

Comparative Studies on the Biology and Filarial Susceptibility of Selected Blood-feeding and Autogenous *Aedes togoi* Sub-colonies

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Blood-feeding and autogenous sub-colonies were selected from a laboratory, stock colony of Aedes togoi, which was originally collected from Koh Nom Sao, Chanthaburi province, Southeast Thailand. Comparative biology and filarial susceptibility between the two sub-colonies (blood-feeding: F₁₁, F₁₃; autogeny: F₃₈, F₄₀) were investigated to evaluate their viability and vectorial capacity. The results of comparison on biology revealed intraspecific differences, i.e., the average egg deposition/gravid female (F₁₁/F₃₈; F₁₃/F₄₀), embryonation rate (F₁₃/F₄₀), hatchability rate (F₁₁/F₃₈; F₁₃/F₄₀), egg width (F₁₁/F₃₈), wing length of females (F₁₃/F₄₀), and wing length and width of males (F₁₁/F₃₈) in the blood-feeding sub-colony were significantly greater than that in the autogenous sub-colony; and egg length (F₁₁/F₃₈) and width (F₁₃/F₄₀), and mean longevity of adult females (F₁₁/F₃₈) and males (F₁₃/F₄₀) in the blood-feeding sub-colony were significantly less than that in the autogenous sub-colony. The results of comparison on filarial susceptibility demonstrated that both sub-colonies yielded similar susceptibilities to Brugia malayi [blood-feeding/autogeny = 56.7%(F₁₁)/53.3%(F₃₈), 60%(F₁₃)/83.3%(F₄₀)] and Dirofilaria immitis [blood-feeding/autogeny = 85.7%(F₁₁)/75%(F₃₈), 45%(F₁₃)/29.4%(F₄₀)], suggesting autogenous Ae. togoi sub-colony was an efficient laboratory vector in study of filariasis.

Key words: *Aedes togoi* - blood feeding - autogeny - biology - filarial susceptibility - *Brugia malayi* - *Dirofilaria immitis*

Lymphatic filariasis, due to *Brugia malayi* and *Wuchereria bancrofti*, is still an important mosquito-borne human disease in many countries of Asia, particularly in India, Indonesia, Malaysia, the Philippines, Sri Lanka and Thailand (WHO 2001).

In order to carry out a variety of research and development concerning lymphatic filariasis, e.g., physiology, biochemistry, pharmacology, pathology, immunology and molecular biology, it is necessary to use infective larvae as a starting point of investigations. This has led to a search for a reliable laboratory vector. Consequently, *Aedes togoi* (Taiwan strain) has been selected and used widely for more than three decades as an efficient laboratory vector in the mass-production of infective larvae. It is easily bred and maintained in an insectarium, is a good blood-feeder and highly susceptible to a wide range of genera and species of filariae, i.e., periodic *B. malayi*, subperiodic *B. malayi*, *B. pahangi*, rural strain of *W. bancrofti*, *Dirofilaria immitis*, *Breinlia* sp. and *Setaria* sp. (Ramachandran et al. 1963). An additional strain of *Ae. togoi* (Thailand strain) was subsequently affirmed (Choochote et al. 1983, 1987), since it was highly susceptible to the nocturnally periodic (urban strain) and subperiodic (rural strain) *W.*

bancrofti, nocturnally subperiodic *B. malayi*, *B. pahangi* and *D. immitis*. The disadvantage in both colonies of *Ae. togoi* is the regular live bloodmeals necessary for routine maintenance. However, the *Ae. togoi* (Thailand strain) exhibited autogenous behavior (Choochote et al. 1987), and to take advantage of this, a selected autogenous sub-colony was established and subsequent generations prove to be an efficient laboratory vector of filariae. This was because the 8th and 17th generations of the selected sub-colony yielded similar susceptibilities (to *B. malayi* and *D. immitis*) to the stock colony (mixed blood-feeding and autogeny) (Riyong et al. 2000). As an addition to the previous study, we report herein, the comparative biology and filarial susceptibility of the 11th and 13th generations of selected blood-feeding, and the 38th and 40th generations of selected autogenous *Ae. togoi* sub-colonies.

MATERIALS AND METHODS

Mosquitoes - The stock colony of *Ae. togoi* was obtained originally from Koh Nom Sao, Chanthaburi province, Southeast Thailand. The colony was established in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand in 1981. Since 1983, it has been maintained continuously for several generations in the insectarium of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Establishment of blood-feeding and autogenous sub-colonies - As declared by Choochote et al. (1987) and Riyong et al. (2000) and, this stock colony-strain of *Ae. togoi* exhibited both blood-feeding and autogenous behavior simultaneously within the colony, thus, the selec-

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tions of sub-colonies were divided into blood-feeding and autogenous individuals. For the blood-feeding sub-colony, approximately 100 egg batches of blood-fed adult females were pooled, the hatched larvae were reared to the next generation, and blood-fed adult females were allowed to oviposit egg again. This process was repeated continuously to establish the selected blood-feeding sub-colony. For the selected autogenous sub-colony, the process for selection mainly followed that for the blood-feeding sub-colony, except egg batches obtained from non-blood feeding adult females were used.

Biological study - In order to determine the viability of selected blood-feeding and autogenous *Ae. togoi* sub-colonies, comparative studies were carried out on feeding (fed on golden hamster, and for autogenous sub-colony: the females that deposited first autogenous batch of eggs were used) and mating abilities by measuring the insemination rate, oviposition capacity, average egg deposition/gravid female, embryonation rate, hatchability rate, egg dimensions, wing dimensions (right wing of each adult; length: axial incision to apical margin excluding the apical fringe; width: maximal width from costa to the opposite wing margin excluding the apical fringe), and female and male adult longevity (fed on 5% sucrose solution). To rule out the variable factors of the above experiments, thus, both sub-colonies of *Ae. togoi* were reared simultaneously under similar conditions; larval density (400 larvae/46 x 35 x 6 cm-sized plastic tray containing 2 l of deionized water); daily larva-feeding; an adult sex ratio of 200 females:400 males in a 30 cm cube cage; and maintenance in an insectarium (12 h illumination, 27 ± 2°C, 70 ± 10% RH). The 11th and 13th generations of a selected blood-feeding sub-colony, and the 38th and 40th selected autogenous sub-colony were used in these studies.

Infection of *Ae. togoi* with filariae - Two species of filariae, which were represented on the sites of development in vectors, were chosen and used. They were the thoracic development of the nocturnally subperiodic *B. malayi*, and the Malpighian tube development of *D. immitis*. Five-day-old adult females of both sub-colonies (F₁₁/F₃₈, F₁₃/F₄₀) (fasted for 12 h, and for autogenous sub-colony: the females that deposited first autogenous batch of eggs were used) were allowed to feed simultaneously on the heparinized blood of an infected cat for nocturnally subperiodic *B. malayi* (microfilarial density = 2.10 mf/mm³ for F₁₁/F₃₈ and 2.55 mf/mm³ for F₁₃/F₄₀), and an infected domestic dog for *D. immitis* (microfilarial density = 3.15 mf/mm³ for F₁₁/F₃₈ and 1.70 mf/mm³ for F₁₃/F₄₀), using the artificial membrane feeding technique, as described by Chomcharn et al. (1980). Fourteen days after feeding, all mosquitoes were dissected in normal saline solution and examined under a dissecting microscope. The harvested infective larvae were killed with hot 70% ethanol and mounted in glycerine on a slide, and body dimensions were assessed under a compound microscope using a camera-lucida drawing.

RESULTS

Biological study - Details of comparison on some biological aspects between blood-feeding and autogenous sub-colonies are presented in Table I.

Results demonstrated that there were some intraspecific differences of the biology that were not related to the behavior of the sub-colonies. The average egg deposition/gravid female [F₁₁/F₃₈: 190.20 ± 39.20/98.27 ± 31.81 (t = 9.97, p < 0.05), F₁₃/F₄₀: 115.24 ± 32.46/85.31 ± 21.99 (t = 4.11, p < 0.05)], embryonation rate [F₁₃/F₄₀: 99.33/77.33 (χ² = 70.45, p < 0.05)], hatchability rate [F₁₁/F₃₈: 94.67/89.33 (χ² = 5.80, p < 0.05), F₁₃/F₄₀: 91.00/74.00 (χ² = 30.03, p < 0.05)], egg width [F₁₁/F₃₈: 197.22 ± 9.52 μm/190.93 ± 11.07 μm (t = 2.36, p < 0.05)], wing length of females [F₁₃/F₄₀: 3.29 ± 0.10 mm/3.23 ± 0.08 mm (t = 2.46, p < 0.05)], and wing length and width of males [F₁₁/F₃₈: length 2.69 ± 0.05 mm/2.65 ± 0.04 mm (t = 3.48, p < 0.05), width 0.71 ± 0.01 mm/0.69 ± 0.02 mm (t = 3.01, p < 0.05)] in the blood-feeding sub-colony were significantly greater than that in the autogenous sub-colony, whereas the egg length and width [F₁₁/F₃₈: length 553.88 ± 19.92 μm/565.78 ± 21.70 μm (t = 2.21, p < 0.05), F₁₃/F₄₀: width 179.83 ± 10.09 μm/186.43 ± 11.01 μm (t = 3.86, p < 0.05)], and mean longevity of adult females and males [F₁₁/F₃₈: female 27.33 ± 13.97 day/35.97 ± 15.06 day, F₁₃/F₄₀: male 29.17 ± 11.63 day/45.70 ± 12.18 day (t = 5.38, p < 0.05)] in the blood-feeding sub-colony were significantly less than that in the autogenous sub-colony.

Filarial susceptibility - Details of infective rates and parasite loads in selected blood-feeding and autogenous *Ae. togoi* sub-colonies are shown in Table II.

For *B. malayi*, the dissection results of all infected mosquitoes on day 14 demonstrated that the selected blood-feeding and autogenous sub-colonies yielded similar filarial susceptibilities [F₁₁/F₃₈: 56.67/53.33 (χ² = 0.07, p > 0.05), F₁₃/F₄₀: 60.00/83.33 (χ² = 2.95, p > 0.05)] and an average number of infective larvae/infected mosquito [F₁₁/F₃₈: 5.82 (1-29)/3.75 (1-22) (t = 0.89, p > 0.05), F₁₃/F₄₀: 11.38 (1-54)/17.76 (1-51) (t = 1.27, p > 0.05)]. The infective larvae obtained from all experimental feedings were very active and found to be distributed in all regions, but they were mainly located on the head and proboscis, where more than 70% of infective larvae could be observed. Nonetheless, intraspecific differences in the body dimensions of infective larvae that were also found from the blood-feeding sub-colony, i.e., F₁₁/F₃₈: body length 1633.50 ± 101.26 μm/1552.83 ± 142.02 μm (t = 2.53, p < 0.05) and body width 25.56 ± 1.50 μm/24.70 ± 1.47 μm (t = 2.23, p < 0.05) were significantly larger than that in the autogenous sub-colony, and the F₁₃/F₄₀: body length 1544.43 ± 143.28 μm/1616.65 ± 111.44 μm (t = 2.18, p < 0.05) obtained from the blood-feeding sub-colony was significantly smaller than that in the autogenous sub-colony.

For *D. immitis*, both sub-colonies yielded the same filarial susceptibilities [F₁₁/F₃₈: 85.71/75.00 (Fisher exact p > 0.05), F₁₃/F₄₀: 45.00/29.41 (χ² = 0.95, p > 0.05)], with an average number of infective larvae/infected mosquitoes [F₁₁/F₃₈: 8.00 (1-21)/6.78 (1-23) (t = 0.57, p > 0.05), F₁₃/F₄₀: 14.44 (2-48)/11.40 (1-35) (t = 0.37, p > 0.05)]. More than 65% of infective larvae could migrate to the head and proboscis, except in the F₁₃ experiment where only 36.15% of infective larvae could migrate to the head and proboscis, and as much as 53.1% of them were found in the abdomen. Nevertheless, body width 23.18 ± 1.23 μm (F₁₃)/23.85 ± 1.02 μm (F₄₀) (t = 2.31, p < 0.05) recovered

TABLE I
Comparative biology of the selected blood-feeding and autogenous *Aedes togoi* sub-colonies

	Experiment 1		Experiment 2	
	SB(F ₁₁)	SA(F ₃₈)	SB(F ₁₃)	SA(F ₄₀)
Feeding rate (No.)	92.50 (37/40)	95.00 (38/40)	85.00 (34/40)	80.00 (32/40)
Insemination rate (No.)	100 (30/30)	100 (30/30)	93.33 (28/30)	100 (30/30)
Oviposition rate (No.)	96.67 (29/30)	86.67 (26/30)	96.67 (29/30)	96.67 (29/30)
Average egg deposition per gravid female (range)	190.20 ± 39.20 (20 - 236)	98.27 ± 31.81 (3 - 129)	115.24 ± 32.46 (63 - 192)	85.31 ± 21.99 (37 - 116)
Embryonation rate (No.)	97.00 (291/300)	98.67 (296/300)	99.33 (298/300)	77.33 (232/300)
Hatchability rate (No.)	94.67 (284/300)	89.33 (268/300)	91.00 (273/300)	74.00 (222/300)
Egg dimensions (µm) ^a (range)				
Length	553.88 ± 19.92 (509.25 - 609.00)	565.78 ± 21.70 (525.00 - 609.00)	564.99 ± 34.55 (506.15 - 650.17)	565.69 ± 24.31 (484.88 - 593.56)
Width	197.22 ± 9.52 (178.50 - 220.50)	190.93 ± 11.07 (168.00 - 215.25)	179.83 ± 10.09 (160.49 - 205.75)	186.43 ± 11.01 (167.20 - 209.00)
Wing dimensions (µm) ^b (range)				
Female				
Length	3.40 ± 0.07 (3.28 - 3.55)	3.38 ± 0.07 (3.18 - 3.52)	3.29 ± 0.10 (3.08 - 3.49)	3.23 ± 0.08 (3.07 - 3.45)
Width	1.04 ± 0.02 (1.00 - 1.08)	1.05 ± 0.03 (0.99 - 1.10)	1.02 ± 0.03 (0.97 - 1.07)	1.01 ± 0.03 (0.94 - 1.07)
Male				
Length	2.69 ± 0.05 (2.61 - 2.80)	2.65 ± 0.04 (2.55 - 2.74)	2.61 ± 0.06 (2.50 - 2.72)	2.60 ± 0.05 (2.48 - 2.71)
Width	0.71 ± 0.01 (0.68 - 0.75)	0.69 ± 0.02 (0.66 - 0.74)	0.69 ± 0.03 (0.60 - 0.73)	0.68 ± 0.02 (0.66 - 0.70)
Mean longevity of adult in days (range) ^c				
Female	27.33 ± 13.97 (5 - 59)	35.97 ± 15.06 (5 - 67)	34.23 ± 6.12 (23 - 43)	35.90 ± 13.06 (10 - 59)
Male	36.63 ± 16.61 (5 - 63)	43.03 ± 15.20 (14 - 69)	29.17 ± 11.63 (9 - 43)	45.70 ± 12.18 (14 - 64)

a: measurement from 30 eggs; b: measurement from 30 wings; c: observation from 30 adults; SB: selected blood-feeding sub-colony; SA: selected autogenous sub-colony; F: generation

from the blood-feeding sub-colony was significantly smaller than that in the autogenous sub-colony.

DISCUSSION

Extensive, comparative studies of autogenous and anautogenous mosquitoes have been reported in several species comprising at least 15 mosquito genera (Eberle & Reisen 1986, Reisen & Milby 1987, Clements 1992, Chambers & Klowden 1994, Su & Mulla 1997, Sawabe & Moribayashi 2000, Olejnicek & Gelbic 2000). Studies are mostly concerned with the intrinsic and extrinsic factors that influence the biology, physiology, biochemistry, mechanism and/or genetic control of autogeny, etc. Except in the study of Trpis (1981), very few studies related the autogeny of the host to parasite infection. The author reported high susceptibility in the autogenous group of the *Ae. scutellaris* complex (*Ae. cooki*, *Ae. kesseli*, *Ae. tongae tabu* and an undescribed *Aedes* sp.) to *B. malayi* and *B. pahangi*, and it was proposed that this sibling species group could serve as an excellent mosquito labo-

ratory model in the study of filariasis. However, no account confirmed the susceptibility comparison of subsequent generations of consecutively selected autogenous sub-colony to both filarial species.

The results of comparative studies on some biological aspects between the selected blood-feeding and autogenous *Ae. togoi* sub-colonies revealed that the viability of the selected autogenous sub-colony was obviously stable up to the 40th generation, although, there were some intraspecific differences in the average egg deposition/gravid female, embryonation rate, hatchability rate, egg length and width, wing length and width, and mean longevity of adults. The marked reduction of average egg deposition/gravid female in the selected autogenous sub-colony was similar to that in previous reports of autogenous *Ae. togoi* (Malaysia strain) (Thomas & Leng 1972) and *Culex tarsalis* (Su & Mulla 1997). The lower egg deposition/gravid female, embryonation rate and hatchability rate of autogenous sub-colony than selected blood-feeding sub-colony were the biological disadvantages of au-

TABLE II

The infective rates and parasite loads in selected blood-feeding and autogenous *Aedes togoi* sub-colonies after artificial feeding on blood infected with *Brugia malayi* and *Dirofilaria immitis*, all dissected 14 days after feeding

	Experiment 1		Experiment 2	
	SB(F ₁₁)	SA(F ₃₈)	SB(F ₁₃)	SA(F ₄₀)
<i>B. malayi</i>				
Infective rate (No.)	56.67 (17/30)	53.33 (16/30)	60.00 (18/30)	83.33 (25/30)
Average No. L3 per Infected mosquito (range)	5.82 (1 - 29)	3.75 (1 - 22)	11.38 (1 - 54)	17.76 (1 - 51)
L3-distribution				
% head (No.)	77.78 (77)	88.33 (53)	74.15 (152)	76.18 (339)
% thorax (No.)	13.13 (13)	5.00 (3)	3.90 (8)	8.31 (37)
% abdomen (No.)	9.01 (9)	6.67 (4)	21.95 (45)	15.50 (69)
Total	99	60	205	445
L3-dimensions (µm) ^a				
Mean body length (range)	1633.50 ± 101.26 (1320.00 - 1815.00)	1552.83 ± 142.02 (1265.00 - 1870.00)	1544.43 ± 143.28 (1166.65 - 1777.76)	1616.65 ± 111.44 (1333.32 - 1777.76)
Mean body width (range)	25.56 ± 1.50 (21.60 - 28.35)	24.70 ± 1.47 (21.60 - 27.00)	24.50 ± 1.87 (21.00 - 28.00)	24.50 ± 1.02 (22.40 - 26.60)
<i>D. immitis</i>				
Infective rate (No.)	85.71 (18/21)	75.00 (18/24)	45.00 (9/20)	29.41 (5/17)
Average No. L3 per Infected mosquito (range)	8.00 (1 - 21)	6.78 (1 - 23)	14.44 (2 - 48)	11.40 (1 - 35)
L3-distribution				
% head (No.)	86.80 (125)	76.23 (93)	36.15 (47)	66.67 (38)
% thorax (No.)	5.56 (8)	5.74 (7)	10.77 (14)	17.54 (10)
% abdomen (No.)	7.64 (11)	18.03 (22)	53.08 (69)	15.79 (9)
Total	144	122	130	57
L3-dimensions (µm) ^a				
Mean body length (range)	973.50 ± 83.16 (770.00 - 1100.00)	999.17 ± 50.21 (880.00 - 1100.00)	1012.95 ± 63.06 (833.33 - 1111.10)	999.99 ± 69.97 (833.33 - 1111.10)
Mean body width (range)	22.95 ± 1.37 (20.25 - 25.65)	23.40 ± 1.56 (20.25 - 27.00)	23.18 ± 1.23 (21.60 - 25.65)	23.85 ± 1.02 (21.60 - 25.65)

a: measurement from 30 larvae; SB: selected blood-feeding sub-colony; SA: selected autogenous sub-colony; F: generation

togenous sub-colony. Nonetheless, the above biological variations did not have an effect on the differences in filarial susceptibility (infective rate and average number of infective larvae/infected mosquito) between the two sub-colonies, suggesting that the selected autogenous *Ae. togoi* sub-colony was an efficient laboratory vector-model for the study of filariasis. Additionally, the similarity in the feeding rates of the two sub-colonies supported the advantages of the selected autogenous sub-colony.

The successful establishment of the selected autogenous *Ae. togoi* sub-colony (F₄₀) that yielded satisfactory susceptibility to both thoracic (*B. malayi*) and Malpighian tube (*D. immitis*) development filariae, will contribute to the low cost and high effectiveness of the system for mass-production of infective larvae, since the utility small-space laboratory animal to provide the mosquito bloodmeal, could be completely excluded. We now propose that our laboratory could provide this selected autogenous sub-colony (F₄₀₊) to other researchers to facilitate the study of filariasis and/or other aspects.

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REFERENCES

- Chambers GM, Klwoden MJ 1994. Nutritional reserves of autogenous and anautogenous selected strains of *Aedes albopictus* (Diptera: Culicidae). *J Med Entomol* 31: 554-560.
- Chomchorn Y, Surathin K, Bunnag D, Sucharit S, Harinasuta T 1980. Effects of a single dose of primaquine on a Thai strain of *Plasmodium falciparum*. *Southeast Asian J Trop Med Public Health* 11: 408-409.
- Choochote W, Abeyewickreme W, Sucharit S, Apiwathnasorn C, Tumrasvin W, Leemingsawat S 1983. *Aedes togoi* Koh Nom Sao, Chanthaburi, a laboratory vector for *Brugia malayi* and *Brugia pahangi* in Thailand. *J Parasit Trop Med Assoc Thai* 6: 25-31.
- Choochote W, Keha P, Sukhavat K, Khamboonruang C, Sukontason K 1987. *Aedes (Finlaya) togoi* Theobald 1907,

- Chanthaburi strain, a laboratory vector in studies of filariasis in Thailand. *Southeast Asian J Trop Med Public Health* 18: 259-260.
- Clements AN 1992. The biology of mosquitoes. In *Development, Nutrition and Reproduction*, vol. 1, Chapman and Hall, London, 509 pp.
- Eberle MW, Reisen WK 1986. Studies on autogeny in *Culex tarsalis*: 1. Selection and genetic experiments. *J Am Mosq Control Assoc* 2: 38-43.
- Olejnick J, Gelbic I 2000. Autogeny in *Culex pipiens quinquefasciatus* Say. *J Vector Ecol* 25: 118-122.
- Ramachandran CP, Wharton RH, Dunn FL, Kershaw WE 1963. *Aedes (Finlaya) togoi* Theobald, a useful laboratory vector in studies of filariasis. *Ann Trop Med Parasitol* 57: 443-445.
- Reisen WK, Milby MM 1987. Studies on autogeny in *Culex tarsalis*: 3. Life table attributes of autogenous and anautogenous strains under laboratory conditions. *J Am Mosq Control Assoc* 3: 619-625.
- Riyong D, Choochote W, Jitpakdi A, Suvannadabba S, Leemingsawat S, Chaitong U 2000. Autogenous *Aedes togoi* sub-colony (Chanthaburi, Thailand strain), an efficient laboratory vector in study of filariasis. *Southeast Asian J Trop Med Public Health* 31: 246-251.
- Sawabe K, Moribayashi A 2000. Lipid utilization for ovarian development in an autogenous mosquito, *Culex pipiens molestus* (Diptera: Culicidae). *J Med Entomol* 37: 726-731.
- Su T, Mulla MS 1997. Selection-dependent trends of autogeny and blood feeding in an autogenous strain of *Culex tarsalis* (Diptera: Culicidae). *J Am Mosq Control Assoc* 13: 145-149.
- Thomas V, Leng YP 1972. The inheritance of autogeny in *Aedes (Finlaya) togoi* Theobald, from Malaysia and some aspects of its biology. *Southeast Asian J Trop Med Public Health* 3: 163-174.
- Trpis M 1981. Susceptibility of the autogenous group of the *Aedes scutellaris* complex of mosquitoes to infection with *Brugia malayi* and *Brugia pahangi*. *Tropenmed Parasitol* 32: 184-188.
- WHO-World Health Organization 2001. *Regional Strategic Plan for Elimination of Lymphatic Filariasis*, New Delhi, 30 pp.

