	1	–	1	–	3	–0·084	2	–	4	–0·088	0·973
PP20	48	1	–	1	–	1	–	3	0·159	2	–0·033	3	–0·086	0·944
PP21	48	1	–	1	–	1	–	2	0·072	2	–0·011	5	0·042	0·716
PP22	25	1	–	1	–	1	–	2	–0·104	2	–0·021	2	1	0·254

July													
City centre													
PP1′	48	1	–	1	–	1	–	2	0·087	1	–	4	–0·147	0·883
PP2′	48	1	–	1	–	2	–	3	0·049	3	0·308	4	0·164	**0·010**
PP3′	48	1	–	2	–0·106	3	–0·176	3	–0·081	2	–0·024	2	–0·014	0·998
PP4(a)′	48	1	–	1	–	1	–	3	0·037	3	–0·031	4	0·493	0·086
PP4(b)′	48	1	–	2	–0·098	2	–0·103	3	–0·208	3	–0·052	4	0·056	0·964
PP5′	48	1	–	1	–	1	–	3	0·254	1	–0·005	4	–0·221	0·560
PP6′	48	1	–	1	–	1	–	2	–0·094	2	–0·106	3	–0·059	0·912
PP7′	48	1	–	1	–	1	–	2	–0·266	3	0·190	4	0·019	0·430
PP8′	24	1	–	1	–	1	–	2	0·073	3	0·281	3	0·019	0·373
PP9′	45	1	–	1	–	2	–0·035	3	0·033	3	–0·138	3	0·352	0·288
Suburbs (Peninsula)														
PP10′	48	1	–	2	–	2	–	3	0·004	3	–0·058	4	**0·407**	**0·031**
PP14(a)′	18	1	–	1	–	1	–	3	–0·067	3	–0·014	3	0·269	0·736
PP14(b)′	48	1	–	1	–	2	–	3	–0·246	3	–0·173	4	0·056	0·265
Suburbs (North)														
PP11′	48	1	–	2	–	2	0·299	3	0·131	2	–0·106	2	0·299	0·094
PP13′	48	1	–	2	–	2	–	3	0·138	2	–0·080	4	0·002	0·945
Suburbs (West)														
PP15′	48	1	–	1	–	1	–	2	–0·326	3	–0·238	4	–0·023	0·098
PP16′	48	1	–	2	–0·011	3	–0·048	3	0·125	3	0·261	4	–0·029	0·809
PP18′	48	1	–	2	–0·044	2	–0·093	4	–0·068	3	0·121	4	0·026	0·306
Suburbs (South)														
PP20′	48	2	–	2	0·152	3	–0·022	3	–0·120	3	**0·209**	3	–0·094	0·067
PP21′	48	1	–	1	–	1	–	3	0·374	2	–0·011	4	–0·147	0·154

N, sample size; N_{all}, number of alleles per locus; F_{IS}, the inbreeding coefficient, measures the reduction of heterozygosity of a subpopulation caused by nonrandom mating; *P*, the probability for rejecting Hardy–Weinberg equilibrium.
The significant values after correction using Bonferroni's method are shown in bold.

predominated and were scattered in the middle of fields. Samples from the city centre were at most 6 km away from one another and the more distant samples in the suburbs were 15 km apart.

Collected samples were reared until the imago stage in insectaries under standardized conditions. After oviposition, the field generation (F0) was stored at –80 °C for allozyme assays. Assessments of vector susceptibility to a dengue virus were performed on females of the F1-offspring.

(ii) *Enzyme polymorphism*

Each adult was homogenized in 25 μl of distilled water and centrifuged for 5 min at 20 000 *g* at +4 °C. The supernatant was loaded in a starch gel using the Tris–malate–EDTA (pH 7·4) buffer system. Seven enzyme systems were studied: glutamate oxaloacetate transaminase (Got-1 and Got-2, EC 2.6.1.1.), glycerol-3-phosphate dehydrogenase (G-3-pdh, EC 1.1.1.8.), hexokinase (Hk-1, Hk-2 and Hk-3, EC 2.7.1.1.), malic enzyme (Me, EC 1.1.40.), malate dehydrogenase (Mdh, EC 1.1.1.37.), phophoglucoisomerase (Pgi, EC 5.3.1.9.) and phosphoglucomutase (Pgm, EC 2.7.5.1.) (for more details, see Paupy *et al*., 2000). 18–48 adults from each sample were analysed for the seven enzyme systems, which provided 11 putative genetic loci. Reference control females of an *Ae. aegypti* strain developed from an isofemale lineage (*Ae. aegypti* Isololo, French Polynesia), were included on each gel. The most common allele at each locus for the control strain was used as the reference standard and given a mobility value of 100. Migration distance for all other alleles was recorded as proportional values of the standard.

Genetic variation, deviations from Hardy–Weinberg (HW) proportions, genotypic linkage disequilibrium and genetic differentiation, were analysed by using the GENEPOP (version 3.3) software (Raymond & Rousset, 1995). Deviations from the HW proportions in each population and at each locus were investigated using an exact approximation proposed by Haldane (1954). Multilocus estimates of significance for HW equilibrium tests were estimated by Fisher's combined probability test (Fisher, 1970). Heterozygote deficits or excess were tested using an exact test procedure (Rousset & Raymond, 1995). Genotypic association between pairs of loci was tested for each sample using Fisher's test on rank × column contingency tables. F_{IS} the inbreeding coefficient and F_{ST} the fixation index were calculated according to the formula of Weir and Cockerham (1984). Genetic differentiation across populations was estimated by calculating the *P* value associated to the F_{ST} estimate. The overall significance of multiple tests was estimated by Fisher's combined probability test (Fisher, 1970). Critical significance levels for multiple testing were corrected using sequential Bonferroni procedures (Holm, 1979). Genetic isolation by geographic distance was tested by estimating rank correlations between $F_{ST}/(1–F_{ST})$ calculated between pairs of samples and Ln distances (Slatkin, 1993).

(iii) *Experimental infection of mosquitoes*

Oral infections were performed by feeding 1-week-old non-blood-fed females on an infectious meal that contained two parts of washed rabbit erythrocytes and one part of dengue-2 virus suspension (Vazeille-Falcoz *et al*., 1999). The feeding mixture had a final titre of $10^{8·2}$ MID_{50} (50% of mosquito infectious dose for *Ae. aegypti*) per ml of a dengue-2 strain isolated in 1974 from a human serum sample collected in Bangkok, Thailand (Vazeille-Falcoz *et al*., 1999). For each sample tested, two assays were performed at 2-day intervals. Fully engorged females were kept for 14 days and, after the extrinsic incubation period, detection of infected females was carried out by indirect immunofluorescence assay (IFA) on head squashes. The Paea *Ae. aegypti* strain was used as an infectivity control (Vazeille-Falcoz *et al*., 1999). Variation in the proportions of infected females was compared using the R × C Fisher exact test (Raymond & Rousset, 1995). Analysis of variance (ANOVA) was used to test the effect of temporal variation on infection rates (Statistica 6.0, Statsoft, Tulsa, USA).

3. Results

(i) *Population genetic analysis*

Significant deviations from HW equilibrium were detected in four out of 163 tests run: Hk-2/PP9 (F_{IS}=0·793), Pgi/PP2 (F_{IS}=0·850), Pgi/PP20′ (F_{IS}= 0·209) and Pgm/PP10′ (F_{IS}= 0·407) (Table 2). When considering global tests (i.e. all loci for each sample), significant heterozygote deficiencies were found in six samples: PP2, PP4, PP8, PP17, PP2′ and PP10′. No significant heterozygote excess was detected in any of the samples tested.

Assessments of the linkage disequilibrium between pairs of loci for each sample showed that only 34 combinations out of 544 were non-randomly associated: ten concerning the combination Hk-1/Hk-2, ten for Hk-1/Hk-3, ten for Hk-2/Hk-3, one for G-3-pdh/Hk-3, one for Pgi/Hk-1, one for Pgi/Hk-2 and one for Pgi/Hk-3 (data not shown). Therefore, the loci Hk-1 and Hk-3 were excluded from our data set.

The genetic differentiation between samples collected on the same date gave low, significant estimates of F_{ST}: F_{ST}=0·027 with $P<10^{-6}$ in February and F_{ST}=0·037 with $P<10^{-6}$ in July (Table 3). Large, significant genetic differentiation was detected when pooling samples according to their location: in the city centre (February-F_{ST}=0·027; July-F_{ST}=0·041),

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Table 3. *Genetic differentiation of* Aedes aegypti *sampled in Phnom Penh in 2001*

Comparison	*N* February	F_{ST} (all loci)	*N* July	F_{ST} (all loci)
All	22	0·027**	20	0·037**
City centre	9	0·027**	10	0·041**
Suburbs				
Peninsula	2	0·015	3	0·048*
North	3	0·028**	2	–0·005
West	4	0·021**	3	0·040**
South	4	0·005	2	0·027**

N, number of samples.
*$P<0·01$.
**$P<0·0001$.

in Peninsula (July-$F_{ST}=0·048$), in the northern suburbs (February-$F_{ST}=0·028$), in the western suburbs (February-$F_{ST}=0·021$; July-$F_{ST}=0·040$) and in the southern suburbs (July-$F_{ST}=0·027$). When comparing F_{ST} values between the two sampling dates for different groupings of samples, the largest temporal differences were found between samples collected in the Peninsula (3·2-fold higher in July), the southern suburbs of Phnom Penh (4·9-fold higher in July) and the northern suburbs (5·6-fold higher in February) (Table 3).

When estimating genetic divergence according to geographic distance, the relation $F_{ST}/(1-F_{ST})=a+b\times\text{Ln distance}$, was significant ($P=0·048$) and positive ($b=+0·013$) for samples collected in February in the city centre. This result indicates that pairs of samples that are situated farther apart geographically show greater genetic distance. This tendency was not detected for other sample groupings (i.e. city centre in July, suburbs in February and suburbs in July).

(ii) *Susceptibility to a dengue-2 virus*

Infection rates of F1 females ranged from 64·5% (PP3a) to 100% (PP11a) for February collections and from 57·0% (PP15′b) to 94·5% (PP7′a) for July collections (Table 4). Rates for the control varied from 63·5% to 89·2%. When comparing rates between the replicates of each sample, they were all similar ($P>0·05$) except for samples PP4 ($P=0·0001$) and PP7′ ($P=0·0004$). Based on these results, only the sample with the higher number of individuals analysed (PP4a and PP7′b) were considered in further analysis. When comparing rates of the Paea control, *P* values were all insignificant ($P>0·05$) indicating homogeneous infection rates of our control (February, $P=0·28$; July, $P=0·196$).

When analysing spatial variation of infection rates, *P* values were highly significant ($P<0·05$) for all samples collected in Phnom Penh on the two dates (Table 5). In February, differences were more related to inter-area differences (i.e. between the city centre and the suburbs, with $P<10^{-4}$). In July, the difference was mainly due to inter-area differences within the suburbs ($P=0·019$). When analysing temporal variations of infection rates, all sample groupings exhibited significant *P* values ($P<0·05$) except for the samples collected in the city centre ($P=0·482$). Taken as a whole, infection rates tended to decrease at the end of the dry season (Fig. 2). The lowest rates were detected for samples collected in the city centre ($76·8\pm8·9$ in February and $76·2\pm6·7$ in July), whereas the highest were obtained for samples from the southern suburbs ($95·3\pm0·1$ in February and $83·2\pm3·4$ in July).

4. Discussion

Our major findings on spatial and temporal variation in genetic structure and susceptibility to a dengue-2 infection of *Ae. aegypti* at a local scale, Phnom Penh and its suburbs, can be summarized as follows. First, we have established there is a low level of genetic exchange between populations collected in the city centre. Despite being separated by less than 6 km, differentiation remained very high whatever the time of collection. Second, populations collected in the suburbs displayed different levels of genetic differentiation depending on the type of environment: the densely populated suburbs (the South), the residential suburbs (the West) and the more rural suburbs (the North and the Peninsula). Different temporal evolutions of genetic structure have been detected. Third, oral susceptibility to dengue-2 virus tended to decrease during the dry season.

(i) *Genetic variation*

The departures from HW equilibrium detected in our study were always associated with heterozygote deficiencies. Three of the six heterozygote deficits were observed in mosquito samples collected in February in the city centre. This could be due to factors such as null alleles, the Wahlund effect or inbreeding. For allozymes, null alleles can be generated by mutations or deletions within a gene and thus do not produce any detectable protein. The occurrence of null alleles in allozymes is very rare. Besides, inbreeding could be excluded as an explanation because deficits would be expected in all loci. A Wahlund effect caused by the pooling of two recently established populations stemming from different genetic sources (Hartl & Clark, 1989) might be a better explanation. At the beginning of the dry season, large reductions of population densities tend to decrease population genetic diversity and at the same time, a limited gene flow caused by a reduction of available breeding site; this favours the establishment of patches of differentiated populations. Furthermore, dispersal occurs

Table 4. *Comparisons of infection rates to a dengue-2 virus of* Aedes aegypti *samples collected in Phnom Penh in 2001*

Comparison	Sample	Replicate	Infection rate (*N*) Assay	Control
February				
City centre				
	PP3	a	64·5 (31)	83·9 (31)
		b	86·7 (30)	81·4 (43)
		P	0·07	1
	PP4	a	66·0 (47)	83·9 (31)
		b	97·4 (38)	81·4 (43)
		P	0·0001	1
	PP6	a	75·8 (29)	71·4 (35)
		b	76·9 (26)	83·3 (54)
		P	1	0·19
	PP8	a	86·2 (65)	81·2 (32)
		b	81·6 (76)	88·7 (62)
		P	0·504	0·35
All centre		*P*	0·002	
Suburbs				
North				
	PP11	a	100 (11)	71·4 (35)
		b	95·1 (41)	83·3 (54)
		P	1	0·19
	PP12	a	87·5 (16)	71·4 (35)
		b	80·0 (25)	83·3 (54)
		P	0·68	0·19
	All	*P*	0·144	
West				
	PP16	a	81·4 (43)	81·2 (32)
		b	90·5 (95)	88·7 (62)
		P	0·17	0·35
	PP18	a	84·6 (13)	89·2 (37)
		b	91·5 (82)	87·9 (66)
		P	0·60	1
	All		0·282	
South				
	PP23	a	95·2 (21)	89·2 (37)
		b	95·4 (87)	87·9 (66)
		P	1	1
	All	*P*	1	
All suburbs		*P*	0·152	
All samples		*P*	$<10^{-4}$	
July				
City centre				
	PP2′	a	84·6 (39)	65·2 (46)
		b	73·2 (86)	78·6 (98)
		P	0·17	0·11
	PP3′	a	85·9 (78)	73·9 (46)
		b	80·0 (110)	73·9 (46)
		P	0·34	0·39
	PP4(b)′	a	69·2 (104)	63·5 (63)
		b	78·0 (105)	74·7 (95)
		P	0·16	0·15
	PP6′	a	78·1 (64)	75·5 (53)
		b	80·0 (105)	83·3 (102)
		P	0·85	0·28
	PP7′	a	94·5 (55)	72·1 (79)
		b	63·6 (99)	73·5 (102)
		P	0·0004	0·87
	PP9′	a	75·9 (108)	63·5 (63)
		b	70·1 (58)	74·7 (95)
		P	0·46	0·15
All centre		*P*	$<10^{-4}$	

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(*Cont.*)

Comparison	Sample	Replicate	Infection rate (*N*) Assay	Control
Suburbs				
Peninsula				
	PP10′	a	88·6 (88)	72·1 (79)
		b	76·8 (82)	73·5 (102)
		P	0·05	0·87
	PP14′	a	85·2 (95)	72·1 (79)
		b	90·0 (101)	73·5 (102)
		P	0·39	0·87
	All	*P*	0·067	
North				
	PP11′	a	78·6 (89)	75·5 (53)
		b	79·4 (68)	83·3 (102)
		P	1	0·28
	PP13′	a	71·4 (63)	73·9 (46)
		b	81·3 (91)	73·9 (46)
		P	0·175	0·389
	All	*P*	0·560	
West				
	PP15′	a	57·5 (40)	63·5 (63)
		b	57·0 (61)	74·7 (95)
		P	0·21	0·15
	PP16′	a	82·6 (46)	73·9 (46)
		b	87·3 (71)	73·9 (46)
		P	0·41	0·39
	PP18′	a	89·1 (92)	75·5 (53)
		b	80·4 (92)	83·3 (102)
		P	0·16	0·28
	All		$<10^{-4}$	
South				
	PP20′	a	87·2 (78)	65·2 (46)
		b	78·9 (57)	78·6 (98)
		P	0·23	0·23
	PP23′	a	83·3 (66)	65·2 (46)
		b	83·5 (67)	78·6 (98)
		P	1	0·11
	All	*P*	0·652	
All suburbs		*P*	$<10^{-4}$	
All samples		*P*	$<10^{-4}$	

preferentially between nearby populations as shown by the pattern of isolation by distance detected.

(ii) *Evolution of genetic structure*

Regarding the evolution of the genetic structure over time, different scenarios can be proposed. In the city centre, mosquitoes limit their dispersal because blood meals and breeding sites are available everywhere. Even if running water is available in most houses, inhabitants store water in jars, where *Ae. aegypti* mainly breeds. A high differentiation was detected over the whole 6 month period investigated. In the suburbs, different patterns could be observed : samples collected in the Chroy Chang Var peninsula and the southern suburbs became differentiated at the end of the dry season, whereas the opposite was observed for samples from the northern suburbs. In the southern suburbs, inhabitants store water in containers as running water is lacking. Moreover, the absence of garbage collecting favours the accumulation of various artificial containers (acting as temporary breeding sites during rainy season) around dwellings. At the beginning of the dry season, temporary breeding sites began to disappear. Only large containers such as jars assigned to store drinking water are maintained, increasing the average distance between available breeding sites. The lack of migration events and genetic exchanges between populations could be detected through a rise of genetic differentiation (Huber *et al.*, 2002a). Curiously, the same pattern was observed for samples collected in the peninsula, which is a rural, lightly populated area with scarcely distributed water storage containers as breeding sites. At the beginning

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Table 5. *Comparisons of infection rates according to spatial and temporal variation*

Variation	February		July	
	N	*P*	*N*	*P*
Spatial				
Phnom Penh	9	**$<10^{-4}$**	15	**0·001**
Centre	4	0·076	6	0·111
Suburbs	5	0·151	9	**0·019**
South	1	–	2	1
West	2	0·270	3	0·095
North	2	**0·034**	2	0·78
Peninsula	–	–	2	1
Temporal				
February vs July				
Centre		0·482		
Suburbs				
South		**0·002**		
West		**0·001**		
North		**0·009**		
Peninsula		–		

N, number of samples; *P*, probability of homogeneity; –, no data.

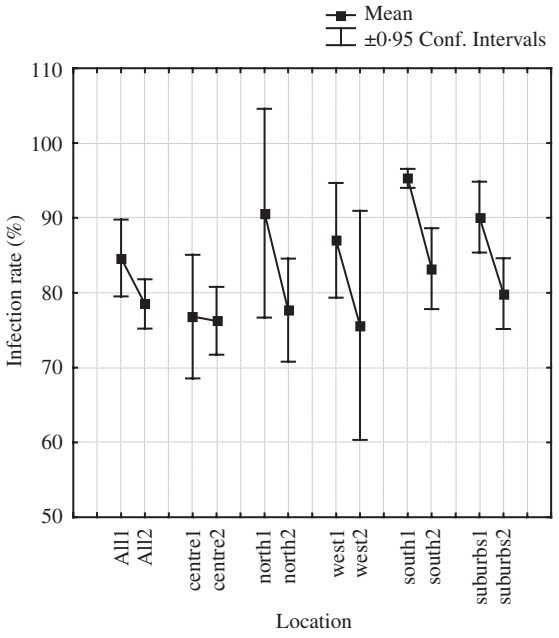

Fig. 2. Variation in infection rates according to location and sampling date. Abbreviations: 1, samples collected in February; 2, samples collected in July; all, all samples; centre, city centre; north, northern suburbs; south, southern suburbs; west, western suburbs.

of the dry season, migration is high enough to counterbalance population genetic differentiation, which completes its establishment at the end of the dry season with the disappearance of temporary breeding sites. In the rural northern suburbs, differentiation decreased, becoming insignificant at the end of the dry season. The lack of structuring could indicate high genetic exchange among populations that remained important enough to overcome the scarcity of breeding sites at the end of the dry season. Thus, adult females tend to exacerbate their dispersal behaviour to reach more distant breeding sites (Reiter *et al*., 1995). As for samples collected in the city centre, those from western suburbs in residential districts remain differentiated whatever the period examined.

In addition to environmental factors, insecticide treatments could shape the genetic differentiation (Lerdthusnee & Chareonviriyaphap, 1999). During 2001, a massive campaign called 'mosquitoblitz' was implemented to prevent the occurrence of the dengue epidemic that usually emerges after the dry season. Water jars were treated with the larvicide temephos. The efficiency of such actions was assessed through the calculation of the Breteau index (number of jars with larvae for 100 houses); this measure decreased from more than 50 to less than 5, one week after the treatment. Unfortunately, the index recovered its pretreatment level seven weeks after the end of the treatment (Chantha, 2002). Recurrent insecticide treatments are responsible for population reductions altering the vector genetic structure. Those modifications were more evident in the suburbs than in the city centre because temporal variations of the genetic structure were better detected in populations from the suburbs.

(iii) *Susceptibility to a dengue-2 virus*

When considering geographic groupings of samples, infection rates were widely homogeneous in the city centre, whereas infection rates in the suburbs were similar in February and became different in July. Moreover, a significant temporal variation of rates was detected: rates were high in February and decreased significantly in July (Table 5). By reducing the availability of some breeding sites and thus acting on mosquito densities (Strickman & Kittayapong, 2002) and population structure (Huber *et al*., 2002a), the dry season could modify vector–host contacts and, indirectly, the pattern of dengue transmission.

Based on our results, we suggest that two types of *Ae. aegypti* populations could be present in Phnom Penh: (1) populations characterized by a lower vector competence and whose larvae usually breed in water jars; and (2) populations characterized by a higher vector competence that are more adapted to small, unintentional containers such as flower pots or abandoned water jars filled with rainwater. Unintentional containers (e.g. unused water jars left outdoors) are usually more productive, containing sufficient nutrition to support mosquito development better (Strickman & Kittayapong, 2003). By contrast, intentionally collected rainwater for domestic purposes

was kept clean and thus larvae suffered from starvation. These two main types of larval habitats tend to affect mosquito size (Briegel, 1990). Small, abandoned containers produced larger mosquitoes than containers used for drinking. Larger *Ae. aegypti* females are apparently better vectors because they are more capable of feeding successfully (Nasci, 1991) and being orally infected (Sumanochitrapon *et al*., 1998). By contrast, small females ingest less blood and thus have less opportunity to acquire dengue infection orally. In the city centre of Phnom Penh, the most common type of larval habitat is intentional water jars (N. Chantha, personal communication). As in the city of Bangkok (Tonn *et al*., 1969), only a small amount of seasonal variation in larval abundance was observed. Water jars were mainly colonized by less-dengue-competent mosquitoes. In the suburbs, where both water jars and unintentional containers are present, *Ae. aegypti* is subjected to population reductions during the dry season because temporary breeding sites tended to disappear. The more dengue-competent populations (i.e. those from small, unintentional containers) are eliminated and thus a significant decrease in vector competence can be detected (Fig. 2).

Over small geographic distances (within the city), low gene flow was measured between populations from the city centre, whereas it remained large for some populations in the suburbs. Moreover, genetic structure is subject to temporal variation. A high rate of gene flow could be exploited to spread genes with defective effects on dengue transmission. The ability of mosquitoes to transmit dengue viruses is proved to be a quantitative genetic trait that is under the control of at least three independently segregating loci (quantitative trait loci) (Black *et al*., 2002; Bosio *et al*., 2000). New methods to control dengue could include blocking transmission by the use of genetically modified mosquitoes that would resist infection by dengue viruses. Manipulation of these sets of genes leading to refractoriness to dengue infections could be an alternative way to control dengue. Are these genes stable enough to be transmitted throughout generations? How effective are they under natural conditions? What are the conditions (i.e. place and time for releases, and number of released mosquitoes) that allow these manipulated mosquitoes to spread efficiently? What would be the real impact of these mosquitoes on dengue transmission? To attempt to answer these questions, more population genetic approaches combined with studies on the interactions between the dengue virus and mosquitoes (and, more precisely, the evolutionary response of the vector to infection) should be encouraged.

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