Indoor-Breeding of *Aedes albopictus* in Northern Peninsular Malaysia and Its Potential Epidemiological Implications

Hamady Dieng¹, Rahman G. M. Saifur¹, Ahmad Abu Hassan¹, M. R. Che Salmah¹, Michael Boots², Tomomitsu Satho³, Zairi Jaal¹, Sazaly AbuBakar⁴

¹ School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia, ² Department of Animal and Plant Sciences, University of Sheffield, Sheffield, United Kingdom, ³ Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan, ⁴ Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia

Abstract

**Background:** The mosquito *Ae. albopictus* is usually adapted to the peri-domestic environment and typically breeds outdoors. However, we observed its larvae in most containers within homes in northern peninsular Malaysia. To anticipate the epidemiological implications of this indoor-breeding, we assessed some fitness traits affecting vectorial capacity during colonization process. Specifically, we examined whether *Ae. albopictus* exhibits increased survival, gonotrophic activity and fecundity due to the potential increase in blood feeding opportunities.

**Methodology/Principal Findings:** In a series of experiments involving outdoors and indoors breeding populations, we found that *Ae. albopictus* lives longer in the indoor environment. We also observed increased nighttime biting activity and lifetime fecundity in indoor/domestic adapted females, although they were similar to recently colonized females in body size.

**Conclusion/Significance:** Taken together these data suggest that accommodation of *Ae. albopictus* to indoor/domestic environment may increase its lifespan, blood feeding success, nuisance and thus vectorial capacity (both in terms of increased vector-host contacts and vector population density). These changes in the breeding behavior of *Ae. albopictus*; a potential vector of several human pathogens including dengue viruses, require special attention.

Introduction

The acquisition of indoor-breeding behavior can potentially increase the biting activity of mosquito vectors that opportunistically bite humans outdoors during the day. This may therefore have important implications to disease transmission. However, despite this epidemiological importance, there have been no previous studies of this issue in dengue vectors.

Dengue viruses infect up to 50 million people each year, causing more than 20,000 deaths [1,2]. These flaviviruses are mainly transmitted by *Aedes aegypti*, but also *Ae. albopictus* [3]. Native to the Oriental Region and some islands in the Indian Ocean [3], *Ae. albopictus* has become well-established in the Western hemisphere where it is the second main vector of dengue [4]. It is also an important vector of yellow fever and various types of encephalitis virus, as well as a competent vector of at least 23 other arboviruses under laboratory conditions [5,6,7]. It is well adapted to peridomestic environments with its larvae breeding in artificial containers and adults aggressively biting humans and different animals during the day [3].

Efforts to control dengue have mainly involved insecticide spraying programs, but this strategy has proven ineffectual [8]. While a vaccine is currently under development, without immediate prospects for success, vector control remains the only viable method to prevent dengue transmission [9,10,11,12]. Improved knowledge regarding egg-laying behavior is relevant because it underpins the primary surveillance method, i.e., ovitrapping [13,14]. However, the most commonly used ovitrap, the CDC gravid trap, is not appropriate for capturing *Ae. albopictus* [15,16].

Blood feeding in mosquitoes represents phenotypic expression of reproductive investment as it is the acquisition of resources specifically for reproduction [17]. Reproductive output represents the energy allocated to egg production and oviposition that could otherwise be allocated to maintenance of somatic function, and the act of oviposition is associated with a risk to survival [18]. There has been a great deal of research regarding the variations of reproductive investment and outcome. Overall, increases in both number and size of blood meals result in increased individual egg mass and number of eggs [18]. Clearly, in the field an increased frequency of blood uptake will tend to require host – mosquito contact and expose hosts to a greater risk of disease transmission.

*Ae. albopictus* has been occasionally incriminated in dengue epidemics in Asian countries [19,20,21]. The first report of a
dengue epidemic in Malaysia was on Penang Island, and dengue fever with hemorrhagic manifestations was also reported from this Island [22]. These epidemics were accompanied by increased populations of *Ae. albopictus*. Increased invasiveness [23] and increased population densities [24] have been recently been reported in the Malaysian peninsula. *Ae. albopictus* females was commonly observed developing in indoor/domestic sites throughout Penang Island during our 2009 entomological survey at Balik Pulau (Sungai Pinang and Sg. Burung), Gelugor, Jelutong, Air Itam (Kampung Relau), and other sites. This shift from outside to inside human dwellings is likely to increase the opportunities for females to obtain blood meals. This indoor immature breeding has raised the question of whether this mosquito may exhibit increased gonotrophic activity (GA) in response to potentially greater blood meal sources.

Given the potentially crucial interactions between reproduction, blood feeding, and vectorial capacity, we examined the GA and fecundity of *Ae. albopictus* using females derived from wild mosquitoes collected from outdoor containers in Kampung Teluk Tempoyak and Balik Pulau, Malaysia, with their daughters after they had spent five generations under laboratory conditions.

### Materials and Methods

#### Occurrence of *Ae. albopictus* larvae in indoor containers

A survey of *Aedes* was carried out from February to June 2009 in Penang province, Malaysia, located between latitudes 5°8′N and 5°35′N and longitudes 100°8′E and 100°32′E [25], covering nine residential areas (townships and villages) on Penang Island (Figure 1). The survey zone is surrounded by hills on the northwest with the rest of the zone opening up into low-lying areas occupied by human populations. Immature mosquito stages were collected in household containers.

#### Statement on ethic issues

This study was carried out in accordance with the principles expressed in the Declaration of Helsinki. The study was approved by the Biological Research Ethics Committee at University Sains Malaysia (Projects # 07-05-16-MG1-GMB15, # 1001/PBIO-LOGI/842004 and Fellowship grant # RU:1001/229/29301/CIPS/AUPE001).

### Colonization of wild *Ae. albopictus*

The *Aedes* mosquitoes used in this study were derived from wild pupae collected from outdoor containers in the survey zone. A colony was established in the insectarium at the School of Biological Sciences, Universiti Sains Malaysia, Penang. Larvae were reared routinely on a diet of dried yeast. Adults had access to 10% glucose solution, and females were blood-fed at two days old on restrained mice. Eggs were processed, dried, and stored under laboratory conditions (temperature 29°C±3°C, relative humidity 75%±10% and photoperiod 13:10 h, 1h dusk provided by an electrical lighting from a 60 and 25-watt incandescent bulbs.

#### Bioassays

Bioassays were carried out using females derived from wild mosquitoes (FWMs) and females derived from FWMs after five generations (*d5FWMs*) under laboratory conditions. In all bioassays, disposable plastic cups (9×11.5 cm) filled with 30 mL...
Experiments

The first experiment was performed to determine the oviposition responses of wild *Ae. albopictus* in the laboratory in relation to blood feeding time. Fifty two-day-old FWMs were blood-fed at 09:00 am on restrained mice. Similarly, a group of fifty two-days old FWMs were offered blood meals on the same day, but at 17:00. In both cases, females were provided with 10% glucose solution for two days. After they became gravid, they were placed individually in oviposition cages holding oviposition cups. Egg deposition was checked two days later at five time points during the day starting at 08:00 (09:00, 11:30, 17:00, 20:30, and 22:30). At each time point, cups with eggs were removed, eggs were counted, and new cups were placed in the cages.

The second experiment was performed to examine the nocturnal biting activity of wild *Ae. albopictus*. Ten newly emerged FWMs were maintained on 10% glucose solution for 3 to 4 days, and thereafter starved for a brief period (12 h). The mosquitoes were then placed in cages containing a restrained mouse and observed continuously. Ten females of *Ae. aegypti* were treated as described above and used as a control group regarding its endophagic behavior [26] and nighttime biting activity [27,28]. Engorgement was checked at eight time points during the night (20:00, 21:00, 22:00, 23:00, 24:00, 01:00, 02:00 and 03:00), and the feeding times were recorded for each female of both species. The experiment was run under laboratory photoperiod conditions.

The third experiment involved determining the effects of *Ae. albopictus* domestication time on the number of gonotrophic cycles (GCs) it can perform in its lifetime. A total of one hundred fully blood-fed FWMs and d5FWMs were used in this experiment. Each gravid female was placed singly in an oviposition cup holding a 10% glucose solution source. Egg deposition was confirmed by examining filter papers under a dissecting microscope at the end of the oviposition period. The same females were again given access to blood re-feeding, and provided with oviposition cups, which was repeated until their death. In addition to the possibility that the generation rank affects the number of times eggs are produced, we also examined whether there was any effect of generation rank on lifetime fecundity. Eggs oviposited in each GC of both FWMs and d5FWMs were counted by examining filter papers under a dissecting microscope at the end of the cages. A rearing cage containing 100 males was used as a mating source for experimental females. Male cage was supplied with individual males from field-collected pupae.

Data collection and analysis

Immature *Aedes* (larvae and pupae) collected from indoor containers were identified morphologically with reference to appropriate taxonomic keys [29]. In all bioassays, females in the experimental group were allowed to feed on blood for one day, to digest it for two days, and to lay eggs for 24 h. Blood feeding was scored based on distention of the abdomen. We recorded the number of GCs for each experimental female (FWMs and d5FWMs). Following previous studies [30], we considered a GC as the time between ingestion of blood and commencement of oviposition. In each GC, the number of eggs deposited was used as the score of fecundity, thus adopting others [31]. Eggs were counted under a dissecting microscope (Meiji EMZ; Meiji Techno Co. Ltd, Tokyo, Japan). We considered percentage female survival as the number of individuals that laid eggs divided by the initial number of individuals tested in each GC trial. As an indicator of body size, the length of one wing per female from the axillary incision to the apical margin excluding the fringe scales was measured under a light microscope (Olympus CX41; Olympus, Tokyo, Japan) as described by others [32]. The rate of oviposition was determined as the number of females that oviposited divided by the initial number of females tested. The differences in the number of GCs, fecundity, and body size between FWMs and d5FWMs were examined by analysis of variance using the statistical software package Systat v.11 (Systat Software, Inc., Richmond, CA, USA) [33]. In the fecundity experiment, Tukey’s test was applied to separated means. In all statistical analyses, *P*<0.05 was taken to indicate statistical significance. Survival rates were compared based on differences in percentages.

Results

Survey *Aedes* inside houses

Indoor containers with immature *Aedes* stages consisted of drums, flower vases, anti-guards, buckets, cement tanks, empty paint cans, wells, bare pots, underground floor, and sinks (Table 1, Figure 2). The majority of mosquitoes collected were *Ae. albopictus*, but *Ae. aegypti* was also found. Between February and May, the sizes of immature stage populations remained high and constant in the five residential areas of the study. In some cases, larval instars of *Ae. albopictus* were persistent until June, suggesting that oviposition events did occur continuously (Table 1). The heterogeneity of larval developmental stages and their persistence suggested that *Ae. albopictus* has established populations within peoples’ houses.

Oviposition activity of wild *Ae. albopictus*

Figure 3 shows the oviposition responses of FWMs of *Ae. albopictus* given blood meals at two different times of the day. In both cases, oviposition activity was low to absent during the night. *Ae. albopictus* exhibited two distinct peaks of oviposition activity for all feeding times: a narrow peak in the morning and a wider peak during the afternoon. Females that took a blood meal in the early morning (09:00, Figure 3A) showed a narrow peak of oviposition at 11:00 two days later. Those that fed on blood in the late afternoon (17:00, Figure 3B) showed a weak and narrow peak around noon (13:00) and a larger wider peak in the afternoon (from 15:00 to 18:00).

Patterns of nocturnal blood feeding

The hourly nighttime biting activities of *Ae. albopictus* and *Ae. aegypti* were examined between 20:00 and 03:00. *Ae. albopictus* showed a constant pattern of biting activity at all time points examined, with higher percentage fed and longer feeding time in comparison to *Ae. aegypti* (Table 2).

Survival and gonotrophic activity period

The survival rate decreased when progressing from first to last GC for both FWMs and d5FWMs, although the pattern of the decrease varied with female generation. In FWMs, more than 50% of the females examined died at the third GC. Among the only two FWMs that survived and achieved a seventh GC, none showed subsequent survival. In d5FWMs, more than 50% of females survived and achieved a fourth GC. More than 20% of d5FWMs
survived and reproduced a seventh time. Among these, six individuals reproduced an eleventh time and one individual reproduced a fourteenth time. Overall, survival decreased with time, but this was more pronounced among FWMs (Figure 4).

Patterns of gonotrophic activity

The number of GCs achieved by Ae. albopictus varied significantly with generation (\( F = 30.06, \text{df} = 1, P < 0.001 \)). The mean number of cycles for FWMs was 2.0±0.10 (range: 1–7), while that in d5FWMs was 4.0±0.10 (range: 1–14). Therefore, it is clear that females from d5FWMs had a greater number of GCs (Figure 5).

Fecundity

Figures 6A and 6B show the egg deposition patterns in FWMs and d5FWMs of Ae. albopictus, respectively. The overall egg production did not differ significantly between females from the two generations (Table 3). Pairwise comparisons using the Tukey test revealed no significant difference in egg production during the first (\( F = 0.015, \text{df} = 1, P = 0.905 \)), second (\( F = 0.041, \text{df} = 1, P = 0.842 \)), third (\( F = 0.195, \text{df} = 1, P = 0.663 \)), fourth (\( F = 0.169, \text{df} = 1, P = 0.684 \)), or fifth (\( F = 0.001, \text{df} = 1, P = 0.975 \)) GC.

In FWMs, the level of egg production was maximal in the first GC (63.25±8.00). The number of eggs laid was not significantly different between the different GCs achieved (\( F = 0.707, \text{df} = 4, P = 0.591 \)). There were more eggs deposited by FWMs at the first than at the last GC, but the difference in mean egg deposition between the first and last GCs was not significant (\( F = 2.001, \text{df} = 1, P = 0.171 \)).

In d5FWMs, the peak of egg production was recorded at the third GC (65.77±8.60), and egg production varied significantly between GCs in these mosquitoes (Table 1). From the third GC, egg production tended to decrease as the rank of GC progressed. The mean number of eggs deposited by d5FWMs was significantly lower at the ninth than at the first GC (\( F = 7.444, \text{df} = 1, P = 0.012 \)).

| Date       | Location          | Household containers types        | First | Second | Third | Fourth | Pupae | Species       |
|------------|-------------------|-----------------------------------|-------|--------|-------|--------|-------|---------------|
| 24/02/09   | Sg. Burung, BP    | Drums, Buckets                    | 300   | 350    | 275   | 200    | 130   | Ae. albopictus|
| 24/03/09   | Kg. TT            | Drums, Cement tanks, Empty paint cans | 63    | 100    | 190   | 268    | 55    | Ae. albopictus|
| 31/03/09   | Sg. Pinang        | Drums, Cement tanks               | 68    | 137    | 49    | 40     | 26    | Ae. albopictus|
| 28/04/09   | JLN Baru, BP      | Ant-guards                         | 60    | 220    | 120   | 200    | 50    | Ae. albopictus|
| 5/5/2009   | Kg. Melayu, Al    | Drums, Buckets                     | 130   | 195    | 75    | 110    | 40    | Ae. albopictus|
| 19/05/09   | Kg. Seronok       | Floor vases                        | 0     | 5      | 10    | 8      | 2     | Ae. albopictus|
| 19/05/09   | Kg. Dua           | Wells                              | 100   | 180    | 70    | 80     | 3     | Ae. aegypti   |
| 26/05/09   | JLN Baru, BP      | Empty paint cans                   | 0     | 10     | 15    | 45     | 0     | Ae. albopictus|
| 23/06/09   | Kg. Seronok       | Buckets                            | 0     | 0      | 10    | 6      | 0     | Ae. albopictus|
| 23/06/09   | Jelutong          | Hare pots                          | 0     | 30     | 20    | 0      | 0     | Mixed with Ae. aegypti|
| 9/6/2009   | Gelugor           | Cement tanks                       | 50    | 35     | 25    | 60     | 0     | Ae. aegypti   |
| 16/06/09   | JLN Baru, BP      | Empty paint cans                   | 20    | 25     | 10    | 0      | 0     | Ae. albopictus|
| 30/06/09   | Jelutong          | Underground floor                  | 200   | 300    | 500   | 570    | 50    | Mixed with Ae. aegypti|
| 9/6/2009   | Jelutong          | Sinks                              | 20    | 35     | 10    | 0      | 0     | Ae. aegypti   |

doi:10.1371/journal.pone.0011790.t001

Figure 2. Indoor breeding of Ae. albopictus. Empty paint containers (A) and a cement tank (B) in residences in the township of Gelugor, Penang, Malaysia contained high numbers of larvae. Red arrows indicate the containers and tank. doi:10.1371/journal.pone.0011790.g002
The mean wing lengths were 2.46±0.04 and 2.51±0.04 mm in FWMs and d5FWMs, respectively. Although d5FWMs tended to be slightly larger than FWMs, there was no significant difference in mean wing length between the two groups (Table 1, Figure 7).

Discussion

The most important observation in the present survey was that *Ae. albopictus* breeds inside peoples’ houses in many parts of Penang Island, Malaysia. Larval populations were heterogeneous and most developmental stages were present over the five-month period of the survey. As this mosquito typically shows outdoor breeding behavior [34,35], the persistence of its larval and pupal stages in indoor containers over a long period suggests that *Ae. albopictus* is being adapted to the indoor environment. A similar observation was recently reported in neighboring Thailand [36], but there have been no studies regarding the epidemiological significance of these observations. Here, we examined the gonotrophic performance of wild *Ae. albopictus* with regard to the crucial interactions between biting activity and vectorial capacity [37].

As the present study was begun with a wild population, it was first necessary to determine whether they could oviposit under laboratory conditions. Oviposition trials indicated that FWMs can lay eggs in the laboratory and that the patterns of egg-laying were associated with blood feeding time. The findings of the present study were consistent with the natural oviposition behavior of this mosquito [38,39,40,41], therefore allowing the long-term experiments required for this study.

We found a major effect of level of adaptation to indoor/domestic environment on the number of GCs in the laboratory. d5FWMs showed a much higher number of GCs than their FWMs counterparts. In mosquitoes, the nutritional history of the parents is influential in determining the fecundity of daughters [42]. This study showed that daughters from parents reared in a food-limited environment produced more eggs than those from parents reared under high food conditions. They suggested that this increased fecundity arose to compensate for expected decreased longevity in stressing environments. Here, females derived from wild mosquitoes (FWMs) and females derived from these FWMs after five generations achieved 7 and 14 GCs, respectively. d5FWMs survival rate was higher than that of FWMs mosquitoes. Although the mechanisms underlying these observations are not yet clear, they could be the result of at least two processes. First, the highly nutritious food conditions in the laboratory could lengthen the mosquito lifespan, and thus increase the probability that it reproduces. Second, the shift from a complex wild environment to a simple environment, such as that in the laboratory, may result in physiological changes that increase the allocation of energy to functions other than egg production, thereby increasing the probability of survival. Longer living females may take more blood meals and reproduce more simply because of the increased availability of meals. Epidemiologically, an increased number of GCs will tend to increase the probability of disease occurrence. With an extended period of GA, females have a higher probability of picking up and transmitting a disease agent as well as an increased lifespan as blood provides an alternative energy source for survival [43]. The increased period of reproduction of the d5FWMs will also lead to a higher mosquito population density.

### Table 2. Nocturnal biting frequencies of FWMs of *Ae. albopictus* and *Ae. aegypti*.

| Time   | % fed FWMs *Ae. albopictus* | Time | % fed FWMs *Ae. aegypti* |
|--------|-----------------------------|------|--------------------------|
| 8pm    | 100                         | 90   |                          |
| 9pm    | 100                         | 90   |                          |
| 10pm   | 100                         | 80   |                          |
| 11pm   | 100                         | 80   |                          |
| 12pm   | 100                         | 80   |                          |
| 01am   | 100                         | 80   |                          |
| 02am   | 100                         | 80   |                          |
| 03am   | 100                         | 80   |                          |

*Time (in minutes after which all 10 individuals have taken a blood meal.*

doi:10.1371/journal.pone.0011790.t002
which is also likely to be associated with increased occurrence of disease. Population levels expressed as larval [44,45,46], pupal [47], and adult indices [48] are often associated with levels of risk for dengue transmission. Dengue outbreaks occurred in Singapore [49] but not in Brazil [50] when the national overall percentage of houses positive for larvae (HI) was below 1%. In Puerto Rico, the incidence of dengue increased one month after larval density peaked [51], whereas in Brazil, dengue seroconversion increased when the HI was above 3% [52]. The present study was prompted by the permanent presence of biting adults and immature stages of *Ae. albopictus* within residences in Teluk Tempoyok and Balik Pulau located in northern peninsular Malaysia and a lack of information regarding the epidemiological implications. Although we did not determine whether these populations were infected with dengue viruses, the focal point of this study was that the presence of larvae strongly suggests that at least a GC has been achieved. Thus, infection would occur if the virus was present. Note that this species is competent for many viruses [6,7], but has only occasionally been incriminated in minor dengue epidemics all the world, e.g., in Hawai in 2001–2002 [53].

In FWMs and *d5FWMs*, the number of eggs oviposited tended to decrease as GC rank progressed, but this effect was most marked in the second group. As in most anautogenous mosquitoes, the production of eggs in *Ae. albopictus* requires the ingestion of blood [54,55]. The female converts about 20% of the ingested blood meal into egg constituents [56]. Several groups have reported that the degree to which eggs are produced depends largely on meal size [32,57,58]. Indeed, a female that ingests a large blood meal size will tend to invest more in egg production than a female with a small blood meal. In the present study, we have used females adapted to laboratory conditions and females derived from wild pupae. It is often assumed that wild insects are subject to much harsher environmental conditions that trigger small body size and that they have lower levels of energetic reserves than laboratory strains. Clearly, in the laboratory, the highly nutritious larval diet will tend to produce large bodied mosquitoes capable of blood feeding for long periods due to little or no host defense behavior from anesthetized hosts [32]. Here, FWMs and *d5FWMs* were similar in body size and had the same feeding time, so differential egg production due to differences in meal size is unlikely. These discrepancies may be explained by differential utilization of blood. Adult mosquitoes feed on blood for...
immediate energy needs [57,59], but in some cases, they use blood as an alternative energy source for survival [43]. There is evidence that colonization alters reproductive traits [60], in particular offspring fecundity [42]. Therefore, it is tempting to suggest that the accommodation of the wild strain to the laboratory environment has occurred in addition to physiological changes relative to blood use. It is possible that the reduced level of egg production observed with increasing generations represented a compensation for better acclimation to the laboratory environment. Presumably, protein use by d5FWMs offsets the costs associated with egg production and facilitates population maintenance in this environment. In support of this suggestion, it has been reported that *Ae. albopictus* may use some blood proteins for maintenance [61].

The pattern of egg production was similar between females of both types, but lifetime fecundity was greater in d5FWMs. This difference was the result of their greater survival. Females with a long lifespan may take more blood meals and reproduce more simply because of the increased availability of meals. Easy access to blood sources in any host – vector interaction can be of crucial epidemiological significance because increased frequency of host-biting may favor the spread of infectious disease present. The presence of *Ae. albopictus* inside houses, that was observed in many residences throughout Penang Island, appears to facilitate human blood feeding. In this context, biting activity during both the day

### Table 3. Statistical analysis by ANOVA of the variations in fecundity and body size between FWMs and d5FWMs of *Ae. albopictus*.

|                | df | F-ratio | P    |
|----------------|----|---------|------|
| Fecundity FWMs| 4  | 0.707   | 0.591|
| d5FWMs        | 8  | 2.39    | 0.014|
| Body size     | 1  | 1.049   | 0.316|

**Figure 6. Numbers of eggs (mean ± SE) laid by FWMs (A) d5FWMs (B) of *Ae. albopictus*.**

doi:10.1371/journal.pone.0011790.g006

**Figure 7. Wing length (mean ± SE) of FWMs and d5FWMs of *Ae. albopictus*.** Bars labeled with the same letter are not significantly different (*P*<0.05).

doi:10.1371/journal.pone.0011790.g007
and might increase mosquito – human contact. In addition, dengue viruses can be transmitted sexually from male to female *Ae. albopictus* [62]. Therefore, the increased fecundity of *d3FWMs* may contribute to virus propagation.

This study emphasizes the invasive properties of *Ae. albopictus* and importantly shows the acquisition of an indoor breeding behavior by this mosquito, a vector of dengue viruses. This behavioral change may lead to increased vectorial capacity. Several parameters come into play when the vectorial capacity of a mosquito for an arbovirus is considered [63]. In particular, host availability and population density are very influential to the competence of a vector. Indeed the more individual vectors are present, the more likely they will be able to transmit a pathogen. Adaptation to the indoor/domestic environment, which triggers increased human-vector contacts, will presumably stimulate feeding behavior. In the neighboring Thailand, 100% of field-collected populations of this mosquito fed on humans [64]. Theoretically, such an affinity for feeding on human blood in a wild context will tend to increase inside residences. *Ae. albopictus* exhibited a high biting activity, showing a shorter feeding time and a greater blood feeding success when compared to *Ae. aegypti* at night. This period is the time when residents exhibit low defensive responses to mosquito feeding. In our study, the epidemiological implications were also approached from gonotrophic performance and survival because biting activity is a pivotal factor in the continuation of both pathogen transmission and vector generation [65]. Furthermore, the mosquito individuals that are most easily infected and most likely to incubate a pathogen to an infectious level are those that live long enough [66]. Clearly, adaption to the indoor/domestic environment may produce more competent vectors, since it favors long and increased lifetime reproductive output.

There is one factor related to our approach that should be discussed in light to keep away from misinterpretations of the obtained results. We have used mice as the blood host. This can appear as a drawback of our method, because the attitudes of a restrained mouse differ from those exhibited by a human under mosquito attacks. We assumed that during sleep, a human may exhibit little defensive responses as a retrained mouse. Experimental mosquitoes were derived from wild pupae collected in a dengue epidemic context. To avoid any infection risks, as no data on dengue infection of *Ae. albopictus* was available, we used mouse as animal model. Although, there are differences in the fitness ramifications for the host species that a mosquito takes blood from, evidence also exist that mice under some conditions mimic well human responses to dengue infection [67,68].

**Acknowledgments**

We thank the team of the Vector Control Unit of the School of Biological Sciences, University Sains Malaysia, the populations of the townships and villages, the survey was carried out. We also thank Steven Neeve of John Innes Centre, Norwich, UK, for his comments on an earlier version of this manuscript. The authors are grateful to the students and drivers of the School of Biological Sciences for their assistance in the field survey.

**Author Contributions**

Conceived and designed the experiments: HD RGMS. Performed the experiments: HD RGMS. Analyzed the data: HD RGMS. Contributed reagents/materials/analysis tools: HD. Wrote the paper: HD RGMS AAH MCS TS MB ZJ SA.

**References**

1. Burke DS, Monath TP (2001) Flaviviruses. In: Knipe DM, Howley PM, et al. (2001) Fields Virology. 4th eds. Philadelphia: Lippincot, Williams & Wilkins. pp 1043–1125.
2. World Health Organization (2006) Dengue haemorrhagic fever: Early recognition, diagnosis and hospital management - an audiovisual guide for health-care workers responding to outbreaks. WHO Western Pacific Region, 1:362-363. http://www.who.int/wer/2006/wer8138/en/index.html.
3. Hawley WA (1958) The biology of *Aedes albopictus*. J Am Mosq Control Assoc 4: 1-14.
4. Knudsen AB, Romi R, Majors J (1996) Occurrence and spread in Italy of *Aedes albopictus*, with implications for its introduction into other parts of Europe. J Am Mosq Control Assoc 12: 177–183.
5. Rosen L, Roseboom EG, Gubler DJ, Lien JC, Chaniotis BN (1985) Comparative susceptibility of mosquito species and strains to oral and parental infection with dengue and Japanese encephalitis viruses. Am J Trop Med Hyg 34: 603–615.
6. Mitchell CJ (1995a) Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. J Vector Ecol 20: 533–537.
7. Mitchell CJ (1995b) The role of *Aedes albopictus* as an arbovirus vector. Parasitology 109: 109–113.
8. World Health Organization (1999) Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines. WHO Regional Publication SEARO 29.
9. Guzman MG, Kouri G (2002) Dengue: an update. Lancet Infect Dis 2: 331–342.
10. Deen JL (2004) The challenge of dengue vaccine development and introduction. J Am Mosq Control Assoc 20: 1–14.
11. Deen JL (2005) Bioecology and vectorial capacity of *Aedes albopictus* in Penang, Malaysia. J Vector Ecol 30: 219–221.
12. Pang T (2003) Vaccines for the prevention of neglected diseases-dengue fever. Lancet Infect Dis 3: 193–199.
13. Guzman MG, Kouri G (2002) Dengue: an update. Lancet Infect Dis 2: 331–342.
14. Ritchie SA, Pyke AT, Smith GA, Northhill JA, Hall RA, et al. (2003) Field experiments: HD RGMS AAH MCS TS MB ZJ SA.
15. Reiter P (1986) A standardized procedure for the quantitative surveillance of mosquito vectors of dengue on Samui Island, Thailand. J Vector Ecol 2: 158–163.
16. Savage HM, Anderson M, Gordon E, McMillen L, Colton L, et al. (2008) Host-seeking heights, host-seeking activity patterns, and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (*Diptera: Culicidae*). J Med Entomol 45: 276–280.
17. Roitberg BD, Sircom J, Roitberg CA, Vanalphen JMM, Mangel M (1993) Life expectancy and reproduction. Nature (Lond.) 364: 106–108.
18. Leinham PT, Sala LM, Juliano SA (2000) Geographic variation in adult survival and reproductive tactics of the mosquito *Aedes albopictus*. J Med Entomol 37: 210–221.
19. Chau YTK, Chan YC, Yong R, Lee KM, Lim BK, et al. (1998) Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus* mosquitoes by a type-specific Polymerase Chain Reaction and cycle sequencing. Am J Trop Med Hyg 58: 578–586.
20. Ali M, Wagaotsama Y, Emch M, Brimva RF (2003) Use of a geographic information system for defining spatial risk for dengue transmission in Bangladesh: role for *Aedes albopictus* in an urban outbreak. Am J Trop Med Hyg 69: 634–640.
21. Almeida AFG, Baptista SGSS, Sousa AGC, Novo MLTM, Ramos HC, et al. (2005) Biorecology and vectorial capacity of *Aedes albopictus* (*Diptera: Culicidae*) in Macao, China, in relation to dengue virus transmission. J Med Entomol 42: 419–428.
22. Rushnick A, Tan EE, Lucas JK, Omar MB (1963) Mosquito borne haemorrhagic fever in Malaysia. Brit Med J 1: 1269–1272.
23. Nur Aida H, Abu Hassan A, Nurita AT, Che Salmah MR, Norashah B (2008) Population analysis of *Aedes albopictus* (Skuse) (*Diptera: Culicidae*) under uncontrolled laboratory conditions. Trop Biomed 25: 117–125.
24. Rozilawati H, Zairi J, Adanan CR (2007) Seasonal abundance of *Aedes albopictus* in Penang, Malaysia. Trop Biomed 24: 83–94.
25. Ahmad F, Ahmad SY, Farooqi MA (2006) Characterization and geotechnical properties of Penang residual soils with emphasis on landslides. Am J Environ Sciences 2: 121–128.
26. Thavara U, Tawasit A, Chaung S, Kong-ngamk W, Pasorwong S, et al. (2001) Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. J Vector Ecol 26: 172–180.
27. Chadee DD, Martinez R (2000) Landing periodicity of *Aedes aegypti* with implications for dengue transmission in Trinidad, West Indies. J Vector Ecol 25: 150–163.
28. Kawada H, Takemura SY, Arikawa K, Takagi M (2005) Comparative Study on Nocturnal Behavior of *Aedes aegypti* and *Aedes albopictus*. J Med Entomol 42: 312–318.
29. Tanaka K, Mimasawa K, Saugstad ES (1979): A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera, Culicidae). Contribut Am Entomol Inst 16: 1-979.
30. Goma LKH (1966): The Mosquitoes. Great Britain: Hutchinson & Co Ltd.
31. Blackmore MS, Lourd CC (2000): The relationship between size and fecundity in Aedes albopictus. J Vector Ecol 25: 212-217.
32. Chadee DD, Beier JC, Mohammed RT (2002): Fast and slow blood-feeding durations of Aedes aegypti mosquitoes in Trinidad. J Vector Ecol 27: 172-177.
33. Systat® 11 software (2004): Systat 11 for windows. Statistics SPSS Inc.
34. Makuya K (1968): Population dynamics of larvae overwintering in southern Japan. Japn J Sanit Zool 19: 225-229.
35. Sota T, Mogi M, Hayamizu E (1992): Seasonal distribution and habitat selection by Aedes albopictus and Aedes rivies) (Diptera : Culicidae) in Northern Kyushu, Japan. J Med Entomol 29: 296-304.
36. Preepachomp W, Mullica J, Jareonsutasin K (2006): The larval ecology of Aedes aegypti and Aedes albopictus in three topographical areas of Southern Thailand. Dengue Bull 30: 204-213.
37. Dye C (1992): The analysis of parasite transmission by blood-sucking insects. Annu Rev Entomol 37: 1-19.
38. McCrae AW (1983): Oviposition by African malaria vector mosquitoes. 1. Temporal activity patterns of caged, wild-caught, freshwater Aedes gambiae Giles sensu lato. Ann Trop Med Parasitol 77: 615-625.
39. Chadee DD, Corbet PS (1990): A night-time role of the oviposition site of the mosquitoes, Aedes aegypti (L) (Diptera: Culicidae). Ann Trop Med Parasitol 84: 429-433.
40. Clements AN (1999): The biology of mosquitoes, volume 2: Sensory reception and behaviour. Wallingford: CABI Publishing pp xvi, 740.
41. Sumba LA, Okoth K, Deng AL, Githure J, Knols BGJ, et al. (2004): The relationship between size and fecundity in Aedes albopictus. J Vector Ecol 25: 212-217.
42. Grech K, Maung LA, Read AF (2007): The effect of parental rearing conditions on offspring life-history in Aedes aegypti (L) (Diptera: Culicidae). J Insect Physiol 21: 1965-1975.
43. Focks DA, Brenner RJ, Hayes J, Daniels E (2000): Transmission thresholds for Aedes aegypti and Aedes albopictus in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. Am J Trop Med Hyg 27: 1225-1231.
44. Preechaporn W, Mullica J, Jaroensutasinee K (2006): The larval ecology of Aedes aegypti (Skuse) in Northern Kyushu, Japan. Am J Trop Med Hyg 62: 371-383.
45. Yang W, Tang J, Wang Z, Shen L, Liang Z, et al. (2002): Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. J Med Trop Med Hyg 7: 757-762.
46. Focks DA, Brenner RJ, Hayes J, Daniels E (2000): Transmission thresholds for Aedes aegypti and Aedes albopictus in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. Am J Trop Med Hyg 27: 1225-1231.
47. Zanotto PM, Bernardini J, Silva A, De Souza M Jr. (1997): Dengue virus infection in SCID mice engrafted with human K562 Cells. J Virol 72: 9729-9737.
48. Wong L-K, Liao C-L, Chen L-K, Yeh CT, Liu CI, et al. (1998): Study of dengue virus infection during an outbreak in Yanes Puerto Rico in 1991. Dengue Bull 30: 204-213.
49. Dengue (1992): Seroprevalence of dengue virus infection. Singapore. Wkly Epidemiol Rec 67: 99-101.
50. Pontes RJ, Freeman J, Oliveira-Lima JW, Hedgon JC, Spielman A (2000): Vector densities that potentiate dengue outbreaks in a Brazilian city. Am J Trop Med Hyg 62: 371-383.
51. Moore CG, Cline BL, Ruiz-Tiben E, Lee D, Romney-Joseph H, et al. (1978): Aedes aegypti in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. Am J Trop Med Hyg 27: 1225-1231.
52. Moore CG, Cline BL, Ruiz-Tiben E, Lee D, Romney-Joseph H, et al. (1978): Aedes aegypti in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. Am J Trop Med Hyg 27: 1225-1231.
53. Effler PV, Pang L, Kitsuntani F, Vornmad V, Nakata M, et al. (2003): Dengue fever, Hawaii, 2001-2002. Emerg Infect Dis 11: 742-749.
54. Sota T, Mogi M, Hayamizu E (1992): Seasonal distribution and habitat selection by Aedes albopictus and Aedesrivies) (Diptera : Culicidae) in Northern Kyushu, Japan. J Med Entomol 29: 296-304.
55. Turley AP, Moreira LA, O’Neill SL, McGraw EA (2009): Wolbachia infection reduces blood-feeding success in the dengue fever mosquito, Aedes aegypti. PLoS Negl Trop Dis 3: e316. doi:10.1371/journal.pntd.0000316.
56. Briegel H (1990): Fecundity, metabolism, and body size in Anopheles (Diptera: Culicidae) vectors of Malaria. J Med Entomol 27: 839-850.
57. Klödnen MJ, Lea OA (1978): Blood meal size as a factor affecting continued host-seeking by Aedes aegypti (L). Am J Trop Med Hyg 27: 827-831.
58. Clements AN (1992): The biology of mosquitoes. Volume 1: development, nutrition and reproduction. London: Chapman and Hall.
59. Foster WF (1995): Mosquito sugar feeding and reproductive energetics. Annu Rev Entomol 40: 433-447.
60. Reisen WK (2003): Lessons from the past: historical studies by the University of Maryland and the University of California, Berkeley. In: Takken W, Scott TW, eds. Ecological applications for application of genetically modified mosquitoes. Dordrecht: Kluwer Academic Publishers, 25-32. Wageningen UR Frontis Series no. 2. [http://library.wur.nl/frontis/malaria/03/reisen.pdf].
61. Bielig H, Timmermann SE (2001): Aedes albopictus: physiological aspects of development and reproduction. J Med Entomol 38: 566-571.
62. Rosen L (1987): Sexual transmission of dengue viruses by Aedes aegypti. Am J Trop Med Hyg 37: 398-402.
63. Reisen WK (1999): Estimation of vectorial capacity: relationship to disease transmission by malaria and arbovirus vectors. Bull Soc Vector Ecol 14: 67-70.
64. Ponlawat A, Harrington LC (2005): Blood feeding patterns of Aedes aegypti and Aedes albopictus in Thailand. J Med Entomol 42: 849-850.
65. Briegel H, Timmermann SE (2001): Aedes albopictus: physiological aspects of development and reproduction. J Med Entomol 38: 566-571.
66. Sumanochitrapon W, Daniel S, Sithiprasana R, Kittayapong P, Innis BL (1998): The association between larval population and dengue virus circulation in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. Am J Trop Med Hyg 27: 1225-1231.
67. Lin Y-L, Liao C-L, Chen L-K, Yeh CT, Liu CI, et al. (1998): Study of dengue virus infection during an outbreak in Yanes Puerto Rico in 1991. Dengue Bull 30: 204-213.