DIFFERENTIAL ENVIRONMENTAL PREFERENCES OF GRAVID FEMALE *AEDES* MOSQUITOES IN OVIPOSITING THEIR EGGS

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Abstract. A prospective field study was carried out to investigate any preferential differences of gravid female *Aedes* mosquitoes in ovipositing their eggs in man-made containers placed in different environmental conditions. The findings of this study show that gravid female *Aedes* mosquitoes preferred to breed in containers found in the outdoor garden than those placed on the patio and or inside the house. The findings also show that if the breeding habitats in the garden were removed, they would favorably use the breeding habitats found on the patio or inside the house as alternatives. An incidental interesting finding in this study shows that ultra-low volume fogging of insecticides using the vehicle-mounted equipment carried out outside the house may promote the gravid female *Aedes* mosquitoes to enter the house to breed.

INTRODUCTION

Dengue fever (DF) and the severe forms, dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) are major causes of morbidity and mortality in Southeast Asia (WHO, 1966, 2000). It is also a growing public health problem in many other parts of the world, especially for those countries situated in tropical and subtropical regions where there is intense dengue virus transmission. Both DF and DHF/DSS are caused by infection with any of four closely related dengue virus serotypes: dengue-1, dengue-2, dengue-3, and dengue-4, which show extensive cross-reactivity in serological tests but do not provide cross-protection (Monath, 1990).

Dengue viruses are transmitted by female *Aedes* species of mosquitoes, such as *Aedes aegypti*, *Ae. albopictus*, *Ae. polynesiensis*, and several species of the *Ae. scutellaris* complex. Both *Ae. aegypti* and *Ae. albopictus* are found in Malaysia, though *Ae. aegypti* is not the indigenous species (Rudnick *et al*, 1965). In Malaysia and also in other Southeast Asian countries, *Ae. aegypti* is regarded as the principal urban vector for the transmission of dengue virus. It is considered a highly domesticated mosquito; highly adapted to living with man, preferring to rest indoors and to feed on humans during daylight hours in an unobtrusive and often undetected manner (Gubler and Meltzer, 1999). The adult female mosquitoes prefer to lay eggs in artificial water containers commonly found in urban areas of tropics and subtropics (Gubler and Meltzer, 1999). Containers found in and around the home, such as those used for water storage, flower vases, old tires, buckets and various plastic containers, and other receptacles that collect rainwater are some of the examples.

A study in Singapore showed that *Ae. aegypti* and *Ae. albopictus* breed in a wide variety of habitats, with 99% of their habitats being artificial and 0.6% natural. Of the artificial habitats, 95% were domestic containers, such as ant traps, earthenware jars, bowls, tanks, tin cans and rubber tries (Chan *et al*, 1971a). Thus, rapid industrial and economic development in most of these Southeast Asian countries over the last two decades has brought about massive infrastructural changes and created a man-made environment conducive for breeding *Aedes* mosquitoes. This is reflected by the finding of most cases of DF and DHF among the urban population (70-80%) with the highest incidence in the working and school going age groups which correlates with the relatively high *Aedes* Index in

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construction sites, factories and schools (Gubler and Meltzer, 1999)

This study is a part of an on-going prospective study on the ovipositing behavior of *Aedes* female mosquitoes in a typical urban environment (Chua *et al*, 2004). This study reports a differential preference of gravid *Aedes* female mosquitoes to breed in habitats placed in different environments.

MATERIALS AND METHODS

Place and duration

This on-going project began on the 1st of March 2002. The study was carried out in an established residential housing estate in the city of Petaling Jaya, peninsular Malaysia (GPS co-ordinate: 03º 05.649N, 101º 37.045E; Elevation: 62M).

Planning and selection of sites for ovitrapping of immature *Aedes* mosquitoes

The study consists of two parts. The first part was to establish any differential preference of gravid female mosquitoes to oviposit their eggs between ovitraps placed inside the house and those in the outdoor garden (Fig 1, House 1). The second part was to compare the field efficiency of oviposition rate by gravid *Aedes* female mosquitoes in ovitraps placed on the patio versus the same type and number of ovitraps placed in the garden (Fig 1, House 2). For the first part, a pair of ovitraps (one with a leaflet of dried *Ptychosterma macathurii* palm leave and another with a plastic strip) were placed at a selected permanent site inside a house (Fig 2a) directly under a window and a similar pair of ovitraps were placed at a distance 7 meters away, in the outdoor garden under the shade of a cluster of *Ptychosterma macathurii* palm trees (Fig 2b). For the second part, another house was selected (Fig 1, House 2). Three permanent sites within the garden and three permanent sites on the patio were chosen for setting up the ovitraps. A pair of ovitraps (one white in color and the other painted black; Fig 2c and Fig 2d),

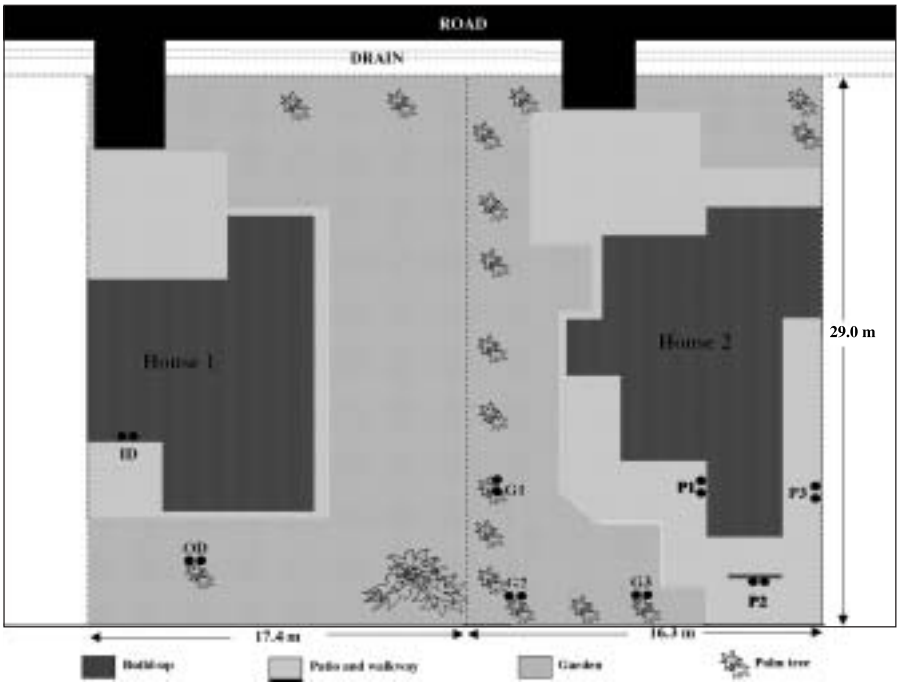

Fig 1–A sketch map (approximately proportional to scale) of two semi-detached houses with their respective adjoining gardens where the field study was conducted. Paired blacked dots (ID, OD, G1, G2, G3, P1, P2, P3) represent the locations where the ovitraps were placed for House 1 (ID = indoor, OD = outdoor) and House 2 (G = garden, P = patio) respectively.

Fig 2–A composite photograph showing respective types of ovitraps used for this field study. Fig 2a and 2b show the type of ovitraps that were respectively placed in the garden (outdoor) and inside the house (indoor) of House 1. Fig 2c and 2d shows the types of ovitraps that were respectively placed in the patio and garden of House 2.

each containing a leaflet of dried *Ptychosterma macathurii* palm leaves, was placed at each site. For both parts, the process of ovitrapping in the garden was discontinued after completing a certain number of cycles of ovitrapping, while the respective cycles of ovitrapping in the house and patio were continued as usual until the end of the study.

Ovitrapping approach for immature *Aedes* mosquitoes

The clean ovitraps were each filled with 1.5 liters of clear rainwater and placed in the respective studied sites as described. Examination of ovitraps for the presence of mosquito larvae was carried out daily. The end-point for harvesting the immature mosquitoes (larvae and pupae) from the ovitraps occured whenever an early mosquito pupa was noted in any of the containers. The larvae in each trapping container were respectively transferred into a smaller transparent plastic container fitted with a perforated screw-cap for ventilation and enumerated accordingly. The larvae in the transparent containers were fed with fish pellets (Azoo Co, Malaysia) until mature adult mosquitoes emerged. Preliminary identification for the species of the collected larvae was made based on the morphological characteristics of the larvae. Final identification for the species of mosquitoes collected was based on the morphological characteristics of the adult mosquitoes. Though both *Ae. aegypti* and *Ae. albopictus* were collected in this study, no attempt was made to count them separately. They were all counted together as *Aedes* species.

After each cycle of ovitrapping, the containers were thoroughly washed and scrubbed with mild detergent, especially the inner surface, to ensure no residual eggs adhering to the surface were carried forward to the next cycle of study. The containers were then rinsed with clean rain or tap water and subsequently refilled with rainwater before being placed back in their respective locations but at a different orientation to reduce possible bias due to relative position of the containers with each other. For statistical analysis, the number of immature mosquitoes collected in each cycle of ovitrapping from both types of ovitraps placed in each site were pooled together and enumerated as the total of immature mosquitoes collected for that specific site.

Statistical analysis

The derived data were tabulated in appropriate worksheets using the Microsoft Excel program and evaluated by chi-square test, paired

t-test (Mann-Whitney test) using the Statistical Program for Social Science (SPSS) and Epi Info 6 (Center for Disease Control and Prevention, Atlanta) free computer program for any statistically significant association. A probability (p) value of 0.05 or less was taken as the level of significant association for each ordinal variable with the relevant adjusting variables. In the analysis, the immature mosquitoes included larvae of all stages of development and the pupae.

RESULTS

This component of an on-going prospective study took place from the 1st of January 2003 to the 31st of September 2004 involving two neighboring semi-detached residential homes in the city of Petaling Jaya. A sketched map representing the layout plan of both houses with locations of major garden trees and sites of ovitraps is shown in Fig 2.

In the first part of this study, 18 cycles of ovitrapping (indoor and outdoor ovitrapping running concurrently) were carried out from 9/2/2003 to 5/10/2003. Following which, outdoor ovitrapping in the garden was discontinued while the indoor ovitrapping process continued as usual for a further 27 cycles extending from 7/10/2003 to 1/9/2004. The number of ovitraps found to have immature *Aedes* mosquitoes and the total number of immature mosquitoes collected in ovitraps placed at each site in each ovitrapping cycle are shown in Table 1. With indoor and outdoor ovitrapping running concurrently, immature *Aedes* mosquitoes were collected in only one ovitrapping cycle out of a total of 18 cycles, whereas the immature mosquitoes were collected in 16 of the 18 ovitrapping cycles. There was a statistically significant difference in gravid female *Aedes* mosquitoes to preferentially deposit their eggs in similar types of ovitraps placed in the outdoor garden (χ^2 = 21.85, p<0.0001). As for the number of ovitraps found to have immature *Aedes* mosquitoes, the immature mosquitoes were collected in only one ovitrap placed indoors, whereas immature mosquitoes were found in 26 ovitraps out of a total of 36 ovitraps set in each site throughout the period. Statistically, gravid female *Aedes* mosquitoes had a significant preference to oviposit eggs in ovitraps placed in the outdoor garden (χ^2 = 34.3, p<0.0001). During the same period, a total of 13 immature *Aedes* mosquitoes were collected in ovitraps placed indoors, whereas 613 were collected in ovitraps placed outdoors (Table 1). These findings support the preference of gravid female *Aedes* mosquitoes to breed in outdoor containers (Mann-Whitney test: Z=4.795, p<0.0001).

During the period when the outdoor ovitrapping was discontinued, immature *Aedes* mosquitoes were collected in ovitraps in 14 out of a total of 27 ovitrapping cycles (Table 1). Comparing the indoor ovitrapping cycles with the indoor and outdoor ovitrapping running concurrently, there was a significant difference in the positive immature mosquito collection rates (χ^2=8.44, p=0.0037). There was also a significantly higher positive rate of getting gravid female *Aedes* mosquitoes to deposit their eggs in ovitraps placed indoors without the presence of outdoor ovitraps (χ^2=10.34, p=0.0013). In terms of the mean number of immature *Aedes* mosquitoes collected, there was also a significantly higher mean number of immature *Aedes* mosquitoes collected in the ovitraps in the absence of outdoor ovitrapping running concurrently (Mann-Whitney test: Z=3.135, p=0.0020).

In the second part of the study, 22 cycles of ovitrapping were carried out with ovitrapping running concurrently with the patio and garden from 2/1/2003 to 5/10/2003. Following which, ovitrapping in the garden was discontinued, while the ovitrapping in the patio was continued as usual for a further 27 cycles extending from 7/10/2003 to 1/9/2004. The number ovitraps with immature *Aedes* mosquitoes and the total number of immature mosquitoes collected in ovitraps placed on the patio versus those in the garden in each ovitrapping cycle is shown in Table 2. With patio and garden ovitrapping running concurrently, immature *Aedes* mosquitoes were found in ovitraps placed on the patio in 15 of the 22 total ovitrapping cycles, whereas the immature mosquitoes were found in ovitraps placed in the garden in all 22 ovitrapping cycles. There was a statistically significant difference in gravid female *Aedes* mosquitoes to preferentially deposit their eggs in similar types of ovitraps placed in the garden (Fisher's exact, p=0.0089). As for the number of ovitraps with immature

Table 1
Comparative numbers of immature *Aedes* mosquitoes collected in ovitraps placed inside the house versus those in the outdoor garden.

Date interval	Immature *Aedes* (N)		Date interval	[a]Immature *Aedes* (N)
	Indoor	Outdoor		Indoor
9/2- 22/2/03	0	5 (1)	7/10- 18/10/03	2 (1)
23/2- 5/3/03	0	16 (1)	20/10- 31/10/03	2 (1)
11/3- 22/3/03	0	22 (2)	31/10- 10/11/03	5 (1)
2/4- 11/4/03	0	67 (2)	14/11- 24/11/03	39 (2)
13/4- 23/4/03	0	99 (2)	25/11- 5/12/03	3 (1)
26/4- 4/5/03	0	27 (2)	6/12- 17/12/03	8 (2)
6/5- 15/5/03	0	17 (2)	19/12- 30/12/03	0
17/5- 26/5/03	0	0	1/1- 12/1/04	0
30/5- 10/6/03	0	20 (2)	14/1- 27/1/04	0
15/6- 26/6/03	0	3 (2)	28/1- 8/2/04	8 (1)
28/6- 8/7/03	0	17 (1)	9/2- 19/2/04	0
11/7- 20/7/03	0	0	19/2- 29/2/04	0
22/7- 1/8/03	0	12 (1)	1/3- 11/3/04	28 (1)
4/8- 14/8/03	0	15 (1)	12/3- 23/3/04	0
17/8- 28/8/03	13 (1)	61 (1)	23/3- 3/4/04	0
29/8- 10/9/03	0	137 (2)	3/4- 13/4/04	0
13/9- 23/9/03	0	57 (2)	15/4- 25/4/04	22 (1)
24/9- 5/10/03	0	38 (2)	26/4- 7/5/04	0
			10/5- 22/5/04	0
			24/5- 1/6/04	17 (2)
			3/6- 14/6/04	0
			17/6- 29/6/04	0
			1/7- 10/7/04	0
			11/7- 21/7/04	15 (1)
			23/7- 3/8/04	18 (1)
			10/8- 19/8/04	17 (1)
			21/8- 1/9/04	40 (2)
Total	13 (1)	613 (26)	Total	224 (18)

[a]Immature *Aedes* (N) = Combined number of immature *Aedes* mosquitoes collected in both ovitraps placed inside the house without any ovitrap placed outside the same house (N= number of ovitraps with immature *Aedes* mosquitoes).

Aedes mosquitoes, the immature mosquitoes were found in ovitraps placed on the patio in 35 of the 132 total ovitraps set up throughout the period, whereas immature mosquitoes were found in ovitraps placed in the garden in 102 of the 132 total ovitraps set up during the same period. Statistically, gravid female *Aedes* mosquitoes had a significant preference to oviposit eggs in ovitraps placed in the garden ($\chi^2 = 74.59$, $p<0.0001$). During the same period, a total of 472 immature *Aedes* mosquitoes were collected in ovitraps placed on the patio, whereas 2,373 were collected in ovitraps placed from the garden (Table 2). These findings support the preference of gravid female *Aedes* mosquitoes to breed in man-made containers placed in the garden over those on the patio (Mann-Whitney test: $Z=4.869$, $p<0.0001$).

During the period when the ovitrapping in the garden was discontinued, immature *Aedes* mosquitoes were collected in ovitraps placed on the patio in 26 of a total of 27 ovitrapping cycles (Table 2). Comparing the patio ovitrapping cycles with the patio and garden ovitrapping running concurrently, there was a significant difference in the positive immature mosquito collection

Table 2
Comparative number of immature *Aedes* mosquitoes collected in ovitraps placed on the patio of a house versus those in the garden.

Date Interval	Immature *Aedes* (N)		Date Interval	[a]Immature *Aedes* (N)
	Patio	Garden		Patio
2/1- 11/1/03	54 (5)	88 (6)	7/10- 18/10/03	24 (2)
12/1- 22/1/03	104 (4)	45 (4)	20/10- 31/10/03	39 (5)
26/1- 5/2/03	47 (2)	56 (5)	31/10- 10/11/03	90 (5)
9/2- 19/2/03	115 (5)	70 (6)	14/11- 24/11/03	130 (6)
21/2- 2/3/03	18 (2)	77 (4)	25/11- 5/12/03	56 (4)
6/3- 16/3/03	36 (2)	137 (6)	6/12- 17/12/03	42 (4)
17/3- 29/3/03	0	141 (6)	19/12- 30/12/03	6 (2)
2/4- 11/4/03	0	140 (5)	1/1- 12/1/04	14 (1)
13/4- 23/4/03	0	78 (3)	14/1- 27/1/04	40 (3)
26/4- 4/5/03	0	189 (6)	28/1- 8/2/04	30 (3)
6/5- 15/5/03	21 (3)	75 (4)	9/2- 19/2/04	8 (2)
17/5- 26/5/03	2 (1)	35 (3)	19/2- 29/2/04	36 (3)
30/5- 10/6/03	19 (2)	185 (5)	1/3- 11/3/04	15 (2)
15/6- 26/6/03	0	185 (6)	12/3- 23/3/04	1 (1)
28/6- 8/7/03	2 (1)	102 (6)	23/3- 3/4/04	9 (2)
11/7- 20/7/03	0	56 (4)	3/4- 13/4/04	0
22/7- 1/8/03	9 (3)	152 (6)	15/4- 25/4/04	124 (4)
4/8- 14/8/03	14 (2)	236 (5)	26/4- 7/5/04	42 (3)
17/8- 28/8/03	17 (1)	62 (3)	10/5- 22/5/04	53 (5)
29/8- 10/9/03	8 (1)	42 (4)	24/5- 1/6/04	18 (3)
13/9- 23/9/03	0	37 (3)	3/6- 14/6/04	52 (6)
24/9- 5/10/03	6 (1)	185 (6)	17/6- 29/6/04	12 (2)
			1/7- 10/7/04	40 (3)
			11/7- 21/7/04	32 (3)
			23/7- 3/8/04	130 (6)
			10/8- 19/8/04	93 (6)
			21/8- 1/9/04	40 (2)
Total	472 (35)	2,373 (106)	Total	1,176 (88)

[a]Immature *Aedes* (N) = Combined number of immature *Aedes* mosquitoes collected in both ovitraps placed on the patio of the same house without any ovitraps placed in the nearby garden (N= number of ovitraps with immature *Aedes* mosquitoes).

rates on the patio without the garden ovitrapping (Fisher exact, p=0.0158). There was also a significantly higher positive rate for gravid female *Aedes* mosquitoes to deposit eggs in ovitraps placed on the patio without the presence of ovitraps in the garden (χ^2=21.98, p<0.0001). In terms of the mean number of immature *Aedes* mosquitoes collected, there was also a significant higher mean number of immature *Aedes* mosquitoes collected on the ovitraps placed on the patio without the presence of ovitraps in the garden (Mann-Whitney test: Z=2.771, p=0.0060).

DISCUSSION

Oviposition is an important component of most mosquito-borne diseases (Wallis, 1954; Bentley and Day, 1989). A tremendous amount has been written concerning the oviposition of mosquitoes, including *the Aedes* species (Bates, 1940; Angerilli, 1980; Bailey, 1981). Selection of oviposition sites by gravid female mosquitoes is a crucial event for the survival of their species. In the past, insects, such as *Aedes* mosquitoes, used spaces in living or dead terrestrial plants

which held water, termed phytotelmata, as breeding habitats (Mogi, 2000). With urbanization and reduction of these natural habitats, the *Aedes* mosquitoes, especially *Ae. aegypti*, have become highly adapted to living with man. Containers found in and around the home, such as those used for water storage, flower vases, old tires, buckets, various plastic containers, and other receptacles that collect rainwater have become the usual breeding habitats. A study in Singapore showed that *Ae. aegypti* and *Ae. albopictus* bred in a great variety of habitats, with 99% of their habitats being artificial types and 0.6% natural types. Of the artificial containers, not all types are equally preferred by *Aedes* mosquitoes for breeding. Our earlier study showed that certain types of artificial containers were preferred by *Aedes* mosquitoes to oviposit (Chua *et al*, 2004).

It was a previously accepted notion that female *Aedes* mosquitoes in an urban environment prefer to rest and breed indoors. The notion was mainly based on the ability to capture these adult mosquitoes indoors and their immature forms were found in indoor man-made containers (Chan *et al*, 1971a, b). This notion may not necessarily be true. In an urban environment, with the reduction of its natural breeding habitats, *Aedes* mosquitoes have adapted to man-made breeding habitats. The findings of this study show that gravid female *Aedes* mosquitoes still prefer to breed in containers found in the outdoor garden, rather than those placed on the patio or inside the house. The findings also show that if the breeding habitat in the garden is removed, they prefer the breeding habitats found on the patio or inside the house.

An incidental interesting finding in this study shows that whenever there was ultra-low volume fogging of insecticides being carried out by the city municipality using vehicle-mounted equipment, immature *Aedes* mosquitoes could be found in the ovitraps placed indoors (Table 1, period of ovitrapping cycle in bold). During the first part of the study when indoor and outdoor ovitrapping was running concurrently, the only ovitrapping cycle that immature *Aedes* mosquitoes were found in the indoor ovitrap was during the period when chemical fogging took place. Further study is needed to investigate whether ultra-low fogging of insecticides outside the house actually promotes female *Aedes* mosquitoes to enter the house to feed and breed.

REFERENCES

Angerilli NPD. Influences of extracts of freshwater vegetation on the survival and oviposition by *Aedes aegypti*. *Can Entomol* 1980; 112: 1249-52.

Bailey DL. Effects of salinity on *Anopheles albimanus* ovipositional behavior, immature development and population dynamics. *Mosq News* 1981; 41: 161-7.

Bates M. Oviposition experiments with anopheline mosquitoes. *Am J Trop Med* 1940; 20: 569-83.

Bentley MD, Day JF. Chemical ecology and behavioral aspects of mosquito ovipositon. *Ann Rev Entomol* 1989; 34: 401-21.

Chan KL, Ho BC, Chan YC. *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) in Singapore City - 2. Larval habitats. *Bull World Health Organ* 1971a; 44: 635-41.

Chan YC, Chan KL, Ho BC. *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) in Singapore City - 1. Distribution and density. *Bull World Health Organ* 1971b; 44: 617-27.

Chua KB, Chua IL, Chua IE, Chua KH. Differential preferences of oviposition by *Aedes* mosquitoes in man-made containers under field conditions. *Southeast Asian J Trop Med Public Health* 2004; 35: 599-607.

Gubler DJ, Meltzer M. Impact of dengue/dengue hemorrhagic fever on the developing world. *Adv Virus Res* 1999; 53: 35-70.

Mogi M. Phylotelmata: cryptic mosquito habitats. In: Ng FSP, Yong HS, eds. Mosquitoes and mosquito-borne diseases: biology, surveillance, control, personal and public protection measures. Kuala Lumpur: Aura Productions Sdn Bhd, 2000: 255-72.

Monath TP. Flaviviruses. In: Fields BW, Knipa DM, Chanock RM, Melnick JL, Roizman B, Shope RE, eds. Field's virology. Vol 1. 2nd ed. New York: Raven Press, 1990: 763-814.

Rudnick A, Tan EE, Lucas JK, Mohamed bin Omar. Mosquito-borne haemorrhagic fever in Malaysia. *Br Med J* 1965; 1: 1269-72.

Wallis RC. A study of oviposition activity of mosquitoes. *Am J Hyg* 1954; 60: 135-68.

WHO. Dengue haemorrhagic fever: diagnosis, treatment and control. Handbook of the World Health Organization. Geneva: WHO, 2000, 1-84.

WHO. Mosquito-borne haemorrhagic fevers of South-East Asia and the Western Pacific. *Bull World Health Organ*. 1966; 35: 1-103.