

COLONIZATION OF *MANSONIA DIVES* SCHINER IN A FIELD INSECTARY

Chang Moh Seng¹, Ho Beng Chuan² and Chan Kai Lok³

¹Vector Borne Disease Control Program, Medical Department, Kuching, Sarawak, Malaysia;

²Department of Microbiology, Faculty of Medicine and ³Department of Zoology, Faculty of Science, National University of Singapore, Republic of Singapore.

Abstract. Successful colonization of *Manonia dives*, the principal vector of subperiodic *Brugia malayi* was established in a field insectary. Mean egg clusters laid on *Eichhornia crassipes*, *Pistia stratiotes*, *Homalomena cordata* and polystyrene strips were 12.0, 10.4, 9.5 and 13.7 respectively. However, the mean number of first instar larvae hatched from each egg cluster laid by females on the three plant substrates (range 51.1 to 58.6) was higher than that laid on the polystyrene strips (41.8). There were no significant differences in the success pupation and adult emergence rates among the three host plants used as attachment substrates. Adult emergence occurred at a mean of 10.8 days. The first adult emergence was observed at the 25th day after hatching and continued till the 50th day. The 50% mortality rates for the adults were estimated as 8 days for the males and 14 days for the females. The mean gonotrophic cycle ranged from 3.8 to 4.3 days with a mean of 4.04 days. 63.6% of *Ma. dives* females oviposited in a medium of rat dung and water. The mean incubation period of eggs ranged from 5.2 to 6.5 days with a mean of 5.7 days. The biology of *Ma. dives* and *Ma. bonnea* is briefly compared.

INTRODUCTION

Brugian filariasis is known to be endemic in Malaysia (Wilson, 1961; Mak, 1976, 1983). *Mansonia dives* Schiner has been incriminated as one of the principal vectors of the subperiodic form of *Brugia malayi* (Wharton, 1957a, 1962). The biology and vector-parasite relationships of this vector are little known due to the difficulty to successfully colonize this mosquito species. Wharton (1957b) succeeded in colonizing a Malaysian strain of *Mansonia uniformis* in small numbers for susceptibility studies of filariae. More recently, colonies of *Ma. uniformis*, *Ma. indiana* and *Ma. bonnea* were successfully established in Malaysia (Cheong, *et al*, 1984; Chiang *et al*, 1985). Wharton (1957b), Breeland *et al* (1981) and Sucharit *et al* (1982), using styrofoam as the oviposition and attachment substrates, clearly demonstrated that successful colonization of *Mansonia* mosquitos can be established in the absence of living aquatic plants. Chiang (1987)

had successfully colonized 11 generations of *Ma. indiana* and *Ma. bonnea* and 12 generations of *Ma. uniformis* in a humidified insectary in Malaysia. Due to high mortality and low adult emergence, Wharton (1962) was only able to maintain a *Ma. longipalpis* (then a mixture of *Ma. bonnea* and *Ma. dives*) colony stock up to the F₁ generation.

The current colonization studies were initiated as part of an extensive study of the biology and control of *Ma. dives* and *Ma. bonnea* in Sarawak, Malaysia. The objective of the present study was to establish a sustained field insectary capable of mass producing immature stages of *Ma. dives* for larval bioassays and filarial infectivity experiments.

MATERIALS AND METHODS

Origin of mosquito colony

The colony of *Ma. dives* was established initially from the mosquitos collected in Kampong

Ampungan, Sarawak. Mosquitos were allowed to feed on albino rats overnight in the laboratory. Lots of 20 engorged females were transferred into each of the paper cups (6 cm × 10 cm). After a holding period of 2 days, 15-20 of the gravid females were transferred into each of the 500 ml glass beakers filled with 250 ml conditioned tap water. Each beaker was provided with 15 polystyrofoam strips (10-15 mm² long and 1-2 mm thick) as oviposition substrate. Five days later, a total of 187 egg clusters were obtained and they were divided into 90 and 97 clusters of *Ma. bonneae* and *Ma. dives* respectively, according to their coloration as described by Wharton (1962). Each egg cluster of *Ma. dives* was allowed to hatch separately, and the F₁ generation of the mosquitos then established.

Rearing procedures in the field insectary

Ma. dives colonies were maintained in an unhumidified mosquito-proof field insectary (120 m²) which was constructed by hatched palm leaf walls and illuminated with natural daylight. Daily average room temperature was 29°C (± 3°C) and relative humidity was 81% (± 5.5%).

Two different types of oviposition chambers (ovipots) were used: (a) six 450 ml plastic cups each filled with 200 ml of conditioned tap water. Several young aquatic plants of *E. crassipes*, *P. stratiotes* and *H. cordata*, which served as attachment substrate for the larvae and pupae, were introduced into three pairs of plastic cups, each pair containing one of the three aquatic plant species. (b) 500 ml glass beaker filled with 250 ml conditioned tap water and a few polystyrofoam strips were provided as attachment substrate.

Ten gravid females of *Ma. dives* were introduced into each ovipot for oviposition.

Egg hatching

Batches of egg clusters obtained from each ovipot were transferred separately into 500 ml glass beakers containing 300 ml conditioned tap water. Hatching was observed daily for 7 days. One-day old larvae were counted daily and transferred to separate trays containing a larval breeding medium of rat dung infusion.

Maintenance of larvae

Larvae were reared in an aqueous medium containing 1% (v/v) albino rat dung infusion. 10 g

of rat dung infusion mixed with 1 liter of deionized water was allowed to ferment for 2 days. This mixture served as a stock solution. The final dilution for the larval medium was made up in the ratio of 1 part of stock solution to 2 parts of conditioned tap water into which 1 g yeast powder was added every 2-3 days.

Field collected young aquatic plants of *E. crassipes*, *P. stratiotes* and *H. cordata* were washed thoroughly to remove any attached *Mansonia* immatures, predators and debris. The roots of the plants were pruned to an uniform length. Several aquatic plants were introduced into 6 polyethylene trays (30 × 16 × 20 cm³), each containing 3 l of the final breeding solution and 200-250 1st-instar stage *Ma. dives* larvae. Both larval breeding medium and host plants were changed every 7-10 days. Daily observations were made for pupation and adult emergence.

Maintenance of adults

All of the daily emerged adults were sexed and placed into separate cages (30.5 × 30.5 × 30.5 cm³). The adults were provided with 10% sugar soaked cotton wicks until mating (stenogamous) took place on the 2nd day. Female mosquitos were allowed to take their first blood meal 72 hours after emergence. This was followed by a second blood meal 4 days later. The individual females were set up for oviposition in individual glass beaker ovipots containing 250 ml deionized water and 15 polystyrofoam strips as oviposition substrates.

Gonotrophic cycle

Laboratory reared F₂ progeny females were fed overnight on a white rat. The following morning, each engorged individual was transferred into a single ovipot containing 250 ml deionized water and 15 polystyrofoam strips. A total of 14 replicates was made and each replicate consisted of 10 isofemale lines. After oviposition, females were removed from ovipots and the hatching of eggs was checked every 12 hours. The interval between blood feeding and oviposition (the gonotrophic cycle), and the duration between oviposition and egg hatching (the hatching interval) were determined.

Adult survivorship

Single cohorts of the 24-hour old females of F₂ generation were used to determine the survivorship

curves of both sexes under laboratory conditions. Approximately 2000 *Ma. dives* pupae were placed in breeding trays together with *H. cordata* host plants. The trays were then placed inside a cage (30.5 × 30.5 × 30.5 cm³) for 7 days. On the 4th day (set as day 0) newly-emerged adult mosquitos, consisting of 233 males and 237 females *Ma. dives*, were transferred into a clean cage. Observations for mortality of males and females were made at 0800 hours daily until the last living adult had died.

RESULTS

Oviposition and larval productivity

Table 1 shows the mean number of egg clusters, egg hatch and larval productivity of *Ma. dives*. The mean egg clusters for each oviposition substrate was 12.0 for *E. crassipes*, 10.4 for *P. stratiotes*, 9.5

for *H. cordata* and 13.7 for polystyrofoam strips. ANOVA tests indicated no significant difference in egg cluster production among host plants (F = 1.05; p = 0.20). However, as shown in Table 1, the mean number of first-instar larvae hatched from each egg cluster laid by *Ma. dives* on the plant substrates was higher than that laid on the polystyrofoam strips.

Success pupation and adult emergence

Table 2 summarizes the success pupation and adult emergence of *Ma. dives* associated with three aquatic plant species. No significant differences were observed among the three host plants (F = 1.33, p = 0.28 for success pupation; F = 1.35; p = 0.27 for adult emergence).

Daily adult emergence rate

Adult emergence of *Ma. dives* from pupae attached to *H. cordata* was observed daily. From the initial stock of 3,000 L₁ larvae, 18.7% adult

Table 1

Mean egg clusters and larval productivity of F₂ *Ma. dives* in four different types of oviposition substrate based on 15 observations in which 10 females were confined in each cage for 7 days.

Oviposition substrate	Egg clusters	First-instar larvae	
	(Mean ± SD)	(Mean ± SD)	(Mean No./egg cluster)
<i>E. crassipes</i>	12.0 ± 3.5	642 ± 252	55.4
<i>P. stratiotes</i>	10.4 ± 3.2	533 ± 178	51.1
<i>H. cordata</i>	9.5 ± 3.4	557 ± 158	58.6
polystyrofoam strips	13.7 ± 3.1	575 ± 144	41.8

Table 2

Success pupation (%) and adult emergence rate (%) of F₂ *Ma. dives* associated with aquatic plant species as attachment substates. (The results were based on 15 observations).

No. of first-instar larvae	Aquatic plant species	Success pupation (%)	Adult emergence rate (%)
		Mean ± SD (Total)	Mean ± SD (Total)
2,750	<i>E. crassipes</i>	24.54 ± 3.7 (675)	17.9 ± 3.2 (492)
2,700	<i>P. stratiotes</i>	26.04 ± 4.0 (703)	19.5 ± 3.0 (532)
3,000	<i>H. cordata</i>	25.07 ± 5.1 (752)	18.7 ± 4.1 (563)
	Overall mean	25.20	18.8

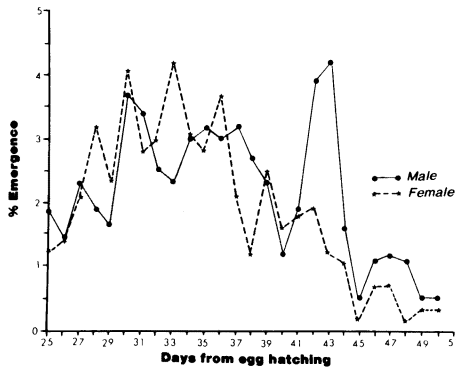


Fig 1—Daily emergence of males and females of *Ma. dives* from day 25 after egg hatching.

emerged (Table 2), with a sex ratio of 1 female : 1 male. The first adult emerged on day 25 after hatching and adult emergence continued till day 50 (Fig 1). Since daily male and female emergence rates (from 25 to 50) were correlated ($p = 0.0000035$), it was justifiable to pool both sexes together to estimate the mean duration of the period of adult emergence. This duration was calculated to be 10.8 days.

Gonotrophic cycle

During the two weeks observation period, 63.6% of *Ma. dives* oviposited. The mean gonotrophic cycles based on each of the 14 independent replicates ranged from 3.8 to 4.3 days with a mean of 4.04 days. Egg hatch was successfully recorded for 89 isofemale lines of *Ma. dives*. Mean incubation period of eggs ranged from 5.2 to 6.5 days with a mean of 5.7 days.

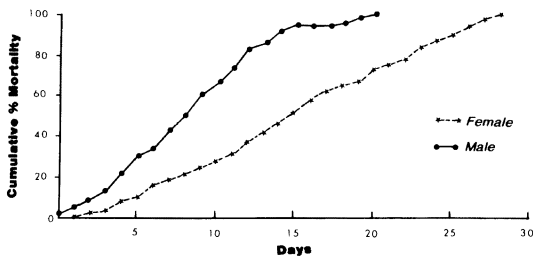


Fig 2—Cumulative percentage mortality of males and females of *Ma. dives* from an insectary colony.

Adult survivorship

As shown in Fig 2, when daily cumulative adult mortality was plotted against days, the fluctuation of cumulative mortality percentage of *Ma. dives* females followed closely that of the males. The 50% cumulative mortality for *Ma. dives* was 8 days for males and 14 days for females.

DISCUSSION

In the present study, successful colonization of *Ma. dives* was established in a field insectary. The advantage in maintaining *Mansonia* colonies in a field insectary is the ready availability of live larvae and adults for bioassay studies. Moreover, the host plants which are essential for the colonization of *Mansonia* mosquitos are abundantly available throughout the year near the field insectary.

The successful colonization of *Ma. dives* throughout two generations may be the first achievement of this kind since Wharton (1957b, 1962) who successfully colonized *Ma. longipalpis* (then a mixture of *Ma. bonneae* and *Ma. dives*) up to the F_1 generation.

In an attempt to develop a more suitable oviposition substrate for *Mansonia* mosquitos, Sucharit *et al* (1982) reported that for the colonization of *Ma. uniformis*, *Ma. indiana* and *Ma. annulifera*, *P. stratiotes* (50% of the total ovipositions) appeared to be the preferred ovipositions substrate over styrofoam (1.3% of the total ovipositions) and five other aquatic plant species (range 0.5-23.6% of the total ovipositions). However, in comparing Wharton's studies (Wharton, 1957b) in which *P. stratiotes* alone was used as the oviposition medium with Sucharit's studies (Sucharit *et al*, 1982) where styrofoam alone was used, they observed a similarity in the egg-laying (95.0% vs 95.8%) and egg hatching (93% vs 91%) characteristics between the two types of ovipots. Our present study showed that the *Ma. dives* oviposited more eggs in polystyfoam strips (mean 13.7 egg clusters) than in *P. stratiotes* substrate (mean 10.4 egg clusters). However, the mean number of eggs per cluster laid by *Ma. dives* on *P. stratiotes* (51.1) was higher than that laid on the polystyfoam strips (41.8) (Table 1). The hatching of L_1 *Ma. dives* in *P. stratiotes* substrate and polystyfoam strips was comparable (533 larvae vs 575 larvae, Table 1).

Table 3

Comparing the biological parameters between *Ma. bonneae* and *Ma. dives* in a field insectary.

Biological parameters	<i>Ma. bonneae</i>			<i>Ma. dives</i>		
	Mean egg clusters	Success pupation (%)	Adult emergence rate (%)	Mean egg clusters	Success pupation (%)	Adult emergence rate (%)
<i>E. crassipes</i>	7.1	27.85	21.1	12.0	24.54	17.9
<i>P. stratiotes</i>	6.3	26.02	19.6	10.4	26.04	19.5
<i>H. cordata</i>	7.4	28.82	23.1	9.5	25.07	18.7
Polystyrofoam	9.3	-	-	13.7	-	-
Sex ratio	1 ♀ : 0.99 ♂			1 ♀ : 1 ♂		
Duration of adult emergence (days)						
Total (from the date after hatching)	27-51			25-50		
Mean	9.5			10.8		
50% cumulative mortality (days)						
Male	9			8		
Female	16			14		
% of females survived beyond 12 days after emergence	68.4			63.0		
Natural infection rate (%) with <i>B. malayi</i> infection	0.19			0.19		
Gonotrophic cycle (days)	3.6-4.9 (mean 4.1 ± 0.32)			3.8-4.3 (mean 4.04 ± 0.14)		
% oviposition of females	60.7			63.6		
Mean incubation of eggs (days)	4.6-6.4 (mean 5.5 ± 0.44)			5.2-6.5 (mean 5.7 ± 0.41)		

In our studies, the biological parameters of the two closely related *Mansonia* species represent the first comparative observations of *Ma. bonneae* and *Ma. dives* in an environment that simulated field conditions. As shown in Table 3, the gonotrophic cycle (mean 4.1 vs 4.04 days, $t = 0.171$; $p = 0.86$) and egg incubation period (mean 5.5 vs 5.7 days, $t = 1.53$; $p = 0.15$) of *Ma. bonneae* and *Ma. dives* are fairly similar. The host plant, *H. cordata*, however, supported significantly higher success pupation and adult emergence rates for *Ma. bonneae* viz-a-viz *Ma. dives* (Table 3) ($\chi^2 = 9.9$; $p < 0.01$ for success pupation; $\chi^2 = 15.9$; $p < 0.001$ for adult emergence rate). This may explain the higher relative abundance of *Ma. bonneae* in the study area (eg Kampong

Ampungan) where *H. cordata* is the major and preferred aquatic plant for this vector species (Chang, 1989).

The first adult emergence of males and females *Ma. dives* occurred on day 25 after hatching and the total duration of adult emergence continued till day 50, while the duration of adult emergence of males and females *Ma. bonneae* occurred between day 27 to day 51 after hatching. There was a similar peak of emergence on day 32 for both males and females *Ma. bonneae*. But there was no distinct peak of emergence for males and females *Ma. dives* (Fig 1). Under field insectary conditions, 63.0% of *Ma. dives* and 68.4% of *Ma.*

bonneae females survived beyond the 12th day after emergence. According to Wharton (1957a), the extrinsic incubation period of *B. malayi* in the *Mansonia* vector is around 12 days. Hence, our studies showed that the two *Mansonia* vectors are able to survive long enough to transmit the disease. This was confirmed by Chang (1989) that the natural infection rate with *B. malayi* infection for *Ma. bonneae* and *Ma. dives* was identical (0.19%).

Our study has therefore fulfilled the main objective of mass producing homogenous stages of larvae and adults of *Ma. dives* from a field insectary. Moreover, extensive studies on the biology, genetics and control measures can now be made for this little known vector of *B. malayi*.

ACKNOWLEDGEMENTS

This study received financial support from the National Research Council, Washington DC, under the Grant No. MVR-MY/1-83-28. We are grateful to the Director of Medical Department, Sarawak for his support and permission to publish this article. Mr John Lah and the entomological team from Vector Borne Disease Control Unit, Medical Department, Sarawak, provided invaluable assistance, and Dr J Hii for valuable advice. We also wish to thank Ms Siti Maryam Masnor for typing the manuscript.

REFERENCES

- Breeland SG, Hackbarth L, Parsons RE, Wilson FL. An artificial attachment medium and submerged air source for larvae and pupae of *Mansonia* and *Coquillettidia*. *Mosq News* 1981; 41 : 800-2.
- Chang MS. Biology and control of *Mansonia* vectors of brugian filariasis in Sarawak. National University of Singapore 1989; 533 pp. PhD Dissertation.
- Cheong WH, Chiang GL, Loong KP, Samarawickrema WA. Laboratory colonization of *Mansonia* in Malaysia : A preliminary report. *Mosq News* 1984; 44 : 72-3.
- Chiang GL. Laboratory and field studies of *Mansonia* and *Coquillettidia* mosquitos in relation to ecology and transmission of filariasis in Malaysia. Universiti Sains Malaysia 1987; 245 pp. PhD Thesis.
- Chiang GL, Cheong WH, Loong KP, Eng KL, Samarawickrema WA. Laboratory colonization of *Mansonia uniformis*, *Ma. indiana* and *Ma. bonneae* in Malaysia. *J Am Mosq Control Assoc* 1985; 1 : 186-90.
- Mak JW. Filariasis : Epidemiology of filariasis in Malaysia. In : Proceeding of the IMR 75th Anniversary Symposium on Dengue, Filariasis, Malaria and Scrub typhus 1976; 78-9.
- Mak JW. Filariasis : An overview of past research. In : Mak JW, ed. Filariasis. *Bull Inst Med Res Malaysia* 1983; 19 : 11-5.
- Sucharit S, Apiwathnasorn C, Rukkeartsukul R, Kerdpi-bule V. Improved oviposition medium for *Mansonia* colonization. *Mosq News* 1982; 42 : 357-9.
- Wharton RH. Studies on filariasis in Malaya : The efficiency of *Mansonia longipalpis* as an experimental vector of *Wuchereria malayi*. *Ann Trop Med Parasitol* 1957a; 51 : 422-39.
- Wharton RH. Studies on filariasis in Malaya : Notes on the breeding of *Mansonia (Mansonioides)* mosquitos in the laboratory. *Ann Trop Med Parasitol* 1957b; 51 : 297-300.
- Wharton RH. The biology of *Mansonia* mosquitos in relation to transmission of filariasis in Malaya. *Bull Inst Med Res Malaysia* 1962; 11 : 1-114.
- Wilson T. Filariasis in Malaya - A review. *Trans R Soc Trop Med Hyg* 1961; 55 : 107-34.