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BIONOMICS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN RELATION TO DENGUE INCIDENCE ON PENANG ISLAND AND THE APPLICATION OF SEQUENTIAL SAMPLING IN THE CONTROL OF DENGUE VECTORS

PHON CHOOI KHIM

UNIVERSITI SAINS MALAYSIA

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by

PHON CHOOI KHIM

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BIONOMIKS NYAMUK *AEDES AEGYPTI* DAN *AEDES ALBOPICTUS* BERHUBUNGAN DENGAN KEJADIAN PENYAKIT DENGGI DI PULAU PINANG DAN PENGGUNAAN PENYAMPELAN BERTURUTAN UNTUK KAWALAN VEKTOR DENGGI

ABSTRAK

Satu kajian telah dijalankan untuk menentukan taburan dua jenis vektor denggi, *Aedes aegypti* dan *Aedes albopictus* di Kampung Paya Teluk Kumbar (luar bandar), Kampung Baru Sungai Ara (pinggir bandar), dan Lorong Mahsuri (bandar), di Pulau Pinang, Malaysia dari Oktober 2003 hingga November 2004. Sejumlah 60 perangkap telur (30 dalam rumah dan 30 luar rumah) telah dipasang di setiap lokasi. Persaingan secara intraspesifik dan interspesifik di antara kedua-dua nyamuk tersebut telah dikaji di dalam makmal dengan menggunakan nyamuk strain Lorong Mahsuri. Hubungan di antara kelimpahan nyamuk, fizikal parameter, dan kejadian denggi di ketiga-tiga lokasi telah dianalisis dengan menggunakan regresi garis lurus. Potensi bagi mengguna penyampelan berturutan kehadiran-ketidakhadiran untuk membuat keputusan bagi kawalan juga dikaji. Keputusan menunjukkan bahawa *Aedes albopictus* adalah nyamuk dominan di dalam dan di luar rumah di Pulau Pinang. *Aedes aegypti* lebih banyak didapati di kawasan bandar (Lorong Mahsuri), dan lebih suka membiak di dalam rumah. Tambahan pula, *Aedes aegypti* telah tersebar secara perlahan-lahan ke bahagian barat daya Pulau Pinang. Berbanding dengan *Aedes aegypti*, *Aedes albopictus* mampu hidup dalam keadaan kurang makanan dan kepadatan larva yang tinggi. Kelebihan daya saing *Aedes albopictus* dalam keadaan bahan sumber yang terhad mungkin menyebabkan kedominannya di Pulau Pinang. Jumlah hujan pada dua bulan lepas telah memberi kesan korelasi positif terhadap populasi *Aedes* di Lorong Mahsuri dan

Kampung Paya Teluk Kumbar. Di Kampung Baru Sungai Ara, jumlah hujan telah memberi kesan korelasi positif yang signifikan kepada kes denggi semasa. Di Kampung Baru Sungai Ara dan Lorong Mahsuri kelimpahan nyamuk telah memberi kesan korelasi positive terhadap kes denggi selepas satu bulan. *Aedes albopictus* memberi kesan korelasi yang lebih jelas terhadap kes denggi berbanding dengan *Aedes aegypti*. Anggaran densiti telur *Aedes* dilakukan dengan menggunakan penyampelan kehadiran-ketidakhadiran, di Lorong Mahsuri dan Kampung Baru Sungai Ara. Dengan menggunakan 60 perangkap telur, di Kampung Baru Sungai Ara, perangkap telur positif sebanyak 75.00% atau kurang menandakan tidak perlu kawalan; sekira-kiranya 83.33% atau lebih, kawalan diperlukan. Manakala di Lorong Mahsuri, sekira-kiranya 73.33% atau kurang perangkap positif, tidak perlu kawalan, manakala 83.33% atau lebih, kawalan diperlukan.

BIONOMICS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN RELATION TO DENGUE INCIDENCE ON PENANG ISLAND AND THE APPLICATION OF SEQUENTIAL SAMPLING IN THE CONTROL OF DENGUE VECTORS

ABSTRACT

A study was carried out to determine the distribution of two dengue vectors, *Aedes aegypti* and *Aedes albopictus* in Kampung Paya Teluk Kumbar (rural area), Kampung Baru Sungai Ara (suburban area), and Lorong Mahsuri (urban area), in Penang, Malaysia from October 2003 to November 2004. A total of 60 ovitraps (30 indoor and 30 outdoor) were placed in each location. Intraspecific and interspecific competitions among these mosquitoes were studied under laboratory conditions using the Lorong Mahsuri strain. The relationship between mosquito abundance, physical parameters, and dengue incidence in all three areas were analyzed using linear regression. The potential use of presence-absence sequential sampling for making control decision was tested. The results showed that *Aedes albopictus* was the dominant species, both indoor and outdoor in Penang Island. *Aedes aegypti* prevails in the urban settlement (Lorong Mahsuri), and prefers to breed indoor. Furthermore, *Aedes aegypti* has begun to spread slowly to the southwestern part of Penang Island. *Aedes albopictus* showed greater survivorship compared to *Aedes aegypti* when food availability is low and larval density is high. The competitive advantage of *Aedes albopictus* in situation of limited resources could be the reason for the dominancy of this mosquito in Penang Island. The previous two months rainfall seems to give a constant positive correlation effect on the *Aedes* population in the Lorong Mahsuri and Kampung Paya Teluk Kumbar. In Kampung Baru Sungai Ara, rainfall had a significant positive correlation effect on the current monthly dengue cases. In Kampung Baru Sungai Ara and Lorong Mahsuri,

mosquito abundance had a positive correlation effect on the dengue cases for the following one month. *Aedes albopictus* gave a better correlation with the incidence of dengue cases compared to *Aedes aegypti*. The density estimation of *Aedes* egg population was made using the presence-absence sequential sampling, in Lorong Mahsuri and Kampung Baru Sungai Ara. In Kampung Baru Sungai Ara, a population estimation using 60 ovitraps showed that when positive ovitraps were 75.00% or less, no control is needed; at 83.33% or higher positive ovitraps, control is needed. While in Lorong Mahsuri, when 73.33% or less ovitraps were positive, no control is needed, but when 83.33% or higher was positive, therefore control is needed.

CHAPTER 1
GENERAL INTRODUCTION

1.0. Introduction

Dengue fever and dengue haemorrhagic fever are caused by the dengue virus, which belongs to the genus *Flavivirus*, family Flaviviridae, that consists of 4 dengue virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), all of which can cause dengue fever and dengue haemorrhagic fever (Miyagi & Toma 2000). Dengue viruses are transmitted from viremic to susceptible human beings by various mosquitoes of the subgenus *Stegomyia*, notably *Aedes aegypti* and *Aedes albopictus*.

The observations over 100 years have shown that the epidemiology of dengue varies a great deal with respect to both geography and time. This is due not only to modifications in human ecology (population increase, urbanization, more frequent travel), but also to ecological adaptations of certain mosquito species in particular with respect to their geographic distribution and abundance which in turn result in changes in their ecology. Over the last two decades, in Malaysia, rapid changes in the urban environment and demographic structure in the country has undoubtedly, influenced changes in the vector ecology and consequently the epidemiology of dengue.

Several studies have reported that *Aedes albopictus* and *Aedes aegypti* may share the same habitat (Gilotra et al. 1967, Chan et al. 1971a, Sprenger & Wuithiranyagool 1986, O'Meara et al. 1993). Because of this association, it has been hypothesized that some parts of Southeast Asia, *Aedes aegypti* has completely replaced the indigenous *Aedes albopictus* in urban areas (Pant et al. 1973, Service 1992). Conversely, observations on the spread of *Aedes albopictus* in the southern coastal states of the United States indicate that the expansion appeared to be occurring at the expense of *Aedes aegypti*. The introduction of *Aedes albopictus* has been accompanied by a drastic and rapid decline of *Aedes aegypti* populations (Nasci et al. 1989, O'Meara et al. 1993). The above studies have brought up some laboratory studies of larval competition. Laboratory studies of larval competition conducted with different Asian strains *Aedes albopictus* and *Aedes aegypti* showed that *Aedes aegypti* is better able to compete than *Aedes albopictus* (Macdonald 1956a, Gilotra et al. 1967, Moore & Fisher 1969, Sucharit et al. 1978, Service 1992). Information obtained by these researches suggested that *Aedes albopictus* would not become established in localities inhabited by *Aedes aegypti* because of the competitive displacement of competitive exclusion. However, Hawley (1988) hypothesized that the apparent spread of *Aedes aegypti* in Southeast Asia has been caused by increased urbanization which favours breeding of this species, which is also prevalent in indoor larval habitats, whereas *Aedes albopictus* breeds more successfully in suburban and rural areas and tends not to colonize in indoor water collections.

From a previous study on Penang Island, *Aedes aegypti* had not spread beyond the city limit of Georgetown to the rest of the Island (Yap 1975a), and *Aedes albopictus* females were found to prefer the outdoor ovitraps than the indoors. Latest information of *Aedes aegypti* distribution on Penang Island is lacking. However, in 1991, a nationwide survey had been carried out in urban towns of Peninsular Malaysia by Lee (1991). He reported that both *Aedes aegypti* and *Aedes albopictus* were found breeding indoor and outdoor in a variety of containers. However, the dominant indoor breeder appeared to be *Aedes aegypti*, while both species showed equal preference for outdoor containers. In his study, he gave the idea that *Aedes aegypti* was beginning to edge out *Aedes albopictus* because of interspecies competition.

Generally, insects are exceedingly sensitive to temperature and rainfall regiments; tropical and temperate species frequently show great variations in seasonal abundance (Vezzani et al. 2004), and many epidemiological studies of mosquito-borne arboviruses have shown that there are relationships between environmental factors, mosquito densities and disease epidemics (Russell 1986). Therefore, by using climatological indicators, vector population increases could than be predicted earlier by routine surveillance, thus increasing the time available for planning and conducting control operations (Moore 1985).

Epidemic haemorrhagic fever is a disease of the rainy season and of the period of *Aedes aegypti* abundance (Halstead 1966). In Chiang Mai, northern Thailand, *Aedes* egg population remained low in the dry season, but increased/decreased exponentially during the first/latter half of the rainy season, respectively (Mogi et al.1988). This seasonal pattern was similar to the seasonal distribution of dengue haemorrhagic fever cases in the area. Foo et al. (1985) analyzed a ten-year data on rainfall and dengue cases; it had provided statistical evidence of an association between increases in dengue cases and heavy rain in Selangor, Malaysia.

The density of insect populations is typically determined by counting the number of individuals in a random sample (Schaalje et al. 1991). Exact counts of insects are often difficult to obtain because of the small size of insect and of the high numbers per sample. Binomial or presence-absence sampling is often useful for estimating population density because the only information needed from a sampling unit is whether or not the organism is absent (Schaalje et al. 1991). An approach was developed for the estimation of insect population density, which eliminates the necessity of counting every individual per sample (Gerrard & Chiang 1970). Using population parameters obtained by preliminary surveys, presence-absence sampling allows density estimation simply from the proportion of positive samples. Danielson & Berry (1978) applied presence-absence sampling on redbacked cutworm by assuming that the insect fitted in the negative binomial distribution. However, Gerrard & Chiang (1970) applied presence-absence sampling on corn rootworm egg without assuming any particular distribution pattern. Many studies on *Aedes* population density estimation whether using ovitraps method or larval survey method, can be made using presence-absence sampling without assuming any distribution pattern (Mogi et al. 1990, Lee 1992a, Lee & Inder Singh 1991, Bellini et al. 1996, Lee 1996, Lee & Chang 1997).

Sequential sampling techniques were developed for quality control in factories as early as 1943 (Lee 1992a). Sequential sampling is especially suited for use in pest management because it allows rapid classification of insect populations into broad levels of infestation (Danielson & Berry 1978). Many researchers have already used this technique in the management of some species of cereal aphids on barley in Southwestern Quebec (Ba-Angood & Stewart 1980) and redbacked cutworm in central Oregon (Danielson & Berry 1978). Subsequently, this technique was applied to dipper counts of rice field mosquitoes (Mackey & Hoy 1978, McLaughlin et al. 1987, Wada et al. 1971). Mogi et al. (1990) showed the potential of these techniques in the surveillance of *Aedes* vectors in Thailand using ovitraps. In Malaysia, control by thermal fogging or ultra low volume is always conducted after dengue cases appeared. A predictive model is needed to estimate the transmission threshold therefore the control of mosquito can be done before an outbreak appears. Lee (1992a) applied such a technique in the analysis of ovitrap data and concluded that an ovitrap index of 10% was critical. Lee and Inder Singh (1991) have estimated the adult *Aedes* vector density threshold using sequential analysis as well. However, it is unknown if the transmission threshold from a particular study site can be generalized and applied to other areas (Lee 1992a).

Based on the above studies, it brings out an idea to study the current distribution of *Aedes aegypti* and *Aedes albopictus* in Penang Island, and the larval competition between these two mosquitoes using wild strain mosquitoes collected from sampling sites. Furthermore, it is interesting to study the relationship between *Aedes* mosquito abundance and physical parameters and the relationship between *Aedes* mosquito abundance, physical parameters and dengue incidence in Penang Island. The sequential sampling of *Aedes* mosquito will be applied in Penang Island as well.

1.1. Specific Objectives

The specific objectives of this study are as follows:

1. To study the relative abundance of *Aedes aegypti* and *Aedes albopictus* in indoor and outdoor ovitraps and in urban, suburban, and rural areas.
2. To study the species competition of wild strain *Aedes aegypti* and *Aedes albopictus* under laboratory conditions.
3. To study the relationship between *Aedes* mosquito abundance and its physical parameters.
4. To study the relationship between *Aedes* mosquito abundance and its physical parameters, and dengue incidence.
5. To study the applicability of sequential sampling in Penang Island.

CHAPTER 2
LITERATURE REVIEW

2.0. Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF)

Dengue is prevalent in over 100 countries and threatens the health of more than 2.5 billion people, living in tropical and subtropical regions (Lee 2005). Dengue is the cause of an estimated 500,000 hospitalizations each year with some 24,000 death each year.

Dengue is caused by four antigenically related virus serotypes which are dengue type 1, 2, 3 and 4 viruses. The dengue virus is in the genus *Flavivirus* (Miyagi & Toma 2000). There are three types of dengue fever, namely classical dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Singh 2000). The term “haemorrhagic fever” was first applied to illness in South-East Asia in the Philippines in 1953 (Halstead 1966).

Dengue fever is seen in syndromes that are age-dependent (Halstead 1980). Infants and children may have undifferentiated febrile illness or mild febrile disease with maculopapular rash. Older children and adults usually have an overt illness characterized by fever, headache, myalgia, and gastrointestinal symptoms, often terminating with a macupapular rash.

Dengue haemorrhagic fever or dengue shock syndrome proceeds through two stages (Halstead 1980). The illness begins with abrupt onset of fever accompanied by dengue-like symptoms; during or shortly after the fall in temperature, the condition of the patient suddenly deteriorates, the skin becoming cold, the pulse rapid, and the patient becomes lethargic and restless. In some children the range of pulse pressure progressively narrows, the patient becomes hypotensive and if not treated, may expire in as little as 4-6 hours.

Aedes aegypti and *Aedes albopictus* are the vectors for dengue fever and dengue haemorrhagic fever. *Aedes albopictus* has been repeatedly incriminated as a vector during dengue outbreaks, particularly in Southeast Asia (Shroyer 1986). Jumali et al. (1979) compared the efficiency in transmission of dengue-3 virus by oral route of *Aedes albopictus* and *Aedes aegypti* and found that both species were equally efficient. However, Rosen et al. (1985) showed that there is a significantly larger proportion of the *Aedes albopictus* that became infected by each dengue virus than *Aedes aegypti*.

2.1. Classification of *Aedes albopictus* and *Aedes aegypti*

Classification of *Aedes albopictus* and *Aedes aegypti* published by Knight & Stone (1977) is as below:

Aedes albopictus	***Aedes aegypti***
Kingdom: Animalia	Kingdom: Animalia
Phylum: Arthropoda	Phylum: Arthropoda
Class: Insecta	Class: Insecta
Order: Diptera	Order: Diptera
Family: Culicidae	Famili: Culicidae
Subfamily: Culicinae	Subfamily: Culicinae
Genus: *Aedes*	Genus: *Aedes*
Species: *albopictus*	Species: a*egypti*

2.2. Distribution of *Aedes albopictus* and *Aedes aegypti*

2.2.1. In Malaysia

Aedes albopictus is an indigenous species in Malaysia. It is believed that this mosquito originated in the tropical forest of Southeast Asia (Smith 1956). The invasion of *Aedes aegypti* was first recorded by Leicester in 1908 and Stanten in 1914 (Lee & Cheong 1987). The distribution of *Aedes aegypti* and *Aedes albopictus* in Peninsular Malaysia, Sabah and Sarawak has been well established (Hii 1977, Chang & Jute 1982, Lee 1991).

In Penang Island, both *Aedes aegypti* as well as *Aedes albopictus* are present; the former is more abundant in shop-houses, while the latter in areas having heavy foliage. Macdonald (1956b) in his survey of Georgetown, examined 25 houses out of which 7 houses had *Aedes aegypti* breeding. From a study conducted by Yap (1975a), result showed that the rest of the Penang Island, with the exception of Tanjung Tokong Lama, was free of *Aedes aegypti*. He indicated that *Aedes aegypti* had not spread beyond the city limit of Georgetown to the rest of the island. Information on a more recent distribution of these mosquitoes is lacking.

2.2.2. Micro-Distribution and Macro-Distribution

In general, *Aedes aegypti* is an indoor breeder while *Aedes albopictus* is an outdoor breeder. *Aedes aegypti* was apparently imported from Africa and has established itself in Malaysia, particularly in urban areas, both inside and outside houses (Lo & Narimah 1984). It is commonly associated with dengue haemorrhagic fever in urban areas. However, *Aedes albopictus* is a local species and more prevalent in peri-urban areas, and is associated with dengue fever. Rudnick et al. (1965) did a mosquito survey and reported that *Aedes aegypti* was present in the urban areas of Penang Island, whereas *Aedes albopictus* was present in the urban, rural and forested areas. From a previous study on Penang Island, *Aedes aegypti* had not spread beyond the city limit of Georgetown to the rest of the Island (Yap 1975a), and *Aedes albopictus* females preferred to breeds in the outdoor ovitraps than indoor ones. However, in 1991, a nationwide survey was done in urban towns of Peninsular Malaysia (Lee 1991). He reported that both *Aedes aegypti* and *Aedes albopictus* were found breeding indoor and outdoor in a variety of containers. However, the dominant indoor breeder appeared to be *Aedes aegypti*, whilst both species showed equal preference for outdoor containers. In his study, he gave an idea that *Aedes aegypti* was beginning to edge out *Aedes albopictus* because of the interspecific competition (Lee 1991). He claimed that the use of household insecticide products such as mosquito coil, liquid vaporizer, mat and aerosol deter the mosquito from breeding in the houses (personal communication). Since then, *Aedes aegypti* choose to breeding outdoor rather than indoor. Therefore, the competition between *Aedes aegypti* and *Aedes albopictus* began.

Aedes aegypti and *Aedes albopictus* mixed breeding was found in 18.9% of water containers surveyed and occurred mainly in outdoor containers of large size (Hwang 1994). He also found that more *Aedes aegypti* than *Aedes albopictus* was observed to breed indoors and outdoors.

2.2.3. In Other Countries

In Asia, *Aedes albopictus* occurs as far north as Beijing, China, at 40º latitude (WHO 1980). *Aedes albopictus* was first documented in the United States in Texas in 1985 (Sprenger & Wuithiranyagool 1986). A year later, this mosquito was found in Florida at a tire dump site near Jacksonville (O'Meara et al. 1995). It has spread to 678 counties in 25 states in a 10 year period (Moore & Mitchell 1997). But the distribution was only concentrated in the southeastern United States. Establishment of this mosquito was less common northwards and westwards, presumably because of the less hospitable environment. The distribution of *Aedes aegypti* currently is limited to urban habitats in southern Texas, Florida and in New Orleans (Lounibos 2002). *Aedes albopictus* was discovered in Italy in 1990 through tire trade (Carrieri et al. 2003). Due to the high biological adaptability and its ability to overwinter in embryonic diapause, it spread rapidly to colonize various areas in the central and northern regions of Italy.

2.2.4. Factors Influencing to Mosquito Distribution

From the global viewpoint, environmental changes, including urbanization, improvement of highway and trunk road networks, and importation or transportation of used tires that are stacked or thrown away, are considered important socio-economic factors in the spread of *Aedes albopictus* (Dutta et al. 1998). Moore & Mitchell (1997) also stated that the dispersion of *Aedes albopictus* appeared to be related to the proximity of a county to interstate highways. The one all-important cause of the present extended distribution of the species is very clearly by man (Christophers 1960). Beside that, there are also several factors that prevent its establishment such as the decrease of mosquito habitats due to abnormally dry weather, abnormally cold weather, and improvements in handling discarded materials such as used tires (Kobayashi et al. 2002).

The density of the vector mosquitoes is related to the climatic condition (Vezzani et al. 2004). *Aedes* prefer clean clear water with low oxygen partial pressure, like rain water to breed (Lo & Narimah 1984). Therefore, the habit of storage of rain water inside homes for domestic use will provide a suitable breeding site for them. This statement is also supported by Christophers (1960) who claimed that a dry climate and domestic habits that lead to the storage of water may also be favourable for mosquito breeding. Both human host and mosquito vectors are affected by the environment in which they thrive. With crowding, squatters and slums, poor sanitary amenities such as water supply, people tend to store water for their daily needs. Thus, it will create potential breeding places for the vector mosquitoes, which are domesticated and tend to follow human movement and development (Lo & Narimah 1984).

2.2.5. *Aedes albopictus* and *Aedes aegypti* Breeding Habitat Preference

Except for some African strains, *Aedes aegypti* lives in close association with man, mainly breeding in artificial containers in the domestic environment. *Aedes albopictus* uses both artificial and natural breeding sites. Sumodan (2003) studied the potential of rubber plantation as breeding source for *Aedes albopictus* in Kerala, India. In the plantations where tapping had been suspended, sap-collecting containers had rainwater collection and also mosquito breeding. *Aedes albopictus* had adapted commensurably to the discardable plastic tea cups, a new man-made breeding site for this vector species (Hiriyan et al. 2003). In Malaysia, *Aedes albopictus* is usually found at forest fringes, in secondary forests, and in green areas in towns (Abu Hassan 1994, Macdonald 1956b). It is one of the most common anthropophilic mosquitoes in Malaysia, and it breeds in man-made containers, tree holes, plant axils, and bamboo stumps. *Aedes aegypti* is an artificial container breeder in the coastal towns. On the Island of Anguilla, West Indies, *Aedes aegypti* is able to breed in rock hole as well (Parker et al. 1983).

2.2.6. Replacement of *Aedes albopictus* by *Aedes aegypti*

There is evidence to suggest that in Southeast Asia, indigenous *Aedes albopictus* has been replaced in many areas by the invasion of *Aedes aegypti* (Service 1992, Rudnick et al. 1967). Laboratory experiments with Southeast Asian populations have shown that *Aedes aegypti* out-competes *Aedes albopictus* (Service 1992). It is quite possible that this phenomenon can be brought about by progressive urbanization which tends to cause reduction in the amount of vegetation, outdoor shade and naturally occurring containers as habitats for mosquito breeding and an increase in artificial containers (Hawley 1988).

2.2.7. Replacement of *Aedes aegypti* by *Aedes albopictus*

Some countries, other than Southeast Asia, have shown a widespread of *Aedes albopictus*. In October 1999, Fontenille & Toto (2001) captured biting *Aedes albopictus* females in Southern Cameroon. By 2000, *Aedes albopictus* was already widespread in Southern Cameroon. In Mobile, Alabama, *Aedes albopictus* was first detected during a CDC sponsored ovitrap survey in the Hótoric District. A comparison of ovitrap and larval survey done in 1957, 1984, 1987 and 1990, indicated that *Aedes albopictus* had replaced *Aedes aegypti* in urban Mobile (Hobbs et al. 1991). In 1985, *Aedes albopictus* was introduced into Houston, Texas (Sprenger & Wuithiranyagool 1986). Then, a well establishment of *Aedes albopictus* in the continental USA. By 1988, large breeding populations were common in Texas, Louisiana and the southern states east of the Mississippi River (Nasci et al. 1989). Live larvae of *Aedes albopictus* had been found entering South Africa in used tire casings imported from Japan on 3 separate occasions. It is suggested that undetected populations of *Aedes albopictus* may have already become established in Africa because large areas of Africa experience climatic conditions similar to those where *Aedes albopictus* is already established (Cornel & Hunt 1991).

In South Florida, the displacement of *Aedes aegypti* by *Aedes albopictus* could be because of larval resource competition based on the result conducted using tires (Juliano 1998). Some reasons have been suggested to explain the replacement of *Aedes aegypti* by *Aedes albopictus*, (a) sterility of offspring from interspecific matings; (b) reduced fitness of *Aedes aegypti* form parasites brought in with *Aedes albopictus* and; (c) superiority of *Aedes albopictus* in larval resource competition (Lounibos 2002).

2.2.8. The Effect of Mosquito Invasion in Public Health

Invaded mosquitoes may affect human health by (i) simultaneous introduction of a novel vector and novel pathogen, (ii) acquisition by an introduced vector of a native pathogen, or (iii) independent introductions of a novel vector and a novel pathogen (Juliano & Lounibos 2005). Novel vector and novel pathogen are defined as a newly invasive vector and pathogen in an area. The introduced vector also defined as a newly invasive vector but there is difference between them. When novel vector invaded to an area, it is the only one vector in that area. While, when introduced vector invaded to an area, it is not the only one vector because there are one or more than one vector which can transmit the same pathogen in a same area.

Simultaneous introduction of a novel vector and a novel pathogen may be sufficient to create a new public health threat, with potential to cause large outbreaks of disease, particularly because the host population will likely have limited immunity. For example, yellow fever originated in Africa and appears to have been spread initially by the slave trade of the 16-17th centuries (Lounibos 2002). Outbreaks of yellow fever occurred in many North American cities, as far as New York City, and it is virtually certain that these outbreaks occurred when both vector and virus arrived via ship (Tabachnick 1991).

If an invasive vector takes on a role in an existing disease transmission cycle, it changes the nature of an existing public health threat, presumably for worse. For example, *Aedes aegypti* is the major vector of urban dengue in tropical Asia, where dengue is likely native. Thus, since its invasion of Asia in the late 19th century, *Aedes aegypti* became the primary vector of a resident pathogen.

If an invasive vector acquires, sometime after establishment, an association with an independently introduced pathogen, susceptible hosts are exposed to a novel pathogen with a new vector. This situation may be particularly prone to unpredictable disease dynamics. In Hawaii, *Aedes albopictus* was established before the introduction of dengue virus in 2001, which probably brought by infected humans (Juliano & Lounibos 2005).

2.3. Species Competitions

The presence of other organisms may limit the distribution of some species through competition. Factors that may contribute to species displacement include changes in physical conditions, environmental modification by one of the competing species, cannibalism, predation, active interference (e.g., inhibition of mating, feeding, or ovipostition), disease, parasitism, and generic drift (Moore & Fisher 1969).

In the context of invasions, effect of competition and predation can be important because they can be impacts of invasive species on native species, and because these processes may act as barriers to invasion (Juliano & Lounibos 2005). In general, the likelihood that exotic species will colonize new regions depends on their capacity to adapt to the new environmental conditions and to compete with the pre-existing species (Carrieri et al. 2003).

Competition among the members of the same species is called 'intraspecific competition', and between individuals belonging to different species is known as 'interspecific competition'. There are many researchers reporting on the interspecific competition between *Aedes aegypti* and *Aedes albopictus*, and yet the results are varied.

Mixed rearing of *Aedes albopictus* with *Aedes aegypti* delayed the development of *Aedes albopictus* larvae. Development of *Aedes albopictus* larvae was slower than that of *Aedes aegypti* larvae under various experimental conditions (Lee 1994, Moore & Fisher 1969). Laboratory experiments on competition between *Aedes aegypti* and *Aedes albopictus* using different detritus resources (e.g. liver powder, yeast, dead insects) have tended to yield approximate competitive equality, or even an advantage for *Aedes aegypti* (Barrera 1996, Daugherty et al. 2000).

In Illinois State, *Aedes albopictus* showed significantly greater survivorship compared with *Aedes aegypti* because *Aedes albopictus* is more superior in resource-harvesting ability (Yee et al. 2004). Another study in Florida showed that *Aedes albopictus* had significantly higher metamorphic success and eggs hatched more rapidly than *Aedes triseriatus* (Lounibos et al. 2001). Also, it was more successful than *Aedes triseriatus* in survival to emergence in the presence of the predatory larvae of the native mosquito *Toxorhynchites rutilus*

Under laboratory conditions at 25°C, Carrieri et al. (2003) observed that *Aedes albopictus* is more successful than *Culex. pipiens* in exploiting artificial microhabitats when food is scarce, because it has greater efficiency in converting food into biomass and rapidity in larval development. However, food-biomass conversion coefficient dropped significantly when the temperature falls below 25°C. Alto & Juliano (2001) also proved that temperature could influence the larval trophic activity.

2.4. Ovitrap

Measurement of *Aedes* populations by adult resting and biting collections is productive only in areas where this species is very abundant. Larval surveys are effective only when rainfall or other moisture is sufficient to flood eggs previously deposited in artificial containers (Hoffman & Killingsworth 1967). Fay & Perry (1965) were the first to use ovitraps for *Aedes aegypti* surveillance, and Fay & Eliason (1966) demonstrated the ovitrap was, in some aspects, superior to larval surveys. Ovitraps were also shown to be useful sampling devices in determining *Aedes aegypti* distribution (Hoffman & Killingworth 1967), seasonal population fluctuation (Jakob & Bevier 1969) and in evaluating the efficacy of aerial ULV malathion application (Kilpatrick et al. 1970). It can be used to estimate fairly well the population of adult mosquitoes in the environment by counting the number of eggs laid on the moist paddle (Ginny Tan & Song 2000). Changes in the species breeding (*Aedes aegypti* to *Aedes albopictus* or vice versa) can also be detected. The first report on the colour preference for oviposition by *Aedes albopictus* was by Yap (1975b). He indicated that *Aedes albopictus* females preferred red and black colour for oviposition in ovitraps.

The exposure duration of the ovitrap is important. Predation from snails and roaches occur when the exposure period is long. However, when the duration is short, most probably the information is not productive enough. Although paddles exposed to a 7 day interval may experience more predation, relative information will be produced as long as the sampling intervals are the same (Kloter et al. 1983). An over-riding consideration is that *Aedes aegypti* ovitrap surveillance on a weekly interval is operationally feasible, whereas a shorter interval is costly and twice as labour intensive.

2.5. Seasonal Abundance of *Aedes* Mosquitoes

In Malaysia, the variation in *Aedes albopictus* larval populations and high densities of biting mosquitoes in various months was related to the rainfall (Abu Hassan et al. 1996). As an example, the high larval population and high densities of biting mosquitoes observed in November 1990 was possibly due to the increase in rainfall in September and October 1990. Deposition of *Aedes aegypti* eggs decreased during periods of no rainfall but sharply increased 2 weeks after significant rainfall in Texas (Hoffman & Killingsworth 1967). In Victoria peak abundance of adult *Aedes sagax* (Skuse) typically followed floods or heavy rains in winter, spring and autumn (Russell 1986).

In Buenos Aires, Argentina, the presence of *Ochlerotatus albifasciatus* was positively related to the amount of rain ($p<0.001$), whereas negatively correlated to air temperature ($p<0.05$) within a 5.2 to 29.7°C range because high temperature is unfavourable to larval development (Fischer et al. 2002). The year-round presence of immature stages of *Ochlerotatus albifasciatus* indicates that the climatic conditions of the city of Buenos Aires are not limiting to the development of this species. And thus, differences in habitat availability may be partially responsible for the detected seasonal fluctuations of mosquito.

Rainfall always provide breeding habitat for mosquitoes. Zyzak et al. (2002) stated that as rainfall-produced surface pools accumulated, an accompanying expansion of oviposition sites occurred resulting in a sustained, elevated *Culex nigripalpus* population in Florida. The extension of dry season and increment of temperature results the reduction of adult mosquito.

2.6. Relationship between Incidence of Dengue Cases and Dengue Vectors

Pattern of dengue transmission are influenced by the abundance, survival, and behaviour of the principal mosquito vector, *Aedes aegypti*; the level of immunity to the circulating virus serotype in the local human population; density, distribution and movement of humans; and time required for development of virus in *Aedes aegypti* (Halstead 1990). Abundant vector populations are often a prerequisite for epidemic and epizootic transmission of arboviruses. The *Aedes aegypti* densities were correspondence to dengue fever incidence (Moore et al. 1978). In Puerto Rico, from 1973-1975, dengue transmission increased when average Breteau indices rose above 20 (Moore et al. 1978). The result was the same as Foo et al. (1985), in Selangor. In year 1966, in Singapore, there was a general correspondence between the epidemic curve and the population fluctuations of *Aedes aegypti* and *Aedes albopictus* (Chan et al. 1971b). The simultaneous rise in the populations of these mosquitoes in February-March was followed by an increase in the incidence of cases, which continued to rise sharply in the subsequent months (Chan et al. 1971b). The slightly fall in the incidence of the cases from July-October was associated with the fall in the populations of the mosquitoes. Another study also suggests that the presence of *Aedes albopictus* in or around household premises was associated with an increased risk of dengue transmission in Dhaka, India (Ali et al. 2003).

2.7. Relationship between Incidence of Dengue Cases and Physical Parameters

Some studies showed that there is an association between rainfall and dengue outbreak. In Selangor, Malaysia, a monthly rainfall of 300mm or more would cause a major dengue outbreak in the state after a lag period of about two to three months (Foo et al. 1985). Abundance of *Aedes albopictus* was significantly correlated with rainfall four weeks before collection dates using ovitraps (Lourenco-de-Oliveira et al. 2004). In Malaysia, the epidemics of dengue from 1973-1982 were related to the two monsoons: SW Monsoon in the first half, and NE Monsoon in the second half of the year (Lo & Narimah 1984). Endemicity is low during the period from January-April, when it begins to rise, reaching a peak in July/August, and then declines. This variation is related to the storage of water during the drought season from January-April, and the drizzling rainfall before the heavy monsoons, which all create a suitable breeding places for the vector (Lo & Narimah 1984). There are some other studies which reported the use of rainfall data to predict malaria epidemics. Mouchet et al. (1998) pointed out that temperature and rainfall influence malaria transmission; the former determines the length of the larval mosquito cycle and the sporogonic cycle of the parasite in the mosquito; the latter determines the number and productivity of breeding sites and consequently the vector density. Rainfall in highland areas has been associated with entomologic transmission parameters (Lindblade et al. 1999) and malaria incidence (Fontaine et al. 1961, Mouchet et al. 1998), and it has been suggested that monitoring rainfall levels could provide sufficiently early warning of highland malaria epidemics. However, elevated temperatures may interact with rainfall to create epidemic conditions (Lindblade et al. 1999), and this interaction must be investigated further before rainfall alone can be used as a suitable indicator of epidemic risk.

2.8. Presence-Absence Sampling

Programmes covering integrated pest management in agriculture often estimate the population of a phytophagous insect by means of binomial or presence-absence sampling. This type of sampling allows estimation of the average population density through the frequency of the positive sampling units, consequently reducing required operating time and cost (Bellini et al. 1996). Binomial or presence-absence sampling is often useful for estimating population density because the only information needed from a sampling unit is whether or not the organism is absent (Schaalje et al. 1991). Binomial sampling requires a statistically estimated relationship between the proportion of positive sample units (p) and mean population density (m).

Generally, there are two assumptions for estimating egg density by presence-absence sampling, negative binomial distribution of insect counts per sampling unit (Mogi et al. 1990, Mackey & Hoy 1978) and no particular distribution pattern (Mogi et al. 1990, Gerrard & Chiang 1970). Generally, negative binomial distribution is used to describe aggregated distribution. This distribution is described by two parameters, the mean (*m*) and the parameter of the negative binomial (*k*), which is a measure of the amount of clumping (Bliss & Owen 1958). *Culex tarsalis* (Mackey & Hoy 1978), redbacked cutworm (Danielson & Berry 1978), cereal aphids (Ba-Angood & Stewart 1980), and *Psorophora columbiae* (McLaughlin et al. 1987) can fit into the negative binomial distribution with a common *k*. Value of *k* is a factor of negative binomial distribution, it will define the shape of the negative binomial distribution and it is also acts as general indicator of aggregation. However, Mogi et al. (1990) found that when estimation of *Aedes* was done using negative binomial model, a common *k* value cannot be justified.

Most of the studies on estimation of *Aedes* density can be done using a model created by Gerrard & Chiang (1970) with an assumption of no particular distribution pattern. Gerrard & Chiang (1970) model relates the natural log of mean (*m*) and natural log of positive samples (*p*) using linear regression (the model was shown in page 25, formula 1). In Malaysia, the Gerrard & Chiang (1970) model was used in ovitrap surveillance of *Aedes* mosquito (Lee 1992a, Lee & Chang 1997), larval surveillance (Lee 1996), and adult surveillance (Lee & Inder Singh 1993). In Italy, Bellini et al. (1996) also concluded that Gerrard & Chiang (1970) model can be used to estimate *Aedes albopictus* in plastic, glass, and metal ovitraps. And, the Gerrard & Chiang (1970) model can be used in estimate *Aedes* density in Thailand (Mogi et al. 1990).

2.9. Sequential Sampling

Sequential sampling allows one to stop sampling as soon as required information has been obtained with predetermined reliability (Mogi et al. 1990). Classification of insect density into broad levels of infestation using this method makes sequential sampling suitable for pest management (Danielson & Berry 1978). For example, when the number of positive samples is below p_1 (predetermined level), no control is needed. When the number of positive samples is over p_2 (predetermined level), control is needed.

A preliminary surveillance must be conducted extensively to determine the level where no control is needed and a level where control is needed. Many researchers have already used this technique in the management of some species of cereal aphids on barley in Southwestern Quebec (Ba-Angood & Stewart 1980) and redbacked cutworm in central Oregon (Danielson & Berry 1978). Subsequently, this technique was applied to dipper counts of rice field mosquitoes (Mackey & Hoy 1978; McLaughlin et al. 1987; Wada et al. 1971).

Mogi et al. (1990) showed the potential of these techniques in the surveillance of *Aedes* vectors in Thailand using ovitraps. In Malaysia, sequential sampling was used in ovitrap surveillance of *Aedes* mosquito (Lee 1992a, Lee & Chang 1997), larval surveillance (Lee 1996), and adult surveillance (Lee & Inder Singh 1993) to determine the transmission threshold or p_2 of dengue epidemic.