

LABORATORY COLONIZATION OF *Aedes lineatopennis*

Atchariya Jitpakdi¹, Anuluck Junkum¹, Benjawan Pitasawat¹, Narumon Komalamisra²,
Eumporn Rattanachanpichai¹, Udom Chaitong¹, Pongsri Tippawangkosol¹, Kom Sukontason¹,
Natee Puangmalee¹ and Wej Choochote¹

¹Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai;

²Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. *Aedes lineatopennis*, a species member of the subgenus *Neomelaniconion*, could be colonized for more than 10 successive generations from 30 egg batches [totally 2,075 (34-98) eggs] of wild-caught females. The oviposited eggs needed to be incubated in a moisture chamber for at least 7 days to complete embryonation and, following immersion in 0.25-2% hay-fermented water, 61-66% of them hatched after hatching stimulation. Larvae were easily reared in 0.25-1% hay-fermented water, with suspended powder of equal weight of wheat germ, dry yeast, and oatmeal provided as food. Larval development was complete after 4-6 days. The pupal stage lasted 3-4 days when nearly all pupae reached the adult stage (87-91%). The adults had to mate artificially, and 5-day-old males proved to be the best age for induced copulation. Three to five-day-old females, which were kept in a paper cup, were fed easily on blood from an anesthetized golden hamster that was placed on the top-screen. The average number of eggs per gravid female was 63.56 ± 22.93 (22-110). Unfed females and males, which were kept in a paper cup and fed on 5% multivitamin syrup solution, lived up to 43.17 ± 12.63 (9-69) and 15.90 ± 7.24 (2-39) days, respectively, in insectarium conditions of $27 \pm 2^\circ\text{C}$ and 70-80% relative humidity.

INTRODUCTION

The distribution of *Aedes* (*Neomelaniconion*) *lineatopennis* (Ludlow) is in Africa and has also extended to Southeast Asia and Australia (Tanaka *et al.*, 1979; Apiwathanasorn, 1986). In Thailand, *Ae. lineatopennis* is distributed throughout the country (Miyagi *et al.*, 1986). This mosquito species is of medical importance and has been incriminated as a potential vector of West Nile (WN) virus in Pakistan (Akhter *et al.*, 1982), epizootic Rift Valley fever (RVF) virus in Kenya (Linthicum *et al.*, 1985), a suspected vector of Japanese encephalitis (JE) virus in the Malaysian peninsular (Vythilingam *et al.*, 1997), and an efficient laboratory vector of the dog heartworm, *Dirofilaria immitis* (Leidy), in Thailand (Tippawangkosol *et al.*, 1998).

Very few research experiments concerning *Ae. lineatopennis* have been conducted, even though this mosquito species is found throughout Thailand and other regions and has been reported as a vicious biter that feeds on humans and animals (Miyagi *et al.*, 1986; Tuetun *et al.*, 2004). This might result from the lack of biological information and/or available laboratory-raised colonies. Until now, *Ae. lineatopennis*, which is indigenous in Thailand, has not been colonized under

laboratory conditions; hence, this study attempted to colonize this mosquito species.

MATERIALS AND METHODS

Wild-caught *Aedes lineatopennis*

Female *Aedes lineatopennis* mosquitoes were collected from a field in San Kamphaeng District, Chiang Mai Province, northern Thailand. The engorged adult females were collected by using a cow-baited trap and kept in paper cups with a pad of cotton wool soaked with water placed on top of a screen. They were stored in a humid chamber by using a picnic cooler to maintain humidity and temperature. Then they were transported to the insectarium of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai Province, Thailand, for colonization and biological studies. All of the experiments were performed in the insectarium at $27 \pm 2^\circ\text{C}$, 70-80% relative humidity, and illuminated with a combination of natural day light from glass-window and fluorescent lighting for approximately 12 hours a day. Identification of specimens was based on the keys of all stages of Tanaka *et al.* (1979).

Searching for a suitable pre-hatch condition

The engorged adult females were reared with 5% multivitamin syrup solution for 4-5 days until gravid. Each gravid female was placed in a small paper cup (13 cm in diameter and 6 cm in depth) containing 30 ml of natural water (brought from a basin that was used for tap-water production) with wet filter paper

Correspondence: Wej Choochote, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail: wchoocho@mail.med.cmu.ac.th

lining the inner side. The eggs that were attached to the filter paper or sunk in the water were counted and collected. Since the biology of the embryonation period and conditions required for *Ae. lineatopennis* egg hatching have never been studied before, the eggs were treated in two conditions, *ie*, air-dried and kept in a moisture chamber from one to 10 days. The chamber was made moist by placing a de-ionized water-soaked Whatman No.1 paper filter (8.5 cm in diameter, Whatman, Hillsboro, OR) over a 0.5 cm thick piece of de-ionized water-soaked cotton wool in a 9-cm-diameter Petri dish covered by a glass plate lid. Eggs in both conditions were processed for three experiments to search for a suitable pre-hatch condition. Firstly, the air-dried and moisture-incubated eggs ranged in age from 1 to 10 days and were inundated in natural and 1% hay-fermented water (prepared by inundation of 10 g of dried hay in one liter of natural water for 24 hours), respectively. Twenty-four hours after the period of inundation, the hatched larvae were counted and 30 eggs in each group were dissected under dissecting microscope to examine the larval development and/or embryonation rates. Secondly, the eggs obtained from the highest yield of embryonation and hatchability from the first experiment were immersed in 0.25, 0.5, 1 and 2% of hay-fermented water. After 24 hours of immersion the embryonation and hatchability were investigated. Thirdly, the newly oviposited eggs were submersed in natural and 1% hay-fermented water for 10 consecutive days. The hatched larvae were counted during a 10-day-period of submergence and the eggs were dissected for embryonation after a 10-day-period of observation.

Searching for a suitable medium for larva rearing

The above methods that yielded the highest embryonation and hatching rates and the hatched, first stage larvae obtained from the best condition were then used for rearing in natural and 0.25, 0.5, 1 and 2% hay-fermented water. The larvae that hatched within 24 hours were transferred to a white plastic tray (25 x 36 x 6 cm) containing 2,000 ml of rearing medium, as mentioned before, and 100 larvae were placed in each tray containing different media. Approximately 0.5 g powder of equal weight of wheat germ, dry yeast and oatmeal suspended in 5 ml of de-ionized water were added every other day as larval food. After pupation, the pupae were removed from the rearing trays each day and transferred to containers of de-ionized water in an emergence cage (30 x 30 x 30 cm) until they emerged as adults. The numbers of pupae, adult females and males were recorded. The adult females and males were provided with 5% multivitamin-syrup solution until use.

Biological study

The adults obtained from the highest yield of the above experiments were used in the study of biology – *ie* feeding ability, ability to mate freely in a 30 x 30 x 30 cm cage, male ability to mate artificially, and adult longevity.

Feeding ability. The experiment was designed to determine the optimal age of female mosquitoes for feeding. The feeding ability of females at different ages ranging from one to 10 days old was examined in the 30 x 30 x 30 cm cage and a paper cup (8.5 cm in diameter and 11 cm in depth). The feeding duration was 19:00-20:00 hours, compatible to the nocturnal feeding habit of *Ae. lineatopennis* collected from the field (Tuetun *et al*, 2004). For feeding in the 30 x 30 x 30 cm cage, each of 30 females at various ages, having fasted for 12-16 hours, were kept in the cage and allowed to feed on blood from an anesthetized golden hamster [anesthetized by intra-peritoneal injection of 0.1 ml Nembutal® (pentobarbital sodium 60 mg/ml) per 100 g body weight] that was put in the cage for 30 minutes. For feeding in the paper cup, each of 30 females at various ages, having fasted for 12-16 hours, were kept in the cup covered with nylon mesh at the top and then allowed to feed on blood from an anesthetized golden hamster that was laid on the nylon mesh for 30 minutes (Fig 1). The number of fed females in both experiments was examined under a dissecting microscope and recorded. A total of 300 females was used in each experiment.

Ability of free mating in a 30 x 30 x 30 cm cage. The experiment was designed to determine the degree of adaptive stenogamy in a 30 x 30 x 30 cm cage. One hundred and fifty and 300 newly emerged females and males, respectively, were transferred into a 30 cm cubed cage and co-habitated for one week. Five percent of multivitamin-syrup solution was provided as adult nutrients. Subsequently, the spermathecae of 100 females were examined for evidence of insemination.

Male ability to mate artificially. The mating ability of males at different ages ranging from 1 to 10 days old, with 5-day-old females was investigated by using the methods of Baker *et al* (1962) and Ow-Yang *et al* (1963). Thirty males of each age were used, with a total of 300 males used in this experiment. The mated females were reared for 24 hours before being dissected and examined for sperm in the spermathecae.

Establishment of the laboratory-colony strain

Suitable conditions from all of the above experiments were selected and used for rearing *Ae. lineatopennis* for at least 10 consecutive generations. The biological aspects of the laboratory-colony strain



Fig 1- Showing forced blood-feeding of *Ae. lineatopennis* on an anesthetized golden hamster.

Table 1

Embryonation and hatchability of 1- to 10 -day-old moisture-incubated eggs of *Ae. lineatopennis* after submersion in natural and 1% hay-fermented water, with all eggs dissected 24 hours after inundation.

Moisture-incubated eggs			
Natural water		Hay-fermented water	
Embryonation ^a rate (No.)	Hatchability ^b rate (No.)	Embryonation ^a rate (No.)	Hatchability ^b rate (No.)
23.33 (7)	0 (0)	20.00 (6)	0 (0)
40.00 (12)	0 (0)	50.00 (15)	0 (0)
80.00 (24)	0 (0)	76.67 (23)	1 (1)
93.33 (28)	0 (0)	83.33 (25)	7 (7)
80.00 (24)	1 (1)	93.33 (28)	6 (6)
83.33 (25)	0 (0)	86.67 (26)	13 (13)
86.67 (26)	12 (12)	86.67 (26)	68 (68)
76.67 (23)	14 (14)	93.33 (28)	62 (62)
73.33 (22)	11 (11)	80.00 (24)	64 (64)
96.67 (29)	16 (16)	83.33 (25)	51 (51)

^aThirty eggs for each age

^bOne hundred eggs for each age

of *Ae. lineatopennis* – ie average eggs deposited per gravid female, embryonation rate, hatchability rate, egg-, larva-, pupa-durations, adult longevity and sex ratio – were recorded on the 1st, 3rd and 8th generations. For longevity, 30 of each newly emerged females and males were kept separately in a small paper cup. Cotton wool pads soaked with 5% multivitamin syrup solution were provided regularly and changed every other day until all of the adult females and males died. Both cups were checked daily and the dead mosquitoes were counted and removed until all of the mosquitoes had died.

RESULTS

The embryonation and hatchability of 1- to 10-day old moisture-incubated eggs of *Ae. lineatopennis*, after being submerged in natural and 1% hay-fermented water for 24 hours, are illustrated in Table 1. The embryonation of 1- to 10-day old air-dried eggs was 0% (data not shown), whereas in 1- to 10-day old moisture-incubated eggs, 23.33-96.67% and 20.00-93.33% embryonated from natural and 1% hay-fermented water, respectively. The hatchability of 1- to 10-day old air-dried eggs was also 0%, while in the

1- to 10-day old moisture-incubated eggs, 0-16% and 0-68% hatched from natural and 1% hay-fermented water, respectively. The hatchability rates of 1-to 6-day old moisture-incubated eggs were very low and erratic from both natural (0-1%) and 1% hay-fermented water (0-13%). The rates increased markedly for 7- to 10-day old eggs, from which 12-16% and 51-68% were obtained from natural and 1% hay-fermented water, respectively. Statistical analysis of the hatchability rates for 7- to 10-day old moisture-incubated eggs, which were inundated in natural and 1% hay-fermented water, demonstrated that the hatchability rates from the latter medium were significantly higher than those from the former medium – ie eggs aged 7 days: natural water/1% hay-fermented water = 12%/68% ($\chi^2 = 65.33$, $p < 0.05$), eggs aged 8 days: natural water/1% hay-fermented water = 14%/62% ($\chi^2 = 48.90$, $p < 0.05$), eggs aged 9 days: natural water/1% hay-fermented water = 11%/64% ($\chi^2 = 59.93$, $p < 0.05$), and eggs aged 10 days: natural water/1% hay-fermented water = 16%/51% ($\chi^2 = 27.49$, $p < 0.05$).

Comparative studies on the hatchability rates of 7-day old moisture-incubated eggs in various concentrations of hay-fermented water provided no statistically significant differences – ie 0.25% (65%); 0.5% (61%); 1% (64%); 2% (66%) and 4% (59%) ($\chi^2 = 1.46$, $p > 0.05$) (Table 2).

The results of newly oviposited eggs after being submerged in natural and 1% hay-fermented water for 10 consecutive days yielded very low embryonation [natural water = 23.34% (88/377), 1% hay-fermented water = 29.76% (106/356)] and hatchability [natural water = 0.27% (1/377), 1% hay-fermented water = 0.84% (3/356)] rates.

The comparison results of larva survival from larvae reared in natural water and various concentrations of hay-fermented water are shown in

Table 3. The pupation rate, emergence rate and adult female/male ratio of *Ae. lineatopennis* reared in natural water and 0.25%; 0.5%; 1%; and 2% hay-fermented water were 79%, 93.67% and 0.95; 87%, 94.25% and 1.05; 91%, 96.70% and 0.91; 89%, 100% and 0.98; 32%, 100% and 1.46, respectively. Comparative pupation rates among 0.25, 0.5 and 1% hay-fermented water manifested a statistically insignificant difference ($\chi^2 = 7.11$, $p > 0.05$).

Feeding ability results of females in the 30 x 30 x 30 cm cage and paper cup at different ages ranging from 1 to 10 days demonstrated that in the cage, adult females of all ages failed to feed on blood from an anesthetized golden hamster, whereas they succeeded in feeding in the paper cup. The results of feeding in the paper cup revealed that 1- to 2-day old females were poor blood-feeders, as a 0% feeding rate was obtained. The rates increased clearly in 3- to 5-day old females, with a maximal rate reached in 5-day old females. [3 days: 26.67% (8/30), 4 days: 36.67% (11/30), 5 days: 86.67% (26/30)]. At the age of 6 and 7 days, the feeding rates of females were still satisfactory – ie 66.67% (20/30) and 56.67% (17/30), respectively. Subsequently, the feeding rates decreased markedly when those of 9- and 10-day old females reached 13.33% (4/30) and 3.33% (1/30), respectively.

In order to determine the adaptive stenogamy of *Ae. lineatopennis*, the newly emerged females and males, at a ratio of 150:300, co-habitated in a 30 x 30 x 30 cm cage for one week. The results indicated that *Ae. lineatopennis* failed to mate freely in the cage at a 0% insemination rate (from experiments repeated three times, data not shown), which strongly suggested eurygamous behavior.

The artificial mating ability of *Ae. lineatopennis* is illustrated in Table 4. In this experiment, 30 male

Table 2

Comparative embryonation and hatchability of 7-day-old moisture-incubated eggs of *Ae. lineatopennis* after submersion in 0.25, 0.5, 1, and 2% hay-fermented water, with all eggs dissected 24 hours after inundation.

Experiments	Embryonation rate (No.) ^a	Hatchability rate (No.) ^b
Hay-fermented water		
0.25%	96.67 (29)	65.00 (65)
0.5%	80.00 (24)	61.00 (61)
1%	93.33 (28)	64.00 (64)
2%	83.33 (25)	66.00 (66)

^aThirty eggs for each experiment

^bOne hundred eggs for each experiment

Table 3
Comparative larva survival of *Ae. lineatopennis* after rearing in natural water and different concentrations of hay-fermented water.

Type of water ^a	No. pupation (%)	No. emergence (%)	No. females and males from total emergence (%)		Sex ratio Female/Male
			Female	Male	
Natural water	79 (79)	74 (93.67)	36	38	0.95
Hay-fermented water					
0.25%	87 (87)	82 (94.25)	42	40	1.05
0.5%	91 (91)	88 (96.70)	42	46	0.91
1%	89 (89)	89 (100)	44	45	0.98
2%	32 (32)	32 (100)	19	13	1.46

^aOne hundred larvae for each experiment

mosquitoes at different ages ranging from 1-10 days were used. In total, 300 male mosquitoes were investigated. The results indicated that 1-day old males could not artificially mate, with a 0% mating rate. The best age for males artificially mating was 5 days, with mating and insemination rates of 80.00% (24/30) and 87.50% (21/24), respectively. However, a satisfactory number of 2- to 6-day old males completed mating and the mating and insemination rates ranged from 40.00-53.33% and 58.33-87.50%, respectively.

According to the results of the above experiments, the procedures to establish a mass-colonization of laboratory-colony strain *Ae. lineatopennis* are as follows: The laboratory-colony strain of *Ae. lineatopennis* was established from a total of 2,075 (34-98) eggs from 30 oviposited females. The newly oviposited eggs were kept in a moisture chamber for 7 days and then inundated in 1% hay-fermented water (for easy preparation) for 24 hours. The hatched larvae were reared in 1% hay-fermented water until they developed to pupae and emerged as adults. The 5-day old adult females were allowed to feed on the blood of an anesthetized golden hamster that placed on a top-screen paper cup, and the fully-engorged females were mated artificially with 5-day old males. The mated, engorged females were reared with 5% multivitamin syrup until gravid, which took approximately 4-5 days. Subsequently, they were allowed to oviposit in a plastic tray (30 gravid females per 25 x 36 x 6 cm tray) containing 2,000 ml of natural water, with wet filter paper lining the inner side. By using this rearing method, the laboratory-colony strain could be successfully colonized for more than 10 generations.

The life duration of larvae, pupae, adult females and males of F₁, F₃ and F₈ of the laboratory-raised *Ae.*

Table 4
Artificial mating ability of *Ae. lineatopennis* males.

Day after emergence ^a	No. successfully mated females (%)	No. insemination (%)
1	0 (0)	-
2	12 (40.00)	7 (58.33)
3	14 (46.67)	12 (85.71)
4	15 (50.00)	13 (86.67)
5	24 (80.00)	21 (87.50)
6	16 (53.33)	14 (87.50)
7	10 (33.33)	9 (90.00)
8	6 (20.00)	2 (33.33)
9	2 (6.67)	1 (50.00)
10	3 (10.00)	2 (66.67)

^aThirty males for each experiment

lineatopennis is summarized in Table 5. The average eggs per deposited female (observation on 30 gravid females) were 62.33 ± 24.42 (22-103), 57.23 ± 19.82 (30-87) and 64.63 ± 21.81 (35-110) in F₁, F₃ and F₈, respectively. The eggs were subjected to incubation in a moisture chamber for 7 days. The larval stage lasted 4.38 ± 0.53, 4.46 ± 0.55 and 4.60 ± 0.51 days; the pupal stage 3.05 ± 0.39, 3.05 ± 0.22 and 3.08 ± 0.37 days; the adult female 45.53 ± 15.28, 44.37 ± 10.77 and 43.80 ± 12.11 days; and the adult male 16.43 ± 7.99, 14.63 ± 6.46 and 16.20 ± 7.62 days in F₁, F₃ and F₈, respectively.

DISCUSSION

In their natural environment, the eggs of *Aedes*

Table 5
Life duration of *Ae. lineatopennis* from the laboratory-raised colony.

Mosquito stages	Generations ^c		
	F ₁	F ₃	F ₈
Day of eggs subject to incubation in moisture	7	7	7
Larvae ^a	4.38 ± 0.53 (4-6)	4.46 ± 0.55 (3-5)	4.60 ± 0.51 (4-6)
Pupae ^a	3.05 ± 0.39 (2-4)	3.05 ± 0.22 (3-4)	3.08 ± 0.37 (2-4)
Adult females ^b	45.53 ± 15.28 (18-69)	44.37 ± 10.77 (11-63)	43.80 ± 12.11 (9-59)
Adult males ^b	16.43 ± 7.99 (2-33)	14.63 ± 6.46 (4-29)	16.20 ± 7.62 (7-39)

^aObservation from 100 larvae and pupae

^bObservation from 100 adults

^cMean duration in day, range in parenthesis

mosquitoes are deposited in breeding places subject to periodic inundation. Some species lay eggs in which the embryo develops quickly and then remains in a state of latency until flooding plus environmental stimuli that activate the pharate larva. This leads to immediate hatching, whereas other species deposit slow embryonic development eggs and need many environmental factors such as temperature, photo period, relative humidity and substrate moisture to favor the conditions for complete embryonation before following the stimulation of egg-hatching (Gerberg *et al.*, 1994; Clements, 1992).

Observation revealed the embryonation and hatchability of the oviposited eggs of *Ae. lineatopennis* in three conditions: (1) air-dried and moisture-incubated eggs with an age range of 1 to 10 days were inundated in natural and 1% hay-fermented water for 24 hours, (2) the moisture-incubated eggs, aged 7 days, were immersed in 0.25, 0.5, 1 and 2% hay-fermented water for 24 hours, and (3) the newly oviposited eggs were submerged in natural and 1% hay-fermented water for 10 consecutive days. The results revealed that *Ae. lineatopennis* deposited slow embryonic development eggs, requiring at least relative humidity and/or substrate moisture in order to complete the peak of embryonation by day 7. These results are generally in agreement with Choochote (1987) and Choochote *et al.* (1993), who studied the embryonation period of *Ae. quasiferinus* (45 days) and *Ae. harinasutai* (15 days). In both mosquito species, the eggs needed to be incubated in a moisture chamber until completely embryonated.

The hatchability of eggs from the above three conditions also demonstrated that the factors affecting the hatching of *Ae. lineatopennis* eggs can be placed into at least two categories. Firstly, pre-hatch conditions as discussed previously, and secondly, the hatching stimuli. The studies of hatching stimuli using two kinds of media – *ie* natural water and various concentrations of hay-fermented water (0.25, 0.5, 1 and 2%) – revealed that 0.25-2% of hay-fermented water was the proper condition in which the eggs could be stimulated to hatch within a 24-hour period. Many previous investigators attempted to find specific environmental conditions which stimulate *Aedes* eggs to hatch. Rozeboom (1934) examined the hatching response of *Ae. aegypti*, and found that embryos would not hatch in sterile water, but hatching occurred when the water was injected with bacteria. Gjullin *et al.* (1939) found that bacteria caused a reduction of dissolved oxygen in the hatching medium, thus, providing the stimulus for hatching in *Ae. sticticus*. Similar results were also observed in the study by Borg and Horsfall (1953) and Judson (1960) that the eggs of *Aedes* mosquitoes hatch in response to a reduction in the level of dissolved oxygen. Horsfall (1956) developed a standard procedure for inducing eggs to hatch by flooding them with nutrient broth, and the subsequent bacterial growth produced a gradual decrease in the dissolved oxygen of the medium. In the case of *Ae. lineatopennis*, even though the fully embryonated eggs were incubated in the moisture chamber for 7 days, their hatching rates in biological water were still very low and erratic. However, when they were immersed in various

concentrations of hay-fermented water, a large number of eggs were hatched within a 24-hour period. From the present results, it could be concluded that the hatching factor using hay-fermented water was necessary and/or at least the most important factor in the stimulation of egg hatching. This was presumably affected by organic and/or inorganic materials or fermented organisms, which reduced oxygen in the medium. It is interesting to note that in the newly oviposited eggs, which by-passed the incubation period in the moisture chamber when immediately inundated in natural and 1% hay-fermented water for 10 consecutive days, very few eggs were hatched and the dissection of eggs demonstrated low embryonation rates. These results were in accordance with Borg and Horsfall (1953) and Horsfall (1956), who reported that although a gradual decrease in the dissolved oxygen content of the medium occurred, hatching was erratic unless the embryos were brought to a uniform state of "conditioning." Thus, it could be concluded that at least two proper conditions were needed in *Ae. lineatopennis* – ie a suitable pre-hatch condition and an appropriate media stimulus.

Observation of the life cycle and developmental period of *Ae. lineatopennis* from the laboratory-raised colony (F_1 , F_3 , F_8) demonstrated that the duration of egg-hatching, including the incubation period in the moisture chamber, was 7 days, in which percentage hatching was 61-66%. The 1st stage larvae were easily reared in 0.25-1% hay-fermented water provided with suspended powder of equal weight of wheat germ, dry yeast, and oatmeal, and it yielded a high adult recovery (87-91%). The larval stages matured in 4-6 days. Pupation duration ranged from 3-4 days, and more than 80% of 1st stage larvae eventually pupated. More than 90% of pupae successfully emerged as adults. The developmental period results in all stages of *Ae. lineatopennis* in the laboratory condition of 27 ± 2 °C, 70-80% relative humidity were generally in accordance with the other aedine-mosquito development (Gerberg *et al*, 1994), except for the eggs of *Ae. lineatopennis*, which required the duration of at least 7 days in order to complete embryonation in the moisture chamber.

Many species of *Aedes* mosquitoes – eg *Ae. aegypti*, *Ae. albopictus* and *Ae. togoi* – are easily reared in the laboratory and also successfully mated in a 30 x 30 x 30 cm sized cage or smaller (Gerberg *et al*, 1994). Observation of the free mating ability of *Ae. lineatopennis* in a 30 x 30 x 30 cm cage revealed that *Ae. lineatopennis* was strongly eurygamous. After emergence from the pupa stage, the male seeks to mate at the first opportunity, which is usually the first evening at dusk in night-mated mosquitoes, and during the day

time in day-mated mosquitoes. In general, the males form dancing swarms at the time of mating and the females may enter these swarms in small numbers. Each female is promptly grabbed by a male, which locates her through the antennae, and the couple can be seen to fall in tandem out of the swarms. In some *Anopheles* species, the swarms of mosquitoes are 15-20 feet above the ground, which is a long distance for the couple to fall (Wharton, 1953; Reid, 1968), whereas many species of *Aedes* and *Mansonia* mate without the males forming swarms (Nielsen and Haeger, 1960). When mosquitoes breed in the laboratory, mating of the swarm forming species is markedly variable according to the limitation of space. Thus, some species of mosquitoes that mate without males forming swarms free-mate easily in small cages such as one of 30 x 30 x 30 cm, whereas others do not. So far, the natural mating behavior of *Ae. lineatopennis* has not been studied, and the observation of mating failure in a 30 x 30 x 30 cm cage possibly indicates the necessity of swarms for *Ae. lineatopennis*. On the other hand, it might also indicate a need for wider space for successful free-mating. It was found that the behavioral stenogamy (mating in a small space) and eurygamy (a wide space required for mating) of mosquitoes are controlled by one or more genes located on the Y-chromosome (Fraccoro *et al*, 1977). Even though the *Ae. lineatopennis* failed to mate in a small cage, the artificial mating technique, using 5-day old males, proved a promising method for induced laboratory-mating. Many *Aedes* species – eg *Ae. triseriatus*, *Ae. hendersoni* and *Ae. harinasutai* – that fail to mate in a small cage can be successfully colonized by artificial mating (Novak and Shroyer, 1978; Choochote *et al*, 1993).

One difficulty and/or failure in colonizing mosquitoes in the laboratory is the subsequent generation's refusal to feed on blood, particularly from small laboratory animals such as guinea pigs, white rats, golden hamsters, etc. This has led to direct feeding from human volunteers, especially at the beginning of the 1st to 5th generations of the colony. However, many reports have declared that *Aedes* spp and *Culex* spp could be capable of transmitting many viruses vertically – eg *Ae. aegypti*, *Ae. albopictus* (Khin and Than, 1983; Rosen *et al*, 1983; Mitchell and Miller, 1990; Bosio *et al*, 1992; Ahmad *et al*, 1997; Thenmozhi *et al*, 2000) and *Cx. tritaeniorhyncus* (Dhanda *et al*, 1989; Rosen *et al*, 1989a,b; Baqar *et al*, 1993). Thus, the use of direct blood feeding of subsequent mosquito progenies from human volunteers is a potentially dangerous technique and should be given up entirely. To solve the feeding failure of *Ae. lineatopennis* on small laboratory animals in a 30 x 30 x 30 cm cage,

and because this species has been reported as a potential vector of some viruses that can be transmitted vertically (Akhter *et al.*, 1982; Linthicum *et al.*, 1985; Vythilingam *et al.*, 1997), force feeding on the blood from an anesthetized golden hamster (laid on the top-screen of a paper cup containing fasted females) was successful in this study. The 4- to 7-day old *Ae. lineatopennis* succeeded in feeding at a rate that ranged from 36.67-86.67%. The golden hamster was chosen as the small animal of choice because of its proven tolerance to Nembutal® (0.1 ml/100 g), when compared with other small animals – *ie* white rats, white mice and Mongolian jirds. From an observation of 20 golden hamsters, with a mean weight of 134.65 ± 12.98 (110-158) g, the mean duration of sleep was 59.90 ± 10.09 (47-77) minutes.

Finally, we hope that the information concerning biological aspects of *Ae. lineatopennis* will prove to be important for the establishment of future laboratory colony strains and/or mass-rearing. This study may help develop a useful procedure for the mass-production of other *Aedes* mosquito species that have previously been difficult to cultivate.

ACKNOWLEDGEMENTS

The authors sincerely thank Professor Supot Vuthikarn (Dean of the Faculty of Medicine, Chiang Mai University, Thailand) of his interest in this research, to the Faculty Endowment Fund for financial support of this research project, and to the Faculty of Medicine Endowment Fund for Research Publication for its financial support in defraying publication costs.

REFERENCES

- Ahmad R, Ismail A, Saat Z, Lim LH. Detection of dengue virus from field *Aedes aegypti* and *Aedes albopictus* adults and larvae. *Southeast Asian J Trop Med Public Health* 1997;28:138-42.
- Akhter R, Hayes CG, Baqar S, Reisen WK. West Nile virus in Pakistan. III. Comparative vector capability of *Culex tritaeniorhynchus* and eight other species of mosquitoes. *Trans R Soc Trop Med Hyg* 1982;76:449-53.
- Apiwathanasorn C. A list of mosquito species in Southeast Asia. Bangkok: SEAMEO-TROPMED National Center of Thailand. Faculty of Tropical Medicine, Mahidol University, Thailand. 1986.
- Baker RS, Frenes WL, Kitzmiller JB. Induced copulation in *Anopheles* mosquitoes. *Mosq News* 1962;22:17.
- Baqar S, Hayes CG, Murphy JR, Watts DM. Vertical transmission of West Nile virus by *Culex* and *Aedes* species mosquitoes. *Am J Trop Med Hyg* 1993;48:757-62.
- Borg A, Horsfall WR. Eggs of floodwater mosquitoes. II. Hatching stimulus. *Ann Entomol Soc Am* 1953;46:472.
- Bosio CF, Thomas RE, Grimstad PR, Rai KS. Variation in the efficiency of vertical transmission of dengue-1 virus by strains of *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* 1992;29:985-89.
- Choochote W. A note on laboratory colonization of *Aedes (Muscidus) quasiferinus* Mattingly 1961, Amphur Muang Chiang Mai, Northern Thailand. *Southeast Asian J Trop Med Public Health* 1987;18:128-31.
- Choochote W, Sukontason K, Tookyang B, Likitvong K, Pakdicharoen A. A note on Laboratory colonization of *Aedes harinasutai*. *J Trop Med Parasitol* 1993;16:25-9.
- Clements AN. The Biology of mosquitoes. Development, nutrition and reproduction, Vol. 1. London: Chapman and Hall, 1992.
- Dhanda V, Mourya DT, Mishra AC, *et al.* Japanese encephalitis virus infection in mosquitoes reared from field-collected immatures and in wild-caught males. *Am J Trop Med Hyg* 1989;41:32-6.
- Fraccoro M, Tiepelo L, Laudani U, Marchia A, Jayakar SN. Y chromosome controls mating behavior of *Anopheles* mosquitoes. *Nature* 1977;256:326.
- Gerberg EJ, Barnard DR, Ward RA. Procedures for laboratory rearing of specific mosquitoes. In: Manual for mosquito rearing and experimental techniques. Louisiana: Allen Press. 1994:41-5.
- Gjullin CM, Yates WW, Stage H. The effect of certain chemicals on the hatching of mosquito eggs. *Science* 1939;89:539-40.
- Horsfall WR. Eggs of floodwater mosquitoes (Diptera: Culicidae), III. Conditioning and hatching of *Aedes vexans*. *Ann Entomol Soc Am* 1956;49:66.
- Judson CL. The physiology of hatching of aedine mosquito eggs: hatching stimulus. *Ann Entomol Soc Am* 1960;53:688.
- Khin MM, Than KA. Transovarial transmission of dengue-2 virus by *Aedes aegypti* in nature. *Am J Trop Med Hyg* 1983;32:590-4.
- Linthicum KJ, Davies FG, Kairo A, Bailey CL. Rift

- Valley fever virus (Family Bunyaviridae, genus *Phlebovirus*). Isolations from Diptera collected during an inter-epizootic period in Kenya. *J Hyg* 1985;95:197-209.
- Mitchell CJ, Miller BR. Vertical transmission of dengue viruses by strains of *Aedes albopictus* recently introduced into Brazil. *J Am Mosq Control Assoc* 1990;6:251-3.
- Miyagi I, Toma T, Tsukamoto M, *et al.* New distribution records of mosquitoes from Thailand with a collection list of 1983-1984 surveys. *Trop Biomed* 1986;3:181-92.
- Neilsen ET, Haeger JS. Swarming and mating in mosquitoes. *Misc Publs Ent Soc Am* 1960;1:71.
- Novak RJ, Shroyer DA. Eggs of *Aedes triseriatus* and *Ae. hendersoni*: A method to stimulate optimal hatch. *Mosq News* 1978;38:515.
- Ow Yang CK, Sta Maria EL, Warton RS. Maintenance of laboratory colony of *Anopheles maculatus* Theobald by artificial mating. *Mosq News* 1963;23:34-35.
- Reid JA. Anopheline mosquitoes of Malaya and Borneo. *Stud Inst Med Res Malaya* 1968;31:520.
- Rosen L, Lein JC, Lu LC. A longitudinal study of the prevalence of Japanese encephalitis virus in adult and larval *Culex tritaeniorhynchus* mosquitoes in northern Taiwan. *Am J Trop Med Hyg* 1989a;40:557-60.
- Rosen L, Lein JC, Shroyer DA, Baker RH, Lu LC. Experimental vertical transmission of Japanese encephalitis virus by *Culex tritaeniorhynchus* and other mosquitoes. *Am J Trop Med Hyg* 1989b;40:548-56.
- Rosen L, Shroyer DA, Tesh RB, Freier JE, Lien JC. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. *Am J Trop Med Hyg* 1983;32:1108-19.
- Rozeboom LE. The effects of bacteria on the hatching of mosquito eggs. *Am J Hyg* 1934;20:496.
- Tanaka K, Mizusawa K, Saugstad ES. A revision of the adult and larval mosquitoes of Japan (including The Ryukyu Archipelago and The Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib Am Entomol Inst* 1979;16:419-22.
- Thenmozhi V, Tewari SC, Manavalan R, Balasubramanian A, Gajanana A. Natural vertical transmission of dengue viruses in *Aedes aegypti* in southern India. *Trans R Soc Trop Med Hyg* 2000;94:507.
- Tippawangkosol P, Choochote W, Jitpakdi A, *et al.* Addition of the potentially indigenous vector of *Dirofilaria immitis* and a selection of highly susceptible laboratory vectors. *J Trop Med Parasitol* 1998;21:55-8.
- Tuetun B, Choochote W, Rattanachanpichai E, *et al.* Mosquito repellency of the seeds of celery (*Apium graveolens* L.). *Ann Trop Med Parasitol* 2004;98:407-17.
- Vythilingam I, Oda K, Mahadevan S, *et al.* Abundance, parity, and Japanese encephalitis virus infection of mosquito (Diptera: Culicidae) in Sepang District, Malaysia. *J Med Entomol* 1997;34:257-62.
- Wharton RH. The habits of adult mosquitoes in Malaya. IV. Swarming of anophelines in nature. *Med J Malaya* 1953;4:285.