flight periods for nearly all occurred in association with sunrise and sunset as shown in Figure 1.

It is concluded that the optimum time to schedule ground or aerial adulticiding treatments is during evening twilight. When early morning treatments are desirable, the most effective treatment time is during morning twilight, or dawn.

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LABORATORY STUDIES OF *TOXORHYNCHITES SPLENDENS* Part I. COLONIZATION AND LABORATORY MAINTENANCE

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ABSTRACT. All known earlier attempts to colonize *Toxorhynchites splendens* (Wiedemann) have been unsuccessful. This report records the first successful laboratory colonization of this species and a description of colony maintenance is given.

INTRODUCTION

The genus *Toxorhynchites* consists of large usually brilliantly colored diurnal, non-bloodsucking species, occurring mostly in the tropics. Because the larvae are predacious on other mosquito larvae in areas where they co-exist in aquatic habitats, their potential usefulness for the biological control of certain vector mosquitoes in the tropics has been favorably discussed (National Academy of Sciences 1973).

The adults of *Toxorhynchites amboinensis* have been successfully used as laboratory hosts for the propagation and assay of

dengue viruses (Rosen, personal communication). In view of this, it was desirable to establish laboratory colonies of *Toxorhynchites* for possible use in the parenteral inoculation technique in our dengue studies in Malaysia.

Toxorhynchites splendens breeds in *Nipa* palm axils, fallen coconuts, and occasionally in tree-holes and artificial containers along the coastal areas of Malaysia. Its distribution includes Sri Lanka, India, eastwards through Thailand, Malaysia and Indonesia to New Guinea, and the Philippines (Macdonald 1958).

A number of workers have contributed biological information on *Tx. splendens* (Paine 1934, Newkirk 1947, Cheong and Ganapathipillai 1964, Jenkins 1964, Chan 1968, Gerberg 1970, Yasuno and Tonn 1970). All known earlier attempts to colonize this species have been unsuccessful (Paine 1934, Newkirk 1947). Peterson (1956) reported the establishment of laboratory colonies of a mosquito identified as this species but later shown to be *Tx. amboinensis*, as noted by Ramalingam and Belkin (1976).

This report records the first successful colonization of *Tx. splendens* and describes colony maintenance. Results of laboratory biological studies will be reported elsewhere.

COLONIZATION AND LABORATORY MAINTENANCE

In November 1976, 145 males and 91 females were collected with sweep nets while they were resting on or hovering about coconut tree trunks, 2–4 m above ground, in Rantau Panjang, Selangor, Malaysia. The wild-caught adults were held in cages and transported to the laboratory where they were released in a 61 x 61 x 61 cm breeding cage and fed 10% honey solution soaked in cotton pads, 10 x 8 x 1 cm. Two such cotton pads were placed on top of the cage and covered with plastic covers to retard water evaporation. Every 48 hr new honey-soaked cotton pads were furnished to the adults. Wet towels were draped along three sides of the cage to maintain a high humidity. The towels were resoaked with tap water every 24 hr. The insectary was maintained at 26.6 ± 4° C and RH of 78 ± 5%. No. attempt was made to regulate photoperiod in the insectary.

Eggs were initially collected from black plastic jars (diameter 8 cm, height 9 cm), that were filled with tap water to within 5 cm of the rim and placed in each corner of the cage. This method of collecting eggs was abandoned after it was observed that a large number of eggs were not being deposited in the oviposition jars but were landing on the floor paper of the cage. This could be attributed to females being haphazard in ovipositing or that several females hovering over the oviposotion jar at the same time created air drafts that caused the eggs to be "blown" out of the jar. Instead, a black surfaced pan, 30 x 25 x 5 cm, filled with tap water to a depth of 15 mm was placed in the center of the cage floor. By this method of egg collection no further loss of eggs was observed. The oviposition pan was removed from the cage after 24 hr and the eggs were left in the pan for another 24 hr. After this period of time the 1st instar larvae and unhatched eggs were poured into an enamelled pan, 35 x 30 x 5 cm, where the larvae were visible for counting. Fifty 1st instar larvae were transferred to a pan, 35 x 30 x 5 cm, containing 500 cc of tap water. First instar larvae of either *Aedes aegypti* or *Ae. albopictus*, at a 10:1 prey-predator ratio, were added to the pan of water. Crushed mouse pellets were added as food for the prey. Each pan was identified as to the generation number, date of egg collection and date of egg hatch, and covered with a clear glass plate to reduce evaporation. Additional pans of prey larvae were maintained to replenish those consumed in the rearing pans.

The 1st instar larvae were retained in the rearing pans until they developed to the 2nd instar. To avoid undue cannibalism, 2nd instar larvae were transferred to individual 120 ml plastic vials supplied with 30 cc of tap water and 10 prey larvae. No food was added for prey

larvae. Rearing vials were placed on a wooden tray which held 49 vials. To conserve space, trays were stacked one upon the other to form three levels, the top tray covered with a clear glass plate. Once every 24 hr each rearing vial was examined for larval development and activity. A 10:1 prey-predator ratio was always maintained in each vial with consumed prey larvae being replenished with larvae of about the same developmental stage as the predator. The water of each vial was changed frequently until pupation occurred.

Pupae were poured from their rearing containers into a round sieve, 15 cm in diameter and 10 cm deep with a mesh opening of 2 mm^2 that was placed in a pan of water. In this fashion, pupae were rapidly separated from the rearing water, accumulated debris and concentrated together. Twenty-five pupae were then transferred with a wide-mouth pipette to a clear plastic cup, diameter 8 cm, height 9 cm, containing 200 cc of tap water. Each cup was identified as to the generation number, date of pupation and number of pupae and placed in a larger breeding cage, 122 cm high x 61 cm x 61 cm.

Emerging adults were supplied with 10% honey solution and wet towels as previously described. After completion of emergence, the plastic cups were removed from the cage. Adults were observed to mate more readily in cages of this size which could accommodate over 4,500 The cage was constructed of 2.5 x 2.5 cm fixed wooden frames covered with wire screening, 18 x 22 mesh, on top and on all sides except the floor. The floor of the cage, 61 x 61 cm, was constructed of plywood, 6 mm in thickness. The front and back of the cage were fitted with 45 cm long surgical stockinette sleeves attached to 20 cm^2 openings.

The first batch of eggs was laid 6–8 days after the first emergence of adults, at which time the population consisted of approximately 50 females and 70 males.

Adults of the 1st 2 generations were slightly smaller than wild-caught adults. Those of subsequent generations were larger or similar in size to wild-caught adults. The colony is now in the 8th generation at the time of this report, and following the procedures described above there should be no reason why the colony could not be maintained indefinitely.

ACKNOWLEDGMENTS

The technical assistance provided by the personnel of the Division of Medical Entomology, Institute for Medical Research, and the Arbovirus Research Unit is duly acknowledged. Special thanks are extended to the Director, Institute for Medical Research, Kuala Lumpur. We are grateful to Professor A. S. Dissanaike and Dr. S. Ramalingam and staff, Department of Parasitology, University of Malaya Faculty of Medicine for their cooperation and assistance given throughout the work.

Supported in part by research grants AI-10051 and AI-11540 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service.

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COLORLESS-EYE, A RECESSIVE AUTOSOMAL MUTANT OF *ANOPHELES STEPHENSI*

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ABSTRACT. A recessive autosomal *colorless-eye* mutant has been found spontaneously occurring in a laboratory colony of *Anopheles stephensi* Liston. Mosquitoes of this genotype express *colorless-eye* at the larval, pupal and adult stage.

INTRODUCTION.

Because of the resurgence of malaria, research is being directed toward the genetics of *Anopheles stephensi* Liston, a major vector of malaria. A mutant with white-eye color has been isolated from our laboratory colony. In addition to the phenotypic expression of this mutant in eye, the body color is also reduced so that individuals can be distinguished from wild type larvae as early as in the 2nd instar, with the naked eye. Eye-color mutants provide an easy and workable expression in genetic studies and have been reported in several mosquito species (Gilchrist and Haldane 1947, Wild 1963, Iltis et al. 1965, Barr and Meyers 1966, Bhalla 1968). A white-eye sex linked mutant in *An. stephensi* var. *mysorenis* has been reported by Aslamkhan (1973). We have designated this mutant as *colorless-eye* which is a recessive autosomal. This paper describes the mode of inheritance of this mutant.

MATERIAL AND METHODS

Specimens were collected from Sonepat (Haryana State) in 1973 and reared following a procedure evolved at this laboratory. Mosquitoes were held in standard laboratory cages, 30 × 30 × 30 cm in size, and maintained at 27-28°C and 70-80% RH. *Colorless-eye* individuals were observed both in rearing pans and in the adult colony. *Colorless-eye* mosquitoes were isolated and established as pure lines. In genetic crosses, *colorless-eye* mutants and wild eye individuals were used.

RESULTS AND DISCUSSION

Results of genetic crosses are given in Table 1. Results revealed that when *colorless-eye* females (*c*/*c*) were crossed with wild eye males (+/+), F_1 progeny consisted of all wild eye individuals (cross 1). When F_1 progeny of phenotypically wild individuals were inbred the F_2 progeny consisted of 366 wild eye and 140 *colorless-eye* individuals i.e. in a ratio of 3:1 ($\chi^2 = 2.08$ n.s.). In the reciprocal cross (cross 2), 347 wild eye and 125 *colorless-eye* mosquitoes were scored, i.e. again in a ratio of 3:1 ($\chi^2 = 0.48$ n.s.). Backcross of F_1 heterozygous females (+/*c*) from the above cross with *colorless-eye* males (*c*/*c*) produced 212 wild