Screening for Synchronized Oviposition of Some Mosquito Species (Diptera: Culicidae) in Thailand

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Abstract

Six genera, comprising 13 species of mosquitoes, which were found indigenously in Thailand, *ie*, *Aedes albopictus*, *Ae. lineatopennis*, *Ae. mediolineatus*, *Ae. togoi*, *Anopheles barbirostris*, *An. maculatus*, *An. minimus*, *An. peditaeniatus*, *An. sinensis*, *Armigeres subalbatus*, *Culex quinquefasciatus*, *Mansonia uniformis*, and *Toxorhynchites splendens*, were screened for synchronized oviposition by placing 5-day-old gravid females on a moist filter paper plate for 1 hour. This revealed that *Ae. albopictus*, *Ae. lineatopennis*, *Ae. mediolineatus*, *Ae. togoi*, and *Ar. subalbatus* could succeed in depositing a satisfactory number of viable eggs within 1 hour. The oviposition rates, average number of eggs per oviposited female, and hatchability rates were 90.0%, 19.4, and 67.3%; 60.0%, 16.0, and 55.7%; 35.0%, 25.4, and 42.7%; 65.0%, 56.2, and 75.0%; 55.0%, 62.4, and 82.0%; per species, respectively.

Keywords: synchronized oviposition, *Aedes*, *Anopheles*, *Armigeres*, *Culex*, *Mansonia*, *Toxorhynchites*

It is well known that an efficient way to inhibit mosquito-borne diseases is to control mosquito vectors. It is accepted that prior to the application of mosquito control strategies in the field, it is necessary to at least evaluate the effect of control agents, *ie*, insecticides, insect repellents, and other natural or synthetic products, on mosquito larvae and adults in the laboratory [1-5]. This has led to the establishment and mass-production of a laboratory colony that provides viable eggs and various mosquito stages. Nonetheless, the exact age of viable mosquito eggs may be needed for some specific investigations, *eg*, the introduction of DNA into the developing embryo in the mosquito egg by using a microinjection technique [6]. Hence, this report affirmed the possible genera and species of mosquitoes from which the eggs could be obtained synchronically.

Six species of wild-caught, fully engorged females, *ie*, Chiang Mai Province, northern Thailand strain: *Aedes lineatopennis*, *Ae. mediolineatus*, *Anopheles peditaeniatus*, *An. sinensis*, *Mansonia uniformis*; Mae Hong Son Province, north-west Thailand strain: *An. barbirostris*, were collected from water buffalo bait. Seven species of laboratory-raised mosquitoes, *ie*, Chiang Mai Province, northern Thailand strain: *Ae. albopictus*, *Armigeres subalbatus*, *Culex quinquefasciatus*, *Toxorhynchites splendens*; Chanthaburi Province, eastern Thailand strain: *Ae. togoi*; Ratchaburi Province,

central Thailand strain: *An. maculatus* (species B); Kanchanaburi Province, central Thailand strain: *An. minimus* (species A), were used. Five-day-old adult females, which had fasted for 12 hours, were allowed to feed on white rats. Immediately after blood feeding, only fully engorged females were selected. Both wild-caught and laboratory-raised fully engorged females were reared with 5% sucrose solution in an insectarium at 27 ± 2 ˚C, 70-80% RH, with 12:12 (L:D) photoperiod.

Five days after blood feeding, selected gravid females were treated in two experiments, *ie*, synchronized and normal ovipositions. For synchronized oviposition, the technique used in this study was slightly modified from that of Suwan *et al* [7]. The gravid females were anesthetized with ether and the wings and legs

Table 1 Oviposition of gravid females under synchronized and normal conditions, collected after 1 hour of experiment.

Mosquito species	Condition	No. females that oviposited eggs (%)	Total eggs	Average no. eggs per oviposited female (range)	Hatchability rate (No.)
Ae. albopictus	S	18 (90)	349	19.4 (3-75)	67.3 (202/300)
	N	-	-	-	56.0 (168/300) [a]
Ae. lineatopennis	S	12 (60)	192	16.0 (1-72)	55.7 (107/192)
	N	-	-	-	61.7 (185/300) [a]
Ae. mediolineatus	S	7 (35)	178	25.4 (3-51)	42.7 (76/178)
	N	-	-	-	45.3 (136/300) [a]
Ae. togoi	S	13 (65)	731	56.2 (1-143)	75.0 (225/300)
	N	-	28	-	70.3 (211/300) [a]
An. barbirostris	S	5 (25)	247	49.4 (15-93)	6.9 (17/247)
	N	-	-	-	89.0 (267/300) [a]
An. maculatus B	S	-	-	-	-
	N	-	-	-	-
An. minimus A	S	-	-	-	-
	N	-	-	-	-
An. peditaeniatus	S	1 (5)	14	14.0	7.1 (1/14)
	N	-	-	-	83.7 (251/300) [a]
An. sinensis	S	-	-	-	-
	N	-	-	-	-
Ar. subalbatus	S	11 (55)	687	62.4 (1-245)	82.0 (246/300)
	N	-	-	-	98.0 (294/300) [a]
Cx. quinquefasciatus	S	1 (5)	29	29.0	0.0 (0/29)
	N	-	-	-	77.7 (233/300) [a]
Ma. uniformis	S	-	-	-	-
	N	-	-	-	-
Tx. splendens	S	2 (10)	4	2.0 (1-3)	100 (4/4)
	N	-	-	-	78.7 (236/300) [a]

S = synchronized oviposition; N = normal oviposition;
[a]oviposited eggs after 1 hour

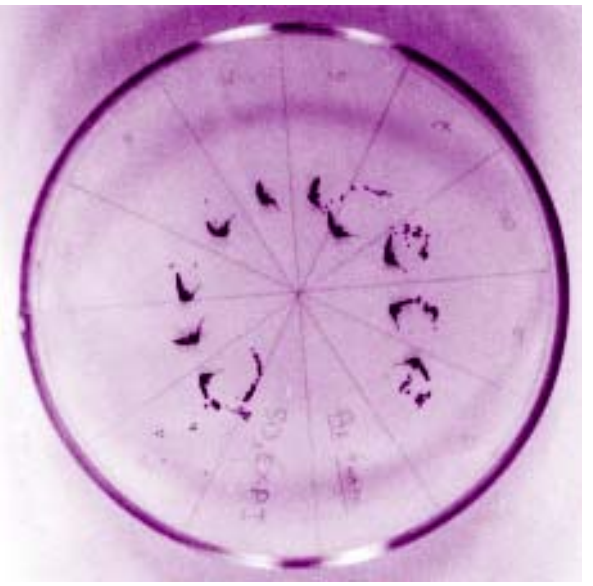

Fig 1 The synchronized oviposition of *Ae. togoi*.

were removed with forceps. The mosquitoes were then placed laterally on a moist filter paper in a moisture chamber (10 mosquitoes per chamber), which was made by laying a Whatman No. 1 filter paper (Whatman Hillsboro, OR) on 0.5 cm thick deionized water-soaked cotton wool in a 9 cm diameter petri dish that was covered by a glass plate lid. After 1 hour, the mosquitoes were removed from the filter paper and the number of eggs was recorded. Some eggs were chosen at random to test for hatchability rates. For normal oviposition, 20 gravid females were introduced into a 30 cm cube cage, with 3 species-specific ovipots, *ie*, moist filter paper laid on 0.5 cm thick deionized water-soaked cotton wool in a 9 cm diameter petri dish for *Aedes* spp, and *Ar. subalbatus*; a 13 cm diameter enamel cup containing approximately 100 ml of natural water taken from an associated breeding place for *Anopheles* spp, *Cx. quinquefasciatus* and *Tx. splendens* [8]; and a 10 cm diameter enamel bowl containing 10 pieces of sliced styrofoam (1.5 x 1.5 x 0.1 cm) floating in about 150 ml of natural water for *Ma. uniformis* [9]. The ovipots were checked for egg depositions after 1 hour of the experiments. Both experiments were performed between 10.00-11.00 am in the insectarium.

The oviposition rates, average number of eggs per oviposited female, and hatchability rates are shown in Table 1. The synchronized oviposition of *Ae. togoi* is illustrated in Fig 1.

For synchronized oviposition, *Ae. albopictus, Ae. lineatopennis, Ae. mediolineatus*, *Ae. togoi,* and *Ar. subalbatus* showed promising results because they provided satisfactory yields of oviposition rates: 90, 60, 35, 65, 55; an average number of eggs per oviposited female: 19.4, 16.0, 25.4, 56.2, 62.4; and hatchability rates: 67.3, 55.7, 42.7, 75.0, 82.0; respectively. For normal oviposition, only an *Ae. togoi* (totalling 28 eggs) succeeded in laying a few eggs within 1 hour. Statistical analysis of the hatchability rate of oviposited eggs between synchronized (S) and normal (N) ovipositions showed significant differences in *Ae. albopictus* (S = 67.3%, N = 56.0%, $\chi^2 = 8.15$, $p < 0.05$), and *Ar. subalbatus* (S = 82.0%, N = 98.0%, $\chi^2 = 42.67$, $p < 0.05$), but did not differ significantly in *Ae. lineatopennis* (S = 55.7%, N = 61.7%, $\chi^2 = 1.71$, $p > 0.05$), *Ae. mediolineatus* (S = 42.7%, N = 45.3%, $\chi^2 = 0.31$, $p > 0.05$), and *Ae. togoi* (S = 75.0%, N = 70.3%, $\chi^2 = 1.64$, $p > 0.05$). Low yields of oviposition and hatchability rates were obtained from *An. barbirostris, An. peditaeniatus, Cx. quinquefasciatus* and *Tx. splendens,* whereas the remainder, *ie*, *An.*

maculatus B, *An. minimus* A, *An. sinensis,* and *Ma. uniformis* provided an oviposition rate of zero.

The oviposition behavior of the mosquito, including all the behavioral and physiological processes of a gravid female that lead up to and terminate in the deposition of eggs in an appropriate biotype, varies considerably according to genera and species, and is dependent upon at least two distinct stages in the process of egg laying, *ie*, pre-oviposition behavior (behavioral responses of gravid females that result in their arrival at potential oviposition sites by an olfactometer to air streams carrying presumed oviposition-site attractants, and that indicate the suitability or acceptability of such sites), and oviposition (the emission of mature oocytes from the ovarian follicles into the calyces and oviducts, and ovulation) [10]. Therefore, it seems very difficult to obtain the same and/or synchronized age of mosquito eggs by breaking through their biological behavior. As for the screening of synchronized oviposition in this present study, only five species of damp substrate egg-laid mosquitoes, *ie*, *Ae. albopictus, Ae. lineatopennis, Ae. mediolineatus, Ae. togoi, and Ar. subalbatus,* successfully deposited viable eggs. The failed genera and species were those mosquitoes that deposited eggs singly onto the water surface (*Anopheles* spp and *Tx. splendens)*, an egg raft on the water surface (*Cx. quinquefasciatus*), and an egg cluster stuck to the underside of an aquatic plant leaf (*Ma. uniformis*) [11]. Even though this present study succeeded in only damp substrate egg-laid mosquitoes, it is hoped that this report will be useful for other research work whenever the mass-production of same and/or synchronized age of mosquito eggs are needed.

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