CROSS -MATING BETWEEN MALAYSIAN STRAINS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN THE LABORATORY

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Abstract. Reciprocal and homologous mating experiments between Malaysian *Aedes aegypti* and *Aedes albopictus* mosquitoes were conducted in the laboratory. Two methods were employed, namely an artificial mating technique and a natural cage mating technique. The study demonstrated there exists a strong reproductive isolation between *Ae. aegypti* and *Ae. albopictus*. Insemination occurred in cross-mating experiments between *Ae. aegypti* females and *Ae. albopictus* males and also between *Ae. albopictus* females and *Ae. aegypti* males. Cross mating between *Ae. aegypti* females and *Ae. albopictus* males produced more eggs than that between *Ae. albopictus* females and *Ae. aegypti* males with both artificial mating and natural cage mating techniques. The matings did not result in the production of viable eggs by the females. No embryonation of these eggs was observed when the eggs were bleached. With homologous mating *Aedes aegypti* produced significantly greater numbers of eggs compared to *Aedes albopictus* mosquitoes, and all the eggs hatched successfully.

INTRODUCTION

The dengue vectors *Aedes aegypti* and *Ae. albopictus* (Rudnick and Lim, 1986) are widely distributed in Malaysia. Dengue fever in Southeast Asia was first recorded in the 19th century, and in Malaysia it was first reported by Skae in 1902. Malaysia is a tropical country which has abounded with these mosquito species since their first appearance in this region. *Ae. albopictus* (Skuse) and *Ae. aegypti* (Linnaeus) are sympatric species that occupy similar ecological niches (Klowden, 1993). In Malaysia both species breed indoors and outdoors in the same habitats and have been found to coexist in the same container (Chen *et al*, 2006). There seems to be no evidence of displacement or competition between these two species as both are abundant in urban as well as rural areas, unlike in the United States where the introduction of *Ae. albopictus* resulted in a decline and virtual disappearance of *Aedes aegypti* (Black *et al*, 1989). Theoretically, interspecific mating may result in the displacement of a species (Nasci *et al*, 1989). This may have happened between *Ae. aegypti* and *Ae. albopictus*. As early as 1913 MacGilchrist reported interspecific mating between *Ae. aegypti* and *Ae. albopictus*, but the matings were unproductive. Numerous studies have been conducted to verify the theory of interspecific mating resulting in viable and nonviable offspring (Toumanoff, 1937; 1950; Huang-Tieh-Try, 1939; Downs and Baker, 1949; Leahy and Craig, 1967; Nasci *et al*, 1989;

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Harper and Paulson, 1994). However, only one study has been conducted in Malaysia by Thomas and Yap in 1973. In this paper we describe the reciprocal and homologous matings between Malaysian *Ae. aegypti* and *Ae. albopictus* by artificial mating and cage incaptivated matings.

MATERIALS AND METHODS

Strain description

Ae. aegypti has been bred in the laboratory for more than 30 years and the generation used in this study was F985. The generation used for *Ae. albopictus* was F26. Both the strains were bred in an isolated room in the insectary.

Rearing methods

All mosquitoes were reared in an insectary maintained at a temperature of 26ºC with 80% relative humidity and 12:12 hour photoperiod. Larvae of both species were fed using liver powder during earlier instars and with pieces of partially cooked cow liver during the 3rd instar stage. The adults of both strains were fed with 10% sugar solution supplemented with vitamin B complex. During the pupal stage, size was used as the distinguishing factor between male and female pupae. The pupae were individually collected and secured in a glass vial with 1 ml of water. Pupae were kept individually in vials to ensure virginity in newly emerged adults. After emergence the adults were placed in different cages for females and males. Before conducting any experiments, the adults were identified carefully to ensure that no males were present in the female cages. If a male was present in the female cage, all the mosquitoes in that cage were discarded. The female mosquitoes were blood fed on white mice prior to commencement of the experiments.

Artificial or forced mating

For this experiment 5-day old females and males were used. The crosses for the reciprocal as well as the homologous controls were carried out using an artificial mating technique as described by Ow Yang *et al* (1963). Ten virgin male mosquitoes were placed in a flask that contained anesthetic ether. The mosquitoes were then removed and laid on a filter paper. The thorax of the male mosquitoes was impaled with the tip of a rod before artificial mating was conducted. While waiting for the male to regain consciousness, 10 virgin females were also anesthetized and placed on a white sheet of paper to conduct the artificial mating. The genitalia of the males and females were brought together at an appropriate angle for genital contact. When the males and females were in the process of copulation, they were allowed to remain so for a few minutes until the two sets of genitalia separated naturally. After artificial mating, individual females were introduced singly into a 0.028 m^3 (1 cubic foot) screened cage with a nylon stockenette sleeve that provided access to the interior of the cage. A solution of 10% sucrose and vitamin B complex was provided as a maintenance diet. The blood fed female mosquitoes were allowed to oviposit after 3 days by placing a damp cone shape filter paper inside a white bowl with a minimum amount of water. They were allowed to oviposit for a period of 5 days. The experiment was repeated with 10 individual females and 10 individual males for all the combination crosses. Eggs oviposited by individual females were counted under a dissecting microscope. The eggs were kept secured to ensure complete embryonation. The filter paper was then immersed in dechlorinated water and the eggs were observed for hatching for a period of 10 days. Eggs that did not hatch were then bleached according to the procedure of Trpis (1970). The females were dissected and the spermathecae examined for the presence of sperm.

Natural case mating experiment

In this experiment thirty 4-5 day old females and forty males were placed in each of 4 breeding cages with four possible combinations consisting of 2 with reciprocal cross combination and 2 with the homologous control populations. The mosquitoes were introduced into a 0.028 m^3 (1 cubic foot) screened cage with a nylon stockenette sleeve that provided access to the interior of the cage. A solution of 10% sucrose with vitamin B complex was provided as a maintenance diet. The mosquitoes were allowed to copulate naturally in the 4 different combination cages for a time period of 2 days. The female mosquitoes were allowed to ovulate for a period of 5 days on a damp cone shaped filter paper placed inside a white bowl with a minimum amount of water. The filter paper with the eggs was removed and the eggs were counted under a dissecting microscope. The eggs were kept secured to ensure complete embryonation. The filter paper was then immersed in water and the eggs were observed for hatching for a period of 10 days. Eggs that did not hatch were then bleached according to the procedure of Trpis (1970). The females were dissected and the spermathecae examined for the presence of sperm.

RESULTS

Artificial mating

The results of artificial mating of the reciprocal crosses and the homologous control crosses are presented in Table 1. From the reciprocal cross between *Ae. aegypti* females and *Ae. albopictus* males, only 2 females (20%) laid eggs from the 10 females

Table 1
Comparison of egg production and viability between reciprocal and homologous crosses by artificial and natural cage mating.

Cross Female x Male	No. of replicates	Mean no. ± SD of eggs	Percentage hatched
Artificial mating[a]			
Reciprocal mating			
Ae. aegypti x *Ae. albopictus*	10	81.50 ± 14.50	0
Ae. albopictus x *Ae. aegypti*	10	26.00 ± 0.00	0
Homologous mating			
Ae. aegypti x *Ae. aegypti*	10	121 ± 3.61	100
Ae. albopictus x *Ae. albopictus*	10	14.0 ± 1.14	100
Natural cage mating[b]			
Reciprocal mating			
Ae. aegypti x *Ae. albopictus*	2	271.50 ± 21.50	0
Ae. albopictus x *Ae. aegypti*	2	123.50 ± 6.50	0
Homologous mating			
Ae. aegypti x *Ae. aegypti*	2	1,984 ± 6.00	100
Ae. albopictus x *Ae. albopictus*	2	201 ± 4.00	100

[a] *t*-test; reciprocal mating: $p = 0.001$; homologous mating: $p = 0.000$.
[b] *t*-test; reciprocal mating: $p = 0.022$; homologous mating: $p = 0.000$.

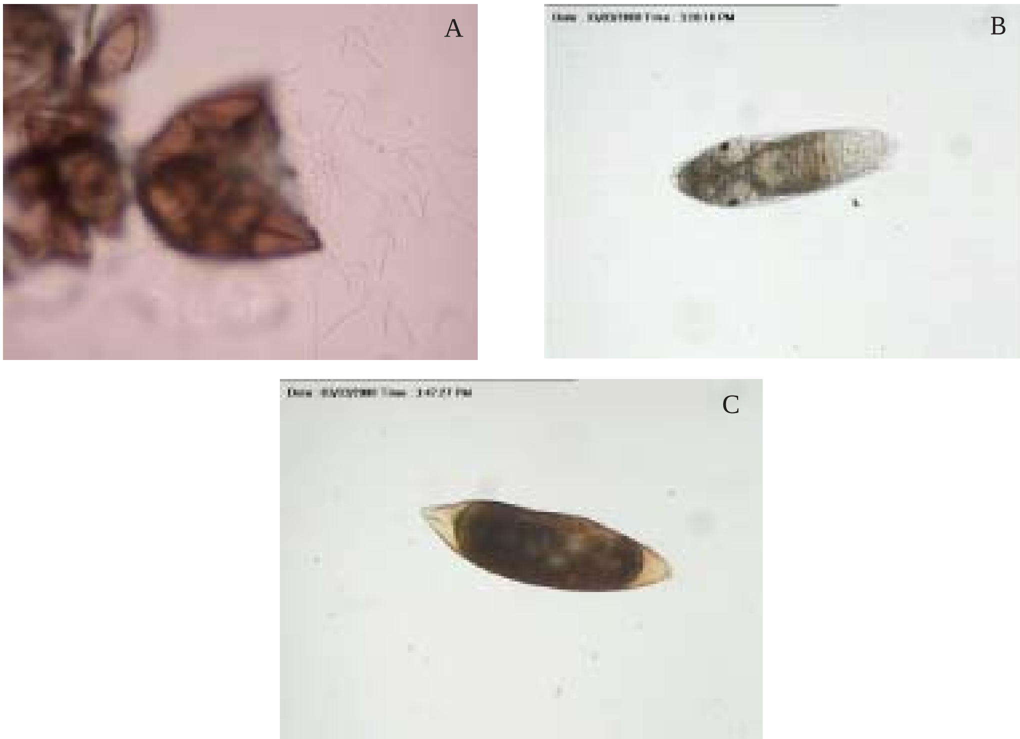

Fig1–(A) The presence of spermatozoa in a mated *Aedes aegypti* female from reciprocal mating (*Ae. aegypti* female x *Ae. albopictus* male). (B) A normal egg with developing embryo from a homologous mating in *Ae. aegypti*. (C) A non-viable egg from reciprocal mating between an *Ae. aegypti* female and an *Ae. albopictus* male. Note the egg yolk mass and the absence of cell differentiation**.**

tested. The mean number of eggs laid by both these females was 81.5 ± 14.5 eggs and none of these eggs hatched, as shown in Table 1. In the reciprocal cross between *Ae. albopictus* females and *Ae. aegypti* males only 1 female (10%) laid 26 ± 0.0 eggs and none of these eggs hatched. Our results show that there was cross insemination in the reciprocal cross between *Ae. aegypti* and *Ae. albopictus* females since the mating pairs appeared to be in perfect copulatory position for transfer of sperm. The females that laid eggs were dissected and sperm were present in the spermathecae (Fig 1A). The eggs from this mating were bleached and the embryonation of homologous matings and reciprocal matings are shown in Fig 1B and Fig 1C.

In the homologous mating all the females of *Ae. aegypti* and *Ae. albopictus* laid eggs and all of these eggs were fertile. The mean numbers of eggs laid by *Ae. aegypti* and *Ae. albopictus* homologous mating were 121 ± 3.61 and 14.0 ± 1.14, respectively. In crosses where *Ae. aegypti* females were used higher numbers of eggs were laid compared to the other reciprocal crosses. It was also noted that single *Ae. aegypti* females produced significantly (*t*-test, *p*-value = 0.00) higher numbers of eggs compared to single *Ae. albopictus* females at a ratio of 8.6:1. It is worth noting that the single female *Ae. albopictus* mosquito in the artificial reciprocal cross mating laid 26 eggs compared to the *Ae. albopictus* in the homologous mating which laid 14.0 ± 1.14.

Natural case mating experiment

The results of the 30 females and 40 females in the natural mating experiments are presented in Table 2. The results show that in the reciprocal crosses beteween *Ae. aegypti* females and *Ae. albopictus* males, 271.5 ± 21.50 eggs were laid and none of these eggs hatched. The cross in the other direction between *Ae. albopictus* females and *Ae. aegypti* males resulted in the female laying a mean number of 123.5 ± 6.50 eggs. The difference between the number of eggs laid was significant (*t*-test, *p*-value was 0.02). In both the crosses, the eggs laid were not fertile. The spermathecae showed the presence of sperm indicating the reciprocal pairs had mated. The homologous natural mating of *Ae. aegypti* females and of *Ae. albopictus* females resulted in 1,984 ± 6.00 and 201 ± 4.00 eggs laid, respectively. The difference between the number of eggs laid in these crosses was highly significant (*t*-test, *p*-value was 0.00). All the eggs that were laid by the homologous pairs were viable and hatched 100% successfully. Eggs that did not hatch from the reciprocal crosses were bleached and observed under light microscope. These eggs were in the form of a mass and there were no cells undergoing further divisions indicating absence of embryonation. Only fertilization occurred with embryonation and eggs shell formation (Fig 1B). Embryonation formation in reciprocal matings did not take place and hence the eggs were not viable (Fig 1C).

DISCUSSION

An older study conducted in Malaysia by Thomas and Yap (1973) showed that in mass mating experiments, a number of females from both reciprocal crosses produced viable eggs and the adults resembled their female parents. Careful examination by them indicated that there was accidental contamination of the *Ae. aegypti* and *Ae. albopictus*. In their single female to single male reciprocal cross experiments, both crosses laid few eggs and none of the eggs were fertile. Mating between *Ae. aegypti* females and *Ae. albopictus* males produced larger numbers of eggs compared to matings between *Ae. albopictus* females and *Ae. aegypti* males. The ratio of egg production between *Ae. aegypti* females and *Ae. albopictus* females in natural mating was 10:1. This has been observed with artificial mating as well. Our findings were similar to those of Nasci *et al* (1989) who reported *Ae. albopictus* males mated equally well with females of both species though they mated more quickly with conspecific females. This observation is also in line with the earlier findings of Thomas and Yap (1973). Our study supports the findings of Harper and Paulson (1994) where cross matings in the Florida strains of *Ae. aegypti* and *Ae. albopictus* did not produce viable eggs. In fact there are few reports of viable offspring produced from these crosses (Downs and Baker, 1949; Toumanoff, 1950), but most crosses in other studies produced no offspring and there appeared to be numerous barriers to the production of viable offspring by these crosses (Leahy and Craig, 1967).

Studies conducted in Hawaii (Bonnet, 1950) showed that crosses between *Ae. aegypti* females and *Ae. albopictus* males gave rise to progeny which appeared exactly like *Ae. aegypti*. Crosses between *Ae. albopictus* females and *Ae. aegypti* males did not produce any fertile eggs. The author gave no explanation for this difference. Gubler (1970) conducted cross-mating studies between *Ae. albopictus* and *Ae. polynesiensis* and found that only eggs produced by females mated with homologous males were fertile. The *Ae. polynesiensis* females who mated with *Ae. albopictus* males laid a considerably larger number of eggs, but none were viable. However, mating between *Ae. albopictus* females and *Ae. polynesiensis* males produced no eggs. Ali and Rozeboom (1971) found that mating between *Ae. albopictus* females and *Ae. polynesiensis* males did not produce any eggs, but eggs were produced in the vice-versa cross mating. Gubler (1970) also found that once a *Ae. polynesiensis* females was mated with a *Ae. albopictus* male, subsequent copulations with her own males did not contribute to the sperm pool retained in the spermathecae and the eggs produced were sterile. He found that *Ae. albopictus* male were effective in sterilizing *Ae. polynesiensis* females. This is in line with a study by Craig (1966). This may be an important factor in the dramatic displacement of *Ae. aegypti* that has been observed since the introduction of *Ae. albopictus* into the southern USA (Nasci *et al*, 1989). In Malaysia, both these vector species share nearly the same habitat, especially in urban areas, although in rural areas *Ae. albopictus* is more prevalent than *Ae. aegypti*. Our study shows that cross-mating between *Ae. aegypti* and *Ae. albopictus* is unlikely, and neither species can displace the other.

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