A Note on Laboratory Colonization of *Toxorhynchites splendens* by Using an Artificial Mating Technique and Autogenous *Aedes togoi* Larva as Prey

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Abstract

An alternative method for the colonization of *Toxorhynchites splendens* was established successfully by using artificial mating for adults and autogenous *Aedes togoi* larva as prey. This current method of rearing could enable a reduction in the large size cage (60 x 60 x 75 cm) for adult rearing and the small laboratory animals as a source of bloodmeal that are used to maintain the colonies of blood-fed prey, eg, *Ae. aegypti*, *Ae. albopictus* and *Culex quinquefasciatus*. The suitable age of adult males for artificial mating ranged from 3-6 days, with 65-90% and 100% mating and insemination rates, respectively. The average number of autogenous *Ae. togoi* larvae consumed by associated stages of *Tx. splendens* were 8.55 ± 2.74, 9.75 ± 3.86, 27.10 ± 7.89 and 61.30 ± 10.62 larvae in stages 1, 2, 3 and 4, respectively.

Keywords: *Toxorhynchites splendens*, colonization, *Aedes togoi*

Toxorhynchites splendens (Wiedemann) was affirmed as a potentially useful biological agent for the control of the dengue hemorrhagic vectors, *Aedes aegypti* and *Ae. albopictus*, and the filarial vector, *Culex quinquefasciatus* [1-3]. This mosquito species is also a very good laboratory host for studies of dengue hemorrhagic virus [4] and lymphatic filariae [5].

Formerly, Chowanadisai *et al* [6] and Horio and Tsukamoto [7] had declared the successful colonization of *Tx. splendens* by using a large-size cage (60 x 60 x 75 cm) for adult copulation and *Ae. aegypti* larvae as larval prey. This method of rearing has been used extensively up to this time, but it needs a spacious insectarium to keep a large size cage as well as a small laboratory animal to provide bloodmeals to maintain *Ae. aegypti* prey. We report herein, an alternative method for rearing *Tx. splendens* by using artificial mating for adults, and non-blood feeding and/or autogenous *Ae. togoi* larva as larval prey.

The laboratory-colony strain of *Tx. splendens* was established by using 73 wild-caught pupae from Wat Phai Hin, Suthep District, Chiang Mai Province, northern Thailand. These had been successfully colonized in the insectarium for several generations since 1987, using the rearing techniques of Chowanadisai *et al* [6] (12 hours illumination, 27 ± 2˚C, 70-80% RH) at the Department of Parasitology, Faculty of Medicine, Chiang Mai University. At the beginning of the year 2000, a selected autogenous *Ae. togoi* sub-colony (Chanthaburi, Thailand strain) had been established successfully in our laboratory for more than 22 generations [8]. Since then, the simplification of the rearing system for *Tx.*

splendens was initiated and autogenous *Ae. togoi* larvae have been used instead of *Ae. aegypti* larvae.

Details of the developmental period and feeding ability of the *Tx. splendens* larvae, which fed on associated stages of autogenous *Ae. togoi* prey that had been reared individually in a plastic container measuring 4.5 cm in diameter, 6.5 cm in depth and containing 50 ml of deionized water, are illustrated in Table 1. A comparison between the mean larval development duration of *Tx. splendens*, which fed on the second stage *Ae. aegypti* prey [6] and those fed on associated stages of autogenous *Ae. togoi* prey in the present study, revealed that the mean duration of larval development using *Ae. aegypti* prey (40.49 days) was longer than autogenous *Ae. togoi* prey (16.95 days). Thus, the use of associated stages of autogenous *Ae. togoi* larvae could reduce the time needed for *Tx. splendens* larval rearing approximately 2.39 times. It is pertinent to note that when 30 first stage larvae of *Tx. splendens* were reared in a 35 x 25 x 6 cm plastic tray containing 2,000 ml of deionized water and excess associated stages of autogenous *Ae. togoi* larvae (approximately predator : prey = 1 : 20 for each day), the mean duration of larval development was 12.97 days, which was shorter than individual rearing (16.95 days), and the survival and pupation rates were 100%. Subsequently, this larval rearing technique was used routinely for mass-rearing *Tx. splendens*.

For adult copulation, a trial to establish a colony capable of free mating in a 30 x 30 x 30 cm cage by the co-habitation of 20 newly emerged females and 30 newly emerged males (the same ratio as co-habitation in a 60 x 60 x 75 cm cage) for 10 consecutive days was not successful, since no oviposition or insemination were observed. A trial was successful with the *Tx. splendens* strain from Palawan Island, the Philippines, by co-habitation of approximately 50 adult females and males in a 20 x 20 x 30 cm cage [9]. Similar results of differing degrees of stenogamous/eurygamous behavior were also observed in various geographic strains of *Cx. tritaeniorhynchus* Giles [10], and *Anopheles minimus* Theobald from Thailand [11-12]. To solve this problem, artificial mating techniques [13-14], that have been used successfully in strongly eurygamous *Anopheles* mosquitoes, were used with *Tx. splendens*. Details of the artificial mating ability of various ages of *Tx. splendens* males with 5-day-old females are shown in Table 2. As determined by the mating rate, all of the females succeeded in mating, with 100% insemination rate. Thus the 5-day-old male should be the age of choice for artificial mating, although there was no statistically significant difference in the mating rate of males aged 5 days from males aged 3, 4, 6 and 7 days (5/3, $\chi^2 = 0.27$, $p > 0.05$; 5/4, Fisher exact, $p > 0.05$; 5/6, Fisher exact, $p > 0.05$; 5/7, $\chi^2 = 2.98$, $p > 0.05$). There was no statistically significant difference in the average number of females mated by one

Table 1 Developmental period and feeding ability of *Tx. splendens* larva on *Ae. togoi* prey.

Experiment	Mosquito stages*				
	L1	L2	L3	L4	Pupa
Average duration of larval development in days (range)	1 (1)	2.65 ± 0.99 (1-4)	4.45 ± 1.19 (3-7)	8.85 ± 1.53 (5-10)	6.45 ± 1.10 (5-8)
Average number of *Ae. togoi* larvae eaten per stage (range)	8.55 ± 2.74 (5-15)	9.75 ± 3.86 (3-17)	27.10 ± 7.89 (16-51)	61.30 ± 10.62 (40-82)	-

*Twenty larvae for each stage

Table 2 Artificial mating ability at various ages of *Tx. splendens* males with 5-day-old females.

Age in day*	Mating rate (No.)	Total females succeeded in mating	Insemination rate (No.)	Average no. of females mated by one male (range)
2	20 (4/20)	7	100 (7/7)	1.75 ± 0.96 (1-3)
3	65 (13/20)	26	100 (26/26)	2.00 ± 1.15 (1-5)
4	90 (18/20)	27	100 (27/27)	1.50 ± 0.71 (1-3)
5	85 (17/20)	46	100 (46/46)	2.71 ± 1.83 (1-7)
6	80 (16/20)	22	100 (22/22)	1.38 ± 0.62 (1-3)
7	55 (11/20)	19	100 (19/19)	1.73 ± 0.65 (1-3)

*Twenty males for each experiment

male from these aged 5 days and those aged 2, 3 and 7 days (5/2, $t = 1.00$, $p > 0.05$; 5/3, $t = 1.22$, $p > 0.05$; 5/7, $t = 2.02$, $p > 0.05$). Observation of 20 mated females, which were reared individually in a 30 x 30 x 30 cm cage, demonstrated that the average duration of oviposition, average eggs per oviposited female and average hatching rates were 14.75 (6-25) days, 173.85 (57-332) eggs and 79.48 (53.91-99.11)%, respectively. The average number of *Tx. splendens* (11.79) eggs laid per female per day was similar to a 10-day-old, force-mated female *Tx. towadensis* (11.5), but obviously more than the *Tx. manicatus yaeyamae* (2.5) and *Tx.* undescribed sp (Okinawa) (1.7) [7].

We hope that our report on the alternative techniques in mass-rearing of *Tx. splendens* by using artificial mating for adult, autogenous *Ae. togoi* larva as larval prey, and a 30 x 30 x 30 cm cage for the maintenance and oviposition of mated females, may indicate a useful way for mass-rearing other *Toxorhynchites* spp, particularly in a small unit that has limited insectarium space. Now, our laboratory can provide a selected autogenous *Ae. togoi* sub-colony (F_{41+}) for other researchers whenever mass-rearing of *Toxorhynchites* mosquitoes is needed.

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