AUTOGENOUS *AEDES TOGOI* SUB-COLONY (CHANTHABURI, THAILAND STRAIN), AN EFFICIENT LABORATORY VECTOR IN STUDY OF FILARIASIS

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Abstract. Comparative filarial susceptibility and biology between stock colony and selectively autogenous *Aedes. togoi* sub-colony were carried out to determine the laboratorial vector-capacity and viability of autogenous sub-colony. The results of susceptibility revealed that the selectively autogenous *Ae. togoi* sub-colony yielded higher susceptibility than the stock colony, *ie Dirofilaria immitis* : susceptibility rates=80.00% [Exp1(F_8)] and 76.19% [Exp2(F_{17})] (autogenous sub-colony), 53.33% (Exp1) and 71.43% (Exp2) (stock colony); *Brugia malayi* : susceptibility rates=83.33% [Exp1(F_{17})] and 84.38% [Exp2(F_{19})] (autogenous sub-colony), 81.25% (Exp1) and 75.00% (Exp2) (stock colony), but not at the level of statistically significant differences except the Exp1 of *D. immitis*, which was significant difference. In addition, the average No. L3 per infected mosquito in the selective autogenous sub-colony (*D. immitis* : Exp1=3.37, Exp2=3.19; *B. malayi* : Exp1=8.80, Exp2=3.37) was also higher than in stock colony (*D. immitis* : Exp1=2.44, Exp2=2.73; *B. malayi* : Exp1=7.85, Exp2=3.02), but not at the level of statistically significant difference. The results of comparisons on some biological aspects demonstrated that most of the cases they have similar biology except the average egg deposition per gravid female of stock colony (130.17±43.33) was significantly more than selectively autogenous sub-colony(F_9) (94.33±13.69), egg length x width 575.62±18.06 μm x 186.15±9.35 μm of stock colony was significantly larger than selectively autogenous sub-colony(F_9) 560.49±18.96 μm x 177.99±8.40 μm, and mean longevity of adult female of stock colony [41.60(6-61)] was significantly longer than selectively autogenous sub-colony(F_9) [35.00(5-39)]. The selectively autogenous sub-colony was established and twenty-two successive generations have been colonized.

INTRODUCTION

Filariasis due to *Brugia malayi* and *Wuchereria bancrofti* are still the public health problems in Thailand. The Annual Report of the Division of Filariasis, Department of Communicable Disease Control, Ministry of Public Health in 1998 stated the microfilaremic rates of *B. malayi* and *W. bancrofti* were 0.09-1.76% and 0.11-18.00%, respectively.

In order to carry out a variety of filarial research aspects, *ie*, biochemistry, pharmacology, physiology, immunology and molecular biology, it is necessary to use the infective larvae as the starting point of investigation models. This led to the establishment of efficient laboratory vector which is proven to be highly susceptible to wide range genera and species of filarial parasite. Consequently, there were at least two mosquito species that used popularly in the mass-production of infective larvae, *ie*, *Aedes aegypti* (Liverpool strain) (MacDonald, 1962) and *Ae. togoi* (Taiwan strain) (Ramachandran *et al*, 1963). Comparison between these two mosquito species, *Ae. togoi* (Taiwan strain) was more susceptible to wide range genera and species of filarial parasites than *Ae. aegypti* (Liverpool strain). It was susceptible to periodic *B. malayi*, subperiodic *B. malayi*, *B. pahangi*, rural strain of *W. bancrofti*, *Dirofilaria immitis*, *Breinlia* sp and *Setaria* sp. Additional strain of *Ae. togoi* (Chanthaburi, Thailand strain) was subsequently declared by Choochote *et al* (1983, 1987), since it was highly susceptible to rural strain of nocturnally subperiodic *W. bancrofti* (Tak and Kanchanaburi strains), nocturnally subperiodic *B. malayi* (Narathiwat strain), *B. pahangi* (Malaysia strain), *D. immitis* (Chiang Mai strain), and urban strain or nocturally periodic *W. bancrofti* (Myanmar strain, unpublished data). It was easily bred and maintained in the laboratory on simple media (deionized tap-water) and larval food (ground animal chow), was a good blood feeder on golden hamster and white rat, mated readily in a 30 cm cube cage, and shown a high survival rate. It was also exhibited autogenous behavior. As an adjunct to the previous report, we report herein the additional benefit of a selectively

autogenous *Ae. togoi* sub-colony (Chanthaburi, Thailand strain) as an efficient laboratory vector in study of filariasis.

MATERIALS AND METHODS

Ae. togoi mosquitos

The origin and/or stock colony of *Ae. togoi* was obtained from Koh Nom Sao, Chanthaburi Province, southeastern Thailand. Larvae of this mosquito species were taken from their breeding places and have been reared in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, since 1981. Subsequently, the colony has been continuously maintained for several generations in the insectary of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, since 1983. As mentioned by Choochote *et al* (1987), this laboratory colony-strain of *Ae. togoi* exhibited autogenous behavior, thus, the selectively autogenous sub-colony was established by egg-batches of non-blood feeding adult females from stock colony. The hatched larvae were reared to the next generation, non-blood feeding adult females were allowed to oviposit eggs again, and the process was repeated continuously to establish the selectively autogenous sub-colony.

Infection of *Ae. togoi* with filariae

Two genera and species of filariae which were represented on the sites of development in vector, *ie*, the thoracic development, nocturnally subperiodic *B. malayi* (Narathiwat strain), the Malpighian tube development, *D. immitis* (Chiang Mai strain) were used to infect *Ae. togoi*. Three-day-old adult females of both stock and selectively autogenous *Ae. togoi* sub-colony (fasted for 12 hours) were allowed to feed simultaneously on heparinized blood of infected cat for nocturnally subperiodic *B. malayi* (microfilarial density ranged from 1.85-2.70 mf/mm^3), and infected domestic dog for *D. immitis* (microfilarial density ranged from 2.90-3.35 mf/mm^3), using artificial membrane feeding technique as described by Chomcharn *et al* (1980). Fourteen days after feeding, all mosquitos were dissected in normal saline solution and examined under a dissecting microscope. The number of mosquitos with one or more infective larvae in head, thorax, and abdomen were recorded. The harvested infective stages were killed with hot 70% ethanol, then cleared in 5% glycerine in 70% ethanol and 20% glycerine in 70% ethanol, respectively. Finally, they were mounted in glycerine on a cavity slide, examined under a compound microscope, and the dimensions of the body were assessed using camera lucida drawings.

Biological study of stock colony and selectively autogenous *Ae. togoi* sub-colony

In order to determine the difference and/or similarity between stock colony and selectively autogenous *Ae. togoi* sub-colony, thus, the comparative studies of some biological aspects, *ie*, feeding ability, oviposition capacity, average egg deposition per gravid female, embryonation rate, hatchability rate, egg dimension under calibrated compound microscope, and adult longevity were performed.

RESULTS

Details of susceptibility, L3-distribution and L3-dimension of stock colony and selectively autogenous *Ae. togoi* sub-colony to *B. malayi* and *D. immitis* are shown in Table 1 and Table 2, respectively.

For *D. immitis*, the results of dissection of all infected mosquitos on day 14 revealed that selectively autogenous sub-colony was more susceptible to *D. immitis* than stock colony, although it was statistically significant difference in only the Exp1 (χ^2=4.80, $p < 0.05$). The infective rates were 80.00% [Exp1(F_8)], 76.19% [Exp2(F_{17})] and 53.33% (Exp1), 71.43% (Exp2) in selectively autogenous sub-colony and stock colony, respectively. The infective larvae obtained from all experimental feedings were very active and found distributed in all regions of the head, thorax and abdomen and they behaved similarly, more than 90% of infective larvae could migrate from the Malpighian tubes to the head and proboscis. The average No. L3 per infected mosquito in the selectively autogenous sub-colony, 3.37 [Exp1(F_8)] and 3.19 [Exp2(F_{17})], were also higher than in stock colony, 2.44 (Exp1) and 2.73 (Exp2), but not at the level of statistically significant differences [t=1.05(Exp1), 0.66 (Exp2), $p > 0.05$]. Statistical analysis of the body dimensions of infective larvae recovered from stock colony and selectively autogenous sub-colony manifested significant difference in body length [t=3.64(Exp1), 4.26 (Exp2), $p < 0.05$], but non-significant difference in body width [t=0.81(Exp1), 1.39(Exp2), $p > 0.05$]. Nonetheless, the body length and width of infective larvae obtained from selectively autogenous sub-

Table 1
The susceptibility, L3-distribution and L3-dimension of stock colony and selectively autogenous sub-colony of *Ae. togoi* after feeding on blood infected with *D. immitis*.

Experiments	Type of colony			
	Stock		Selective autogeny	
	Exp1	Exp2	Exp1(F_8)	Exp2(F_{17})
Susceptibility				
Infective rate (No.)	53.33(16/30)	71.43(15/21)	80.00(24/30)	76.19(16/21)
Average No.L3 per infected mosquito (range)	2.44(1-7)	2.73(1-6)	3.37(1-9)	3.19(1-8)
L3-distribution				
% Head (No.)	95.23(40)	90.24(37)	92.59(75)	92.16(47)
% Thorax (No.)	2.38(1)	7.32(3)	7.41(6)	3.92(2)
% Abdomen (No.)	2.38(1)	2.44(1)	0	3.92(2)
Total	42	41	81	51
L3-dimension(μm)[a]				
Mean body length (range)	916.67±85.43 (700.00-1,100.00)	987.92±78.91 (842.08-1,105.23)	978.33±36.40 (900.00-1,050.00)	1,056.10±60.14 (947.34-1,157.86)
Mean body width (range)	24.83±1.03 (22.40-26.60)	24.80±1.62 (20.55-27.40)	25.06±1.18 (23.80-28.00)	25.35±1.43 (23.29-30.14)

[a]Measurement from thirty larvae, Exp = experiment, F = generation
Exp1 : susceptibility $\chi^2 = 4.80$, $p < 0.05$ average No. L3 per infected mosquito $t = 1.05$, $p > 0.05$, body length $t = 3.64$, $p < 0.05$, body width $t=0.81$, $p > 0.05$
Exp2 : susceptibility $\chi^2 = 0.08$, $p > 0.05$ average No. L3 per infected mosquito $t = 0.66$, $p > 0.05$, body length $t = 4.26$, $p < 0.05$, body width $t = 1.39$, $p > 0.05$

colony were larger than from stock colony in the two comparative experiments, *ie*; length 978.33±36.40 μm and width 25.06±1.18 μm [Exp1(F_8)], length 1,056.10±60.14 μm and width 25.35±1.43 μm [Exp2(F_{17})] (selectively autogenous sub-colony); length 916.67±85.43 μm and width 24.83±1.03 μm (Exp1), length 987.92±78.91 μm and width 24.80±1.62 μm (Exp2) (stock colony).

For *B. malayi*, similar results of susceptibility, L3-dimension and L3-distribution as in *D. immitis* experiments were also observed. The susceptibility rates of selectively autogenous sub-colony were 83.33% [Exp1(F_{17})] and 84.38% [Exp2(F_{19})] of which higher than in stock colony, 81.25% (Exp1) and 75.00% (Exp2), but not at the level of statistically significant difference [χ^2=0.09 (Exp1), 0.82 (Exp2), $p > 0.05$], and more than 80% of infective larvae could migrate from the thorax to the head and proboscis except in the Exp1 from selectively autogenous sub-colony of which 32.84% and 58.96% of infective larvae could migrate to the head and abdomen, respectively. The average No. L3 per infected mosquito in the selectively autogenous sub-colony, 8.80 [Exp1(F_{17})] and 3.37 [Exp2(F_{19})], were also higher than in stock colony, 7.85 (Exp1) and 3.02 (Exp2), but not at the level of statistically significant difference [t=0.23 (Exp1), 0.27 (Exp2), $p > 0.05$]. Statistical analysis of the body dimensions of infective larvae obtained from both colonies manifested non-signifcant differences in body length [t=1.18(Exp1), 0.46 (Exp2), $p > 0.05$], but significant differences in body width [t=2.56(Exp1), 2.97(Exp2), $p < 0.05$]. Nevertheless, the body length and width of infective larvae recovered from selectively autogenous sub-colony were larger than from stock colony in the two comparative experiments, *ie*; length 1,538.70±130.12 μm and width 26.63±1.92 μm [Exp1(F_{17})], length 1,531.60±156.05 μm and width 26.05±1.39 μm [Exp2(F_{19})] (selectively autogenous sub-colony); length 1,503.50±98.52 μm and width 25.42±1.74 μm (Exp1), length 1,515.50±103.78 μm and width 24.93±1.53 μm (Exp2) (stock colony).

Details of comparison on some biological aspects between stock colony and selectively autogenous *Ae. togoi* sub-colony(F_9), *ie*, feeding rate,

Table 2
The susceptibility, L3-distribution and L3-dimension of stock colony and selectively autogenous sub-colony of *Ae. togoi* after feeding on blood infected with *B. malayi*.

Experiments	Type of colony			
	Stock		Selective autogeny	
	Exp1	Exp2	Exp1(F_{17})	Exp2(F_{19})
Susceptibility				
Infective rate (No.)	81.25(13/16)	75.00(21/28)	83.33(15/18)	84.38(27/32)
Average No.L3 per infected mosquito (range)	7.85(1-28)	3.02(1-13)	8.80(1-47)	3.37(1-11)
L3-distribution				
% Head (No.)	88.35(91)	96.10(74)	32.84(44)	80.22(73)
% Thorax (No.)	4.85(5)	2.60(2)	8.20(11)	15.38(14)
% Abdomen (No.)	6.80(7)	1.30(1)	58.96(79)	4.40(4)
Total	103	77	134	91
L3-dimension(μm)[a]				
Mean body length	1,503.50±98.52	1,515.50±103.78	1,538.70±130.12	1,531.60±156.05
(range)	(1,368.38-1,684.16)	(1,368.38-1,684.16)	(1,315.75-1,789.42)	(1,263.12-1,789.42)
Mean body width	25.42±1.74	24.93±1.53	26.63±1.92	26.05±1.39
(range)	(21.52-26.90)	(21.52-26.90)	(22.86-30.94)	(21.52-26.90)

[a]Measurement from thirty larvae, Exp = experiment, F = generation
Exp1 : susceptibility χ^2= 0.09, $p > 0.05$ average No. L3 per infected mosquito $t = 0.23$, $p > 0.05$, body length $t = 1.18$, $p > 0.05$, Body width $t = 2.56$, $p < 0.05$
Exp2 : susceptibility χ^2= 0.82, $p > 0.05$ average No. L3 per infected mosquito $t = 0.27$, $p > 0.05$, body length $t = 0.46$, $p > 0.05$, Body width $t = 2.97$, $p < 0.05$

Table 3
Comparison on some biological aspects of stock colony and selectively autogenous sub-colony(F_9) of *Ae. togoi*.

Experiments	Type of colony		
	Stock	Selective autogeny	Statistical test
Feeding rate (No.)	95.00(38/40)	97.50(39/40)	$\chi^2 = 0$, $p = 1$
Insemination rate (No.)	88.89(24/27)	100(30/30)	$\chi^2 = 1.64$, $p > 0.05$
Oviposition rate (No.)	96.67(29/30)	100(30/30)	$\chi^2 = 0$, $p = 1$
Average egg deposition per gravid female (range)	130.17±43.33 (63-212)	94.33±13.69 (69-136)	$t = 4.25$, $p < 0.05$
Embryonation rate (No.)	94.12(96/102)	89.58(86/96)	$\chi^2 = 1.37$, $p > 0.05$
Hatchability rate (No.)	86.27(88/102)	85.42(82/96)	$\chi^2 = 0$, $p = 0.97$
Eggs dimension (μm) (range)[a]			
Length	575.62±18.06 (535.50-612.00)	560.49±18.96 (525.30-601.80)	$t = 3.17$, $p < 0.05$
Width	186.15±9.35 (173.40-204.00)	177.99±8.40 (163.20-193.80)	$t = 3.56$, $p < 0.05$
Mean longevity of adult in days (range)[b]			
female	41.60(6-61)	35(5-39)	$t = 3.47$, $p < 0.05$
male	32.70(6-40)	33.80(17-38)	$t = 0.60$, $p > 0.05$

[a]Measurement from thirty eggs; [b]Observation from thirty adults.

insemination rate, oviposition rate, average egg deposition per gravid female, embryonation rate, hatchability rate, egg dimension and adult longevity are shown in Table 3. The results from comparison revealed that most of the cases they have similarity of biological aspects except the average egg deposition per gravid female of stock colony (130.17±43.33) was significantly more than selectively autogenous sub-colony (94.33±13.69) ($t=4.25$, $p < 0.05$), egg length x width 575.62±18.06 μm x 186.15±9.35 μm of stock colony was significantly larger than 560.49±18.96 μm x 177.99±8.40 μm of selectively autogenous sub-colony [$t=3.17$ (length) and 3.56 (width), $p < 0.05$], and mean longevity of adult female from stock colony [41.60 (6-61)] was significantly longer than selectively autogenous sub-colony [35.00 (5-39)] ($t=3.47$, $p < 0.05$).

DISCUSSION

The establishment of a laboratory mosquito vector that is easy to handle and highly susceptible to wide range genera and species of filarial parasites is still needed for various aspects of research work, since it reduces cost and time-consume in the mass-production of infective larvae that used in the starting point of filarial research-models. Trial have been made by several, previous investigations to establish the efficient laboratory vector of filariae. The popular pioneers were MacDonald (1962) and Ramachandran *et al* (1963), the former established *Ae. aegypti* (Liverpool strain) and the latter constructed *Ae. togoi* (Taiwan strain). The drawback of both mosquito strains are that they need blood meal for egg production and this lead to the maintenance of small laboratory animal as a source of blood meal. The successful establishment of selectively autogenous *Ae. togoi* sub-colony that yielded satisfactory susceptibility to both thoracic (*B. malayi*) and Malpighian tubes (*D. immitis*) development filariae will contribute to the low cost and effective system for mass-production of infective larvae, since the small laboratory animal which is the important source of mosquito blood meal could be partial or complete reduction.

Extensive study of autogeny has been reported in many species distributed among at least 15 mosquito genera (Eberle and Reisen, 1986; Clements, 1992; Chambers and Klowden, 1994; Su and Mulla, 1997), but no account was taken off autogenous mosquito-parasite benefit, and only pure physiology, biology, mechanism control of autogeny, etc were declared. The linkage of autogenous mosquito-parasite benefit is reported for the first time. The results of the studies, *ie*; filarial susceptibility; L3-recovery; L3-dimension; some biological aspects: feeding ability, oviposition capacity, adult longevity have indicated that the selectively autogenous *Ae. togoi* sub-colony(F_{19}) (Chanthaburi, Thailand strain) was stable and could be served as an efficient laboratory vector-model for study of filariasis in Thailand, and now our laboratory can provide this autogenous sub-colony(F_{22+}) to other investigators whenever the study of filariasis and/or other biological fields are in need.

Additional interesting points are, firstly, the body dimensions (body length and width) of infective larvae of *B. malayi* and *D. immitis* obtained from selectively autogenous *Ae. togoi* sub-colony were larger than from stock colony. Similar results were also found in the infective larvae of *D. immitis* recovered from *in vitro* cultivation, which is bypassed the mosquito refractory mechanism. The long term withdrawal of blood meal in many consecutive generations of selectively autogenous *Ae. togoi* sub-colony may temporarily or permanently rule out the regular refractory mechanism(s) that against the blood parasites and lead to the favorable sites for filarial developments than stock and/or blood-feeding colony. Secondly, the inheritance of autogeny in *Ae. togoi* was controlled by a system of multiple genes (Thomas and Leng, 1972), whereas the f^m (filarial susceptibility, *B. malayi*) and f^t (filarial susceptibility, Malpighian tubes) genes were controlled by simple sex-linked alleles with refractoriness being dominant to susceptibility (MacDonald, 1976; Sulaiman and Townson, 1980). Judged from the results of susceptibilities of *B. malayi* and *D. immitis* in both stock colony (75.00-81.25% in *B. malayi*, 53.33-71.43% in *D. immitis*) and selectively autogenous *Ae. togoi* sub-colony (83.33-84.38% in *B. malayi*, 76.19-80.00% in *D. immitis*), it could be provisionally concluded that autogenous and filarial susceptibility genes were not separated and/or coexisted.

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