LOW ORAL RECEPTIVITY FOR DENGUE TYPE 2 VIRUSES OF Aedes albopictus FROM SOUTHEAST ASIA COMPARED WITH THAT OF Aedes aegypti

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Abstract. Dengue hemorrhagic fever has been a major health problem in Asia since the 1950s. During this period, the former principal vector of dengue viruses in Asia, Aedes albopictus, was replaced by Aedes aegypti in most major cities of the area. Aedes aegypti is now considered the main vector of dengue viruses in Asia. Surprisingly, however, this mosquito has been described as having a relatively low oral receptivity for dengue viruses compared with Aedes albopictus. In the present study, we compared the relative oral receptivities of Aedes aegypti and Aedes albopictus collected in southeast Asia from both sympatric and allopatric breeding sites. In all instances, the oral receptivity of Aedes aegypti to the dengue type 2 virus used was significantly higher than that of Aedes albopictus. We also compared the relative oral receptivity of Aedes aegypti and Aedes albopictus for two other low-passage strains of dengue 2. In all instances, Aedes aegypti was significantly more receptive than Aedes albopictus. It should be noted, however, that the difference was found only for Aedes albopictus recently collected from the field (Ta Promh strain, Cambodia, 2001) and not for an Aedes albopictus strain that had been colonized for many years (Oahu strain, Hawaii, 1971). We also observed a significant increase in the infection rate of Aedes albopictus of the Ta Promh strain with increasing generations in the laboratory. These observations demonstrate the importance of considering the colonization history of mosquitoes when assessing their susceptibility to infection with dengue viruses and, perhaps, other arboviruses.

INTRODUCTION

Dengue is a mosquito-transmitted disease caused by infection by one of the four serotypes of dengue viruses (Flaviviridae: Flavivirus). It was described more than 200 years ago as an influenza-like disease with a pattern of high fever, myalgia, arthralgia, rash, and severe headache.1 But the classic symptoms of dengue fever (DF) are sometimes associated with thrombocytopenia, hemorrhage, and excessive plasma leakage, leading in some cases to a shock syndrome. These manifestations, which can lead to severe disease, especially for children, have been termed dengue hemorrhagic fever (DHF). Although DHF was relatively uncommon until the 1950s, clinical signs and symptoms compatible with it were first described in the Philadelphia epidemic of 1780, and cases of DHF were subsequently associated with outbreaks in Australia (1897), Beirut (1910), Taiwan (1916 and 1931), and Greece (1928).1,2 The epidemic of DHF in Manila in 1953–542 began a period in which it started to be seen throughout southeast Asia and, subsequently, in other parts of the tropical world.2 It is now the most important arboviral disease of humans in terms of both morbidity and mortality.

The emergence of DHF has been linked to: 1) economic and ecologic changes that affected Asia during and after the World War II, and 2) the consequent dramatic expansion of the urbanized vector of dengue viruses, Aedes aegypti. In the second half of the 20th century, industrialization and rapid human population growth led to uncontrolled urbanization in Asia. In the absence of proper water supply, residents had to store water for domestic use, creating the ideal ecologic niche for Aedes aegypti. Except for some West African strains, this mosquito prefers to feed on humans and to lay its eggs in artificial breeding sites. Finally, the growth of air travel also contributed to the expansion of DHF, providing the means for viremic people to move very rapidly from one place to another.2 This was well documented on the island of Tahiti in French Polynesia, where the interepidemic periods shortened and DHF appeared after the international airport opened.4 Should Aedes aegypti be held responsible for the emergence of DHF? Endemic vectors were present in Asia and French Polynesia before the expansion of Aedes aegypti. Several studies have compared the oral receptivity for dengue viruses of Aedes aegypti with that of endemic vectors such as Aedes albopictus in Asia and Aedes polynesiensis in French Polynesia. Those studies led to the same conclusion: Aedes aegypti was not a good host for dengue viruses, i.e., it needed more virus to become infected than did Aedes albopictus and Aedes polynesiensis. It has been suggested that Aedes aegypti may have selected strains of virus producing a higher viremia in humans, assuming that a high viremia might be associated with severe clinical manifestations and hemorrhagic symptoms.5

In previous studies, we tested the oral receptivity of Aedes aegypti mosquitoes from different parts of the world where dengue is endemic (South Vietnam),6 or epidemic (French Polynesia, French Guiana).7,8 We found a very high oral receptivity with all samples. When we infected Aedes albopictus with the same virus strain, the oral receptivity was always lower (La Réunion, Albania).9,10 To study the relative importance of the two mosquito species in dengue transmission, we tested, whenever possible, Aedes aegypti and Aedes albopictus from the same geographical area and, perhaps more important, from the same breeding site. This was done for mosquitoes from both South and North Vietnam and from Thailand where the two species are sympatric. Moreover, to complete the study, we compared the oral receptivity of the two species derived from old laboratory strains (generations higher than 40) and from strains recently collected from the field (generations up to five) for different strains of dengue type 2 collected in Bangkok (Thailand, 1974), (Phnom Penh, Cambodia 2001), or the Seychelles archipelago (Indian Ocean, 1977). In all instances, we found a lower oral receptivity for Aedes albopictus recently collected from the field than for the strain reared in the laboratory for many years.

MATERIALS AND METHODS

Mosquitoes. Mosquitoes were collected from the field in Southeast Asia in 1999 and 2000. Date and area of sampling
are shown in Table 1 for the four sympatric breeding sites and in Table 2 for the four samples collected from allopatric breeding sites. Mosquitoes were collected as larvae and/or pupae. These field-collected mosquitoes (F0 generation) were maintained in the laboratory at 25 ± 1°C with 80% relative humidity and a 16h:8h photoperiod. Adults were given 10% sucrose solution, and females were allowed to feed on a restrained mouse to produce eggs. All F0 adults were identified morphologically. For infection experiments, the entire egg batch was hatched and larvae reared to the adult stage (F1 generation) in pans with tap water and yeast tablets. Depending on the sample size, we tested F1 or F2 females.

Besides field-collected mosquitoes, laboratory strains also were used. The Isohanoi strain of *Ae. aegypti* originated from an isofemale lineage of a sample (AENV1) collected in Hanoi (North Vietnam) in September 2000 in a sympatric breeding site along with ALNV1. The Ta Promh strain of *Ae. albopictus* originated from a sample collected in 2001 at a temple in Angkor, Cambodia. Females from the F4 and F5 generations of the Isohanoi strain of *Ae. aegypti* and of the Ta Promh strain of *Ae. albopictus* were used for feeding experiments. The Paea strain of *Ae. aegypti*, provided by the Institut Louis Malardé (Tahiti, French Polynesia) and reared in Paris since 1994, was used as a control of mosquito oral receptivity. The Oahu strain of *Ae. albopictus* originated from a sample collected in 1971 in Honolulu, Hawai’i.

**Virus.** The dengue type 2 Bangkok virus strain, provided by L. Rosen, was isolated in 1974 from human sera from Bangkok, Thailand. This virus had been passed only in different mosquito species by intrathoracic inoculation: two passages in *Ae. albopictus*, two passages in *Toxorhynchites ambioensis*. We used the fourth mosquito passage to inoculate females of *Ae. aegypti*, Paea strain (pool D2BanP5-AA), and females of *Ae. albopictus*, Oahu strain (pool D2BanP5-AL).

The dengue type 2 Seychelles virus strain, also provided by L. Rosen, was isolated from human sera from the Seychelles archipelago in 1977, in an epidemic propagated by *Ae. albopictus*.13 This virus had been passed three times by intrathoracic inoculation in *Tx. ambioensis*. We used the third mosquito passage to inoculate *Ae. aegypti*, Paea strain, females (pool D2SeyP3-AA) and *Ae. albopictus*, Oahu strain, females (pool D2SeyP3-AL).

The Cambodia strain was isolated from human sera collected in 2001 in Phnom Penh, Cambodia. This serum was inoculated in *Ae. aegypti*, Paea strain, females (pool D2CamP1-AA) and in *Ae. albopictus*, Oahu strain, females (pool D2CamP1-AL).

The inoculated mosquitoes were incubated 14 days at 28°C, then triturated in heated (56°C for 30 min) fetal calf serum (FCS). The supernatant fluid recovered after low-speed centrifugation was used either as a source of virus in the mosquito blood-meals or, after filtration through 220-nm pores, as an inoculum for the production of virus in *Ae. albopictus* C6/36 clone cells.12 Viral stocks were produced by inoculating C6/36 cells with triturated infected *Ae. aegypti*, Paea strain. The mosquito cells were maintained at 28°C on RPMI-1640 medium supplemented by non-essential amino acids, penicillin, streptomycin, and 10% heated FCS. The percentage of infected cells was monitored during the incubation period by the indirect fluorescent antibody (IFA) assay.13 When 100% of cells were infected, the supernatant fluid was collected and the pH adjusted to 7.5 with 10% sodium bicarbonate. The virus stock was divided into aliquots and stored at ~80°C until used.

Viral stocks produced on C6/36 were D2Ban-C6/36 for the D2 Bangkok strain (inoculum = pool D2Ban P5 AA) and D2Sey-C6/36 for the D2 Seychelles strain (inoculum = D2Sey P3 AA).

Titrations of virus stocks produced in culture cells and ground up mosquito pools were carried out in *Ae. aegypti*, Paea strain, by inoculating serial dilutions of the supernatant intrathoracically.14 Mosquito infection was detected by IFA assay on head squashes. Titters were calculated by the 50% endpoint method and expressed as mosquito infectious doses (MID50) per mL.15

**Oral infection of mosquitoes.** The oral susceptibility of females was tested by a feeding protocol described elsewhere.7 Briefly, 5- to 7-day-old females were deprived of sucrose so-

### Table 1

Infection rates of *Aedes aegypti* and *Aedes albopictus* collected from sympatric breeding sites in different locations 14 days after oral infection with dengue type 2 virus, Bangkok strain (titer of each meal, 10−8 MID50/mL)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Geographic origin</th>
<th>Collection date</th>
<th>Mosquito species</th>
<th>G</th>
<th>% of infected females (n)</th>
<th>P</th>
<th>Assay/control</th>
<th>assay/albopictus</th>
</tr>
</thead>
<tbody>
<tr>
<td>AESV1</td>
<td>South Vietnam</td>
<td>1999</td>
<td><em>Ae. aegypti</em></td>
<td>F1</td>
<td>100 (21) 97.1 (69)</td>
<td>1.00</td>
<td>&lt; 10−4</td>
<td></td>
</tr>
<tr>
<td>ALSV1</td>
<td>Binh Long</td>
<td>1999</td>
<td><em>Ae. albopictus</em></td>
<td>F1</td>
<td>21.2 (33) 97.1 (69)</td>
<td>&lt; 10−4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AESV2</td>
<td>South Vietnam</td>
<td>1999</td>
<td><em>Ae. aegypti</em></td>
<td>F1</td>
<td>93.2 (88) 97.1 (69)</td>
<td>0.461</td>
<td>&lt; 10−4</td>
<td></td>
</tr>
<tr>
<td>ALSV2</td>
<td>Binh Long</td>
<td>1999</td>
<td><em>Ae. albopictus</em></td>
<td>F1</td>
<td>27.3 (11) 97.1 (69)</td>
<td>&lt; 10−4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AENV1</td>
<td>North Vietnam</td>
<td>2000</td>
<td><em>Ae. aegypti</em></td>
<td>F2</td>
<td>96.6 (116) 92.2 (51)</td>
<td>0.248</td>
<td>&lt; 10−4</td>
<td></td>
</tr>
<tr>
<td>ALNV1</td>
<td>Hanoi</td>
<td>2000</td>
<td><em>Ae. albopictus</em></td>
<td>F2</td>
<td>45.8 (48) 92.2 (51)</td>
<td>&lt; 10−4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AET1</td>
<td>Thailand</td>
<td>2000</td>
<td><em>Ae. aegypti</em></td>
<td>F2</td>
<td>99.0 (101) 82.9 (35)</td>
<td>0.001</td>
<td>&lt; 10−4</td>
<td></td>
</tr>
<tr>
<td>ALT1</td>
<td>Chiang Mai</td>
<td>2000</td>
<td><em>Ae. albopictus</em></td>
<td>F2</td>
<td>7.4 (27) 82.9 (35)</td>
<td>&lt; 10−4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**a**: tested generation
**n**: number of females
**b**: predominant species in the breeding site
**P**: probability of homogeneity from Fisher’s exact test.

**Note:** The samples were tested in triplicate.
lution 24h before the infectious meal and then allowed to feed for 20 min through a chicken skin membrane covering an apparatus containing the feeding mixture maintained at 37°C. The infectious meal consisted of two-thirds washed rabbit erythrocytes, one-third virus suspension, and ATP (as a phagostimulant) at a final concentration of 5 × 10⁻³ M. Each meal yielded 10⁸.2 M ID₅₀/mL for the samples in Tables 1 and 2 and 10⁸.1 M ID₅₀/mL for the strains tested in Table 3 and Table 4. Only fully engorged females were transferred to small cardboard containers and maintained at 28 ± 1°C for 14 days. Surviving females were killed and tested for the presence of dengue virus by an IFA assay on head squashes.¹⁶

**Statistical analysis.** Variations in the proportions of surviving infected females at 14 days postinfection were compared using the rxc Fisher’s exact test. The subprogram STRUC was used to compute an unbiased estimate of the exact P value.¹⁶

**RESULTS**

**Infection rates of Ae. albopictus and Ae. aegypti samples from sympatric breeding sites.** Infection rates of each sample and of the corresponding Paea control strain are shown in Table 1. Rates ranged from 93.2–100.0% for Ae. aegypti and from 7.4–45.8% for Ae. albopictus. When compared with the infection rate of the corresponding control using the Fisher’s exact test, only one sample of Ae. albopictus exhibited a significant difference (AET1, P = 0.0013) because of a lower rate of infection of the corresponding control. Otherwise, all Ae. albopictus samples exhibited a high significant difference (P < 10⁻⁴) compared with the Ae. aegypti control strain. When considering each of the four breeding sites, infection rates of Ae. aegypti and Ae. albopictus, were highly and significantly different (P < 10⁻⁴). Infection rates were similar among the four Ae. aegypti samples (P = 0.177) but different among the four Ae. albopictus samples (P < 10⁻⁴). Infection rates of Ae. albopictus and Ae. aegypti samples from allopatric breeding sites. Infection rates of each sample and of the corresponding Paea control strain are shown in Table 2. Rates were 97.3% and 98.7% for Ae. aegypti, and 24.6% and 28.8% for Ae. albopictus. Compared with the infection rate of the corresponding control using Fisher’s exact test, only one sample of Ae. aegypti exhibited a significant difference (AET2, P = 0.015) related to a lower rate of infection for the corresponding control, whereas the two Ae. albopictus samples exhibited a highly significant difference (P < 10⁻⁴). Differences in infection rates between Ae. aegypti and Ae. albopictus were highly significant (P < 10⁻⁴). Infection rates were similar when comparing the two Ae. aegypti samples (P = 0.614) and the two Ae. albopictus samples (P = 0.697).

**Infection rates of Ae. albopictus and Ae. aegypti from old or recent laboratory strains for the Bangkok strain of dengue**

**Table 3**

<table>
<thead>
<tr>
<th>Virus pool</th>
<th>Replicate</th>
<th>% of Ae. albopictus infected females (n)</th>
<th>% of Infected Ae. aegypti females (n)</th>
<th>P</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Isahao⁴</td>
<td>Paea⁵</td>
<td></td>
<td>Isahao⁴</td>
</tr>
<tr>
<td>D2Ban-C6/36</td>
<td>1</td>
<td>16.0 (25)</td>
<td>90.0 (20)</td>
<td>92.0 (25)</td>
<td>83.3 (30)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.3 (19)</td>
<td>80.5 (41)</td>
<td>95.7 (46)</td>
<td>95.4 (65)</td>
</tr>
<tr>
<td>D2BanP5-AA</td>
<td>1</td>
<td>25.0 (4)</td>
<td>100 (13)</td>
<td>88.9 (18)</td>
<td>100 (11)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.0 (15)</td>
<td>100 (23)</td>
<td>77.8 (18)</td>
<td>84.6 (13)</td>
</tr>
<tr>
<td>D2BanP5-AL</td>
<td>1</td>
<td>20.7 (29)</td>
<td>95.1 (41)</td>
<td>96.9 (32)</td>
<td>94.6 (37)</td>
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<tr>
<td></td>
<td>2</td>
<td>21.7 (23)</td>
<td>96.3 (27)</td>
<td>96.7 (30)</td>
<td>97.6 (41)</td>
</tr>
</tbody>
</table>

**Note:**

- n: number of females
- F: recent collected strain (F4 for replicates 1 and 2)
- H: old laboratory strain (> F40)
- P: probability of homogeneity from Fisher’s exact test.
**Infection rates of laboratory strain of Aedes aegypti and Aedes albopictus from the Ta Promh strain of C6/36, Aedes aegypti considering infection rates of the two Oahu and Aedes albopictus species than the Ta Promh strain.**

**Infection rates of laboratory strain of Aedes aegypti and Aedes albopictus from the Ta Promh strain of C6/36, Aedes aegypti considering infection rates of the two Oahu and Aedes albopictus species than the Ta Promh strain.**

**Infection rates were similar for the two replicates when tested with the same virus pool (P > 0.05). Infection rates also were similar when comparing the three pools of the D2Ban strain for each species: P = 0.063 for the Aedes albopictus Ta Promh strain, P = 0.063 for the Aedes albopictus Oahu strain, P = 0.139 for the Aedes aegypti Isohanoi strain, and P = 0.122 for the Aedes aegypti Paea strain. Infection rates were not different when comparing the two Aedes aegypti strains, Isohanoi and Paea, for each virus pool (P = 0.169 for D2Ban-C6/36, P = 0.441 for D2BanP5-AA, and P = 0.928 for D2BanP5-AL) but were different between the two Aedes albo-pictus strains, Ta Promh and Oahu (P < 10^-4). When considering infection rates of the two Aedes aegypti strains and of the Oahu strain of Aedes albopictus, homogeneity of infection rates was accepted for each virus pool: P = 0.087 for D2Ban-C6/36, P = 0.058 for D2BanP5-AA, P = 0.993 for D2Ban-AL. The homogeneity was rejected (P < 10^-4) when data from the Ta Promh strain of Aedes albopictus were included.

Results obtained for F4 and F5 strains included in Table 4. As observed for the D2Ban virus strain, the old laboratory species (Aedes albopictus Oahu and Aedes aegypti Paea) and the recently colonized Aedes aegypti Isohanoi strain exhibited higher infection rates than the Ta Promh Aedes albopictus strain. However, when examining individuals from the Ta Promh strain of Aedes albopictus, an increase in infection rates was observed between the F4 and F5 generations. This increase was significant in all cases (P < 0.05) except for infections with D2SeyP3-AL (P = 0.414) where the rate of infection of F4 individuals from the Ta Promh strain of Aedes albopictus was the highest observed (60.7%). This tendency also was observed for the Isohanoi Aedes aegypti strain, for which the increase was significant when infected with D2CamP1-AL (P = 0.001). Infection rates of F4 individuals from the Ta Promh strain of Aedes albopictus were not different when considering the two mosquito pools, D2BanP5-AL and D2CamP1-AL (P = 0.939), but were different when results obtained with D2SeyP3-AL were added or when considering infection rates of D2SeyP3-AL with one pool or the other (P < 10^-3).

**DISCUSSION**

This work is, to our knowledge, the first report comparing the oral receptivity of Aedes aegypti and Aedes albopictus females from samples recently collected from the field in the same geographical area and, especially, from sympatric breeding sites. Our data show a much higher oral receptivity for a dengue type 2 virus strain for Aedes aegypti collected in Southeast Asia than for Aedes albopictus. These results confirm those obtained in a preliminary study we conducted on both mosquito species in Nha Trang (South Vietnam).17 These results contradict the generally accepted belief that Aedes aegypti is much less receptive to oral infection with dengue viruses of all four types than are most other Aedes capable of transmitting such viruses.5,18 It should be stressed, however, that this conclusion was based mainly on data obtained in one study, published in 1985, on females that originated from field collections made before 1971 for Aedes albopictus and before 1974 for Aedes aegypti.18 Therefore, the infection rates obtained with these individuals could not, in our opinion, allow conclusions to be drawn as to the real status of the competence of the two species. Furthermore, all the experiments were not performed with the same amount of virus, and a control strain was not included in each meal to allow a more accurate comparison of the data. We demonstrated in a previous study that with virus titers below 10^8 MID_50/mL, a small decrease in the titer of the meal led to a large decrease in the infection rate.7

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**Table 4**

Infection rates of laboratory strain of Aedes aegypti and Aedes albopictus 14 days after oral infection with two strains of dengue type 2 virus (titer of each meal, 10^8 MID_50/mL)

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Virus pool</th>
<th>Replicate</th>
<th>% of Infected Aedes albopictus females (n)</th>
<th>% of Infected Aedes aegypti females (n)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ta Promh</td>
<td></td>
<td></td>
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<tr>
<td>Seychelles</td>
<td>D2Sey-C6/36</td>
<td>1</td>
<td>41.3 (63)</td>
<td>90.9 (44)</td>
<td>10^-4</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>67.2 (58)</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P</td>
<td>0.006</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>D2SeyP3-AL</td>
<td>1</td>
<td>60.7 (61)</td>
<td>100 (52)</td>
<td>10^-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>69.4 (49)</td>
<td>nd</td>
<td></td>
</tr>
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<td></td>
<td>3</td>
<td>nd</td>
<td>nd</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>0.414</td>
<td>nd</td>
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</tr>
<tr>
<td>Cambodia</td>
<td>D2CamP1-AA</td>
<td>1</td>
<td>37.9 (29)</td>
<td>100 (4)</td>
<td>0.032</td>
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<tr>
<td></td>
<td></td>
<td>2</td>
<td>72.7 (44)</td>
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<td>nd</td>
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<td>P</td>
<td>0.004</td>
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<td>88.9 (18)</td>
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<td></td>
<td></td>
<td>P</td>
<td>0.028</td>
<td>nd</td>
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</tr>
</tbody>
</table>

n: number of females

*: recently collected strain (F4 for replicate 1, F5 for replicates 2 and 3)

*: old laboratory strains (>F40)

P: probability of homogeneity from Fisher’s exact test.

**Type 2 Virus.** Infection rates of each strain of mosquitoes (results on the F4 generation for Ta Promh and Isohanoi strains) with three different pools of the D2Ban strain are shown in Table 3. Females of the Ta Promh strain of Aedes albopictus exhibited lower infection rates (5.3–25.0%) for the three pools of virus compared with that of the old laboratory colony of Aedes albopictus, Oahu (80.5–100%) or of the two Aedes aegypti strains (77.8–96.9% for the Isohanoi strain and 83.3–100% for the Paea strain). Infection rates were similar for the two replicates when tested with the same virus pool (P > 0.05). Infection rates also were similar when comparing the three pools of the D2Ban strain for each species: P = 0.063 for the Aedes albopictus Ta Promh strain, P = 0.063 for the Aedes albopictus Oahu strain, P = 0.139 for the Aedes aegypti Isohanoi strain, and P = 0.122 for the Aedes aegypti Paea strain. Infection rates were not different when comparing the two Aedes aegypti strains, Isohanoi and Paea, for each virus pool (P = 0.169 for D2Ban-C6/36, P = 0.441 for D2BanP5-AA, and P = 0.928 for D2BanP5-AL) but were different between the two Aedes albopictus strains, Ta Promh and Oahu (P < 10^-4). When considering infection rates of the two Aedes aegypti strains and of the Oahu strain of Aedes albopictus, homogeneity of infection rates was accepted for each virus pool: P = 0.087 for D2Ban-C6/36, P = 0.058 for D2BanP5-AA, P = 0.993 for D2Ban-AL. The homogeneity was rejected (P < 10^-4) when data from the Ta Promh strain of Aedes albopictus were included.

Results obtained for F4 and F5 strains infected with D2Cam and D2Sey are shown on Table 4. As observed for the D2Ban virus strain, the old laboratory species (Aedes albopictus Oahu and Aedes aegypti Paea) and the recently colonized Aedes aegypti Isohanoi strain exhibited higher infection rates than the Ta Promh Aedes albopictus strain. However, when examining individuals from the Ta Promh strain of Aedes albopictus, an increase in infection rates was observed between the F4 and F5 generations. This increase was significant in all cases (P < 0.05) except for infections with D2SeyP3-AL (P = 0.414) where the rate of infection of F4 individuals from the Ta Promh strain of Aedes albopictus was the highest observed.
One puzzling question is why our infection rates were consistently higher than those found in other studies. We have shown previously that the quality of erythrocytes used in the meal is an important factor. When using erythrocytes collected and washed 72 h, instead of 24 h, before the blood-meal, the rate of infected females for the control strain dropped dramatically from 90–19.35%.7 It also should be noted that we used a feeding mixture with a much higher concentration of erythrocytes (two-thirds instead of one-third) than in the earlier studies. In one unpublished experiment, conducted with the D2SeY-C6/36 pool, the infection rate dropped from 97% (65/67) to 80% (20/25) for the Paea strain of Ae. aegypti fed on a mixture containing two-thirds or one-third proportions of erythrocytes, respectively. Otherwise, highly dengue-susceptible Ae. aegypti populations are commonly found in Southeast Asian, South American, and South Pacific regions, leading us to conclude that such characteristics are related to the domestic form of Ae. aegypti. Conversely, the native form of Ae. aegypti (i.e., the formosus form) shows lower infection rates.21

To determine if our results were due to a particularity of the Bangkok viral strain, e.g., adaptation or selection in the course of the different passages in the laboratory, or to the genotype of this viral strain;22 we tested two other dengue type 2 strains. We chose one strain from the Seychelles archipelago (isolated in 1977 during an epidemic transmitted by Ae. albopictus) and one collected in 2001 in Phnom Penh, where Ae. aegypti is responsible for dengue transmission. We used supernatant fluid from cell cultures and also triturated infected mosquitoes as a source of virus for the mosquito blood-meals, as was done in previous studies.18–20 In all instances, we observed a significantly lower oral receptivity of the Ta Promh strain Ae. albopictus strain collected in 2001 compared with all other mosquito strains tested. We also observed a significant increase in the rate of infected females in the Ta Promh strain from one generation to the next. This demonstrates the importance of using individuals recently collected from the field to better estimate the “natural” oral receptivity to dengue viruses. We observed the same phenomenon in one population of Ae. aegypti formosus collected in Africa, which showed an increase in the infection rate from 52.0% at the F2 generation to 87.3% at the F20 generation (Vizeille, unpublished data).23

We have shown that the mode of production of the virus (cell culture, various number of passages on mosquitoes), and even the dengue strain itself (old or recently collected) did not alter the fact that the Ta Prohm strain of Ae. albopictus recently collected from the field was less receptive to oral infection than Ae. aegypti. However, the D2SeY-P3-AL pool was able to infect the F4 females of this mosquito strain to a significantly higher degree than did the D2BanP5-AL or the D2CamP1-AL. It has been suggested that Southeast Asian genotypes of dengue 2 replicate more efficiently than American genotypes in field-derived mosquito strains.22 In our study, the Ae. albopictus strain seemed to be more receptive to infection with a virus collected during an epidemic transmitted by the same species. This result should, of course, be confirmed to better appreciate the impact of the genetic variability of virus strains, i.e., the variability in their ability to infect and replicate in a specific vector, in dengue epidemiology.

In view of these results, we conclude that Ae. albopictus populations now present in Southeast Asia are less susceptible to infection with dengue type 2 virus than are Ae. aegypti populations. This result is supported by another study showing that both infection and transmission rates for dengue type 1 were higher for Ae. aegypti than for Ae. albopictus in Taiwan.24 We did not evaluate transmission rates but only infection rates. However, prior studies have already demonstrated that if Ae. aegypti strains differ in the level of oral receptivity (or midgut receptivity) once the virus has disseminated from the midgut, the infected mosquito will always transmit the virus.22,25

We conclude that in Southeast Asia, Ae. aegypti is a very efficient vector for dengue viruses, highly receptive to oral infection, well adapted to an urban environment (e.g., laying eggs only in artificial containers), and feeding exclusively on humans. It is the perfect vector in endemic and epidemic situations. However, it does not transmit the virus to its progeny vertically very efficiently and may not thus contribute to the maintenance of the virus during interepidemic periods. On the other hand, Ae. albopictus, which is not highly orally receptive to dengue type 2 virus, is present mainly in rural areas and does not feed exclusively on humans. However, male Ae. albopictus can transmit dengue virus sexually in the course of mating, and females can transmit it vertically more efficiently than can Ae. aegypti females.26,27 These two mechanisms could explain the maintenance of the virus in nature between epidemics in non-endemic areas where susceptible human or primate populations are not always present. Ae. albopictus also could bridge a putative sylvatic and an urban cycle of dengue since it colonizes both rural and periurban breeding sites. Even when the only vector present is Ae. albopictus, it can be responsible for dengue epidemics, as shown for the outbreaks in the Seychelles,11 Japan,28 and the recent small outbreak in Hawaii (2001). It also is also important to stress that all Ae. aegypti in the story reported here were Ae. aegypti aegypti, the light domestic form. When we compared Ae. aegypti formosus (dark sylvatic form) from Madagascar with sympatric Ae. albopictus, we found opposite results, i.e., a much higher oral receptivity for Ae. albopictus for dengue type 2 virus.29 On this island, Ae. aegypti formosus is scarce and found only in rural breeding sites, whereas Ae. albopictus is the species encountered in cities.

The two dengue vectors in Southeast Asia, Ae. albopictus and Ae. aegypti, exhibit differences in their ecology and their oral receptivity. However, in a suburban environment, they can share a breeding site. Could this larval competition affect the oral receptivity of adult females? When pooling data from the four sympatric breeding sites, we observed a homogeneity of infection rates for Ae. aegypti samples whereas infection rates among Ae. albopictus were heterogeneous. This raises the question of the role of selection in the differentiation of Ae. albopictus populations in the drastic conditions encountered at sympatric breeding sites. Of course, further investigations are needed to better analyze the consequence of larval competition on the oral receptivity of Aedes mosquitoes for dengue viruses or arboviruses in general.

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Aedes albopictus


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