LABORATORY COLONIZATION OF *MANSONIA* MOSQUITOES WITH AN EMPHASIS ON *MA. ANNULATA* AND *MA. BONNEAE*

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Abstract. The present study records the first successful colonization of *Mansonia annulata* and describes colony maintenance with modification of rearing medium and host plants. Three species of *Mansonia* mosquitoes (*Ma. uniformis, Ma. indiana* and *Ma annulifera*) were successfully reared in ambient environments with adult emergence rates > 50%, while *Ma. bonneae* and *Ma. dives* yielded emergence rates > 30%. Colonization of *Ma. annulata* was modified and improved so that they were successfully raised to adult with emergence rates of 23%. Tube sedge, *Lepironia articulata,* was utilized as a host plant and peat swamp water was used as a rearing medium. Yeast and small lizard droppings were added daily to the larval medium to maintain microorganisms and pH in the infusion. However, identifying suitable culture medium remains an obstacle to establishing colonies of *Ma. annulata*, as the culture medium is difficult to mimic in the laboratory. Further study, focusing particularly on larval attachment substrates and rearing medium, is needed to develop a standardized and practical rearing technique for *Mansonia* mosquitoes.

INTRODUCTION

Information on the basic aspects of the mosquito life cycle, such as oviposition, developmental biology of immature stages, adult longevity, and behavior, are important parameters for understanding the biology of insect vectors of tropical diseases, and transmission related to disease control. In many instances (*eg* most Culicidae) such biology can be obtained from field observations; however, in other vectors, such as phlebotomine sandflies, information is only partly or rarely available, owing to the difficulty of obtaining immature stages from breeding habitats. In such cases, colonization in the laboratory represents the most feasible and accessible source of information on these stages. In addition, the vectorial status of some insects can only be determined and confirmed through laboratory strains. All these concerns have contributed to growing efforts to colonize various species of insect vectors, including mosquitoes. The colonies are then used to study vector-parasite relationships, bionomics, physiology, genetics, and insecticide susceptibility of individual species and local varieties.

Mansonia mosquitoes have proven difficult to rear in the laboratory because of problems with food availability in the rearing medium and their dependence on host plants (Laurence, 1964). Since *Mansonia* larvae feed while attaching to the roots and stems of aquatic plants, the major difficulty in maintaining them has been the preparation of aquatic culture media with adequate food for larval

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development, and the provision of host plants for attachment of immature stages. Moreover, the life cycle of *Mansonia* mosquitoes is normally > 20 days at 28-29ºC (Chiang, 1993).

Attempts have been made to colonize these mosquitoes in different insectaries since 1952. Several authors developed rearing techniques by providing various kinds of larval medium and attachment sources, either naturally or artificially (Jayewickreme and Niles, 1952; Smith, 1956; Laurence and Smith, 1958; Samarawickrema, 1958; Ramachandran, 1960). Wharton (1962) described a technique using rabbit dung infusion and *Eichhornia crassipes* for rearing six species of *Mansonia* mosquitoes. In his experiment, an outdoor insectarium was arranged to prolong the survival of host plants in the rearing infusion. The overall adult emergence rate was highest (40%) in *Ma. uniformis* and lowest (0.3%) in *Ma. annulata*. Apiwathnasorn (1987) reported a mass-rearing technique for *Ma. annulifera, Ma. indiana* and *Ma. uniformis,* which was adapted from Wharton (1962), using *Ludwigia adscendens* as the host plant and a 1:4 diluted infusion of guinea-pig-dung powder as a rearing medium, with a daily provision of yeast under insectary and verandah conditions. Styrofoam slices were used instead of aquatic plants for oviposition (Sucharit *et al*, 1982). The average emergence rate was >50% in all species and the colonies were maintained for >7 years. Chiang *et al* (1985) reported successful colonization of *Ma. uniformis, Ma. indiana* and *Ma. bonneae* by replacement of aquatic plants with a special kind of paper ("Keaycolor" ruffia snow-white paper, manufactured by Wiggins Teape Paper, Ltd). This system also worked for *Ma. dives* (Chan *et al*, 1991). However, the adult emergence rates of those 3 species were <30%. In addition, there was no report of successful colonization of *Ma. annulata.*

The present study aimed to demonstrate development and improvement of rearing techniques to establish a laboratory colony of *Mansonia* mosquitoes and to raise successfully *Ma. annulata,* Narathiwat strain.

MATERIALS AND METHODS

The rearing technique for *Mansonia* mosquitoes used in the present study was modified from Apiwathnasorn (1987). Wild-caught *Ma. annulata, Ma. bonneae* and *Ma. dives* females were obtained from cow-baited trap collections in Narathiwat Province and those of *Ma. annulifera, Ma. indiana* and *Ma. uniformis* from Phra Nakhon Si Ayuthaya Province. Fully engorged females of each species were kept separately in paper drinking cups (HARN CUP, 20 cm^3, NISSHO SANGYO Japan) covered with screening material; they were fed with 5% solution of multi-vitamin syrup with 2% sucrose for 5 days. They were then transferred to an oviposition pot (plastic container, 9.5 cm in diameter and 9.0 cm high) covered with a netting lid containing polystyrene strips (1.5 x 1.5 cm,1 mm thick) lying on the entire surface of distilled water, for egg laying. The females were held in the oviposition pot until they oviposited (usually 2-3 days) and then removed and fed on an anesthetized hamster to obtain another oviposition.

Egg clusters of each species were collected and transferred to separate plastic cups containing distilled water. After hatching (5-7 days), 300 larvae were put into a plastic jar (20 cm^3) containing 2 liters of larval rearing medium [ground dried guinea-pig dung for stock and working (diluted) larval media] and fresh aquatic plants (*Ludwigia adscendens* and *Lepironia articulata*). Each aquatic plant species was tested for suitability as a host plant by determining tolerance to rearing infusion and mosquito emergence rate. A small amount of powdered yeast (Fermipan® red DSM Bakery Ingredients Mijlweg 77, Dordrecht, Holland) was added daily to each rearing jar. After 10 days, the rearing medium

and aquatic plants were searched for larvae and the medium was replaced with fresh rearing infusion and fresh plants. The infusion was slightly acidic at first, (pH 6.5-6.9) and was neutral to slightly alkaline (pH 7.0-7.2) by the time it was refreshed. Yeast was provided daily until adult emergence. Adult male and female mosquitoes were then transferred by aspirator to a paper cup, provided with a 5% solution of multi-vitamin syrup, where mating occurred. A few days after emergence, the females were provided with a blood meal and were ready to oviposit within 5-6 days.

The insectary was maintained at 24 ± 2ºC, 80% RH. It was illuminated with a combination of natural daylight and fluorescent lighting for approximately 12 hours for 2 days.

Experiment setup

Treatment 1. Rearing mosquitoes under insectary and ambient conditions as described by Apiwathnasorn (1987). Six species of *Mansonia* (*Ma. annulata, Ma. annulifera, Ma. bonneae, Ma. dives, Ma. indiana*, and *Ma. uniformis*) were used. The number of 1st-instar stage larvae used was 300/jar/experiment. The rearing medium was 1:4 diluted guinea-pig infusion (5 g per 1,000 ml tap water). Creeping water primrose, *Ludwigia adscendens,* was used as the host plant during treatment. The mosquitoes were reared under insectary conditions and partially shaded verandah (28-41ºC).

Treatment 2. Rearing mosquitoes in swamp water under field conditions. Three species of *Mansonia* (*Ma. annulata, Ma. bonneae*, and *Ma. dives*) were used. The number of 1st-instar larvae used was 300/jar/experiment. The rearing medium was swamp water plus small lizard droppings (10 pieces daily). *Lepironia articulata* was used during treatment as the host plant. The mosquitoes were reared in a partially shaded verandah at Pikun Thong Development Study Center, in the peat-swamp forest, Narathiwat Province, Thailand.

RESULTS

In Treatment 1, the rearing jars were held under insectary conditions for 10 days and later on a partially shaded verandah until adult emergence. It demonstrated that the combination of insectary and verandah conditions, using plants with strong stems (*L. adscendens*) resulted in the satisfactory rearing of *Ma. uniformis, Ma. indiana* and *Ma. annulifera,* which were open-swamp dwellers, with adult emergence rates > 50%, but less successful for the swamp-forest dwellers, *Ma. bonneae* and *Ma. dives* (Table 1).

As indicated in Table 1, *Mansonia* mos-

Table 1
Laboratory colonization of *Mansonia* mosquitoes under insectary and ambient conditions, using *L. adscendens* as host plant.

Mansonia species	No. of experiment	Mean percentage of survival to day 10	Mean percentage of adult emergence (range)	Developmental time from larva to adult (days)
uniformis	10	81.3	78.6 (72.6-84.2)	16-24
indiana	10	82.4	63.8 (58.8-69.4)	16-26
annulifera	10	65.6	57.3 (52.5-62.5)	16-25
bonneae	10	37.3	24.6 (20.4-30.1)	16-25
dives	10	31.2	21.7 (17.3-26.6)	15-24
annulata	10	11.3	0	-

Table 2
Laboratory colonization of *Mansonia* mosquitoes using natural rearing medium and local host plants from peat-swamp forest.

Mansonia species	No. of experiment	Mean percentage of survival to day 10	Mean percentage of adult emergence (range)	Developmental time from larva to adult (days)
bonneae	10	37.6	33.5 (27.3-40.3)	20-26
dives	10	31.7	31.0 (26.7-36.7)	29-37
annulata	10	30.3	23.0 (16.3-27.7)	41-52

Table 3
Summary of laboratory colonization of *Mansonia* mosquitoes.

Mosquito species	Treatment	Adult emergence rate			
		Range	Mean	Standard deviation	Confidence level (95%)
Ma. uniformis	1	72.6-84.2	78.62	4.43	3.17
Ma. indiana	1	58.8-69.4	63.83	3.81	2.72
Ma. annulifera	1	52.6-62.5	57.30	3.68	2.63
Ma. bonneae	1	20.4-30.1	24.60	3.37	2.41
	2	27.3-40.3	33.50	4.38	3.13
Ma. dives	1	17.3-26.6	21.70	3.04	2.17
	2	26.7-36.7	31.02	3.31	2.37
Ma. annulata	1	0	0	0	0
	2	16.3-27.7	23.0	3.71	2.65

quito breeding in Treatment 1 achieved varying success, with the highest adult emergence rates for *Ma. uniformis* (78.6%) and the lowest for *Ma. annulata* (0%). No adult emergence occurred for *Ma. annulata*.

Treatment 2 was an attempt to raise *Ma. bonneae, Ma. dives* and *Ma. annulata* under a partially shaded verandah, replacing rearing infusion with swamp water (pH 4.5-6.0), and *L. adscendens* with the local plant, *Lepironia articulata*. In addition, lizard droppings were added daily instead of yeast. To obtain fresh rearing medium (swamp water), this trial was conducted at a field station in the peat-swamp forest of Narathiwat Province. Colonization of these 3 species was satisfactory. Adult emergence rates of *Ma. bonneae* and *Ma. dives* increased to >30% and *Ma. annulata* to >20% (Table 2).

Table 3 summarizes the emergence rates obtained from laboratory colonization of *Mansonia* mosquitoes. The wide range and high standard deviation of all results revealed that the rearing procedures were not adequately standardized. Variations were possibly due to rearing medium, ambient temperature, or host plants. However, the mosquito colonization yields were satisfactory.

DISCUSSION

An attempt was made to improve laboratory colonization of *Mansonia* mosquitoes,

to increase yields from rearing techniques, measured by either larval survival or adult emergence rates, with a focus on rearing medium and aquatic host plants. Although Chiang *et al* (1985) and Chan *et al* (1991) improved colonization of *Mansonia* mosquitoes by using paper for larval attachment, adult emergence rates were < 30%. In the present study, the rearing technique was based on using local aquatic plants under local environmental conditions.

Owing to the long duration of the life cycle of all *Mansonia* species, the main obstacle to colonization was the maintenance of host plants in fresh condition in the larval medium, and larval infusion. The creeping water primrose, *Ludwigia adscendens,* was found to be a suitable host plant, as shown in Table 1, yielding optimized developmental times and adult emergence rates for *Ma. annulifera, Ma. indiana,* and *Ma. uniformis,* but less success for *Ma. bonneae* and *Ma. dives*. *L. adscendens* is a perennial creeper herb that can be found along the shoreline, floating on the water surface, or growing upright. Its round, soft, yellowish green or reddish-purple stems have a spongy bladder-like structure, and rooting at nodes provides suitable sites for larval attachment. The plants are common, and easy to collect, clean, and maintain in the laboratory. *L. adscendens* remained fresh after immersion in larval infusion for several days.

Successful colonization of *Ma. annulata* to the adult stage was obtained by rearing in their swamp forest environments, using either swamp water as breeding medium or the local host plant, *Lepironia articulata*. However, the low emergence rate (23%) for *Ma. annulata* reflected the micro-environment in nature and hence limited the ability to propagate this species. *L. articulata* is an evergreen, slow-growing wetland sedge that occurs naturally in the low-lying acidic areas of peat-swamp forest in Narathiwat Province, particularly in an open environment. The tube sedge is a reed-like, perennial plant in the Family Cyperaceae. The plant can grow up to 2.3 m tall with tubular, hollow, bright-green stems 2-3 cm in diameter. The stems arise from creeping, underground rhizomes in clumps. It is typically grown in swamps with strongly acidic sulfate soil. The pH of the water (4.0-5.5) and plants with a particular root system of short taproots, stilt roots and strong, widespread lateral roots to support themselves on the soft ground in the peat swamp forest (Phlengklai, 1989), may play an important role in rearing *Ma. annulata* and *Ma. bonneae.*

A suitable culture medium for establishing *Ma. annulata* in the laboratory is needed, as it is difficult to mimic in the laboratory. Further study focusing particularly on larval attachment substrates and rearing medium is essential to develop a more standardized rearing technique for *Mansonia* to yield higher larval survival and adult emergence rates, as well as reducing the development period.

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