NOTES ON A LABORATORY COLONY OF *ANOPHELES BALABACENSIS* BAISAS, 1936[1]

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INTRODUCTION. Interest in malaria in Southeast Asia has been stimulated since 1961 by the appearance of strains of *Plasmodium falciparum* in Viet Nam, Malaysia, Cambodia and Thailand which are highly resistant to all or most of the synthetic anti-malarial drugs. In almost all cases these strains were detected in areas where *Anopheles balabacensis* Baisas, 1936 appeared to be the sole or principal vector (Sandosham, 1963). This species was implicated as a malaria vector in several areas of Asia during and after the Second World War, but its importance was most clearly demonstrated by McArthur (1950), who also reviewed the earlier records of infection in the species, under the more commonly used name *Anopheles leucosphyrus* Donitz, 1901. Colless (1956, 1957) studied the *A. leucosphyrus* group in detail and showed that *A. balabacensis* was the member of the group present in most of mainland Southeast Asia, including the countries listed above. In Thailand, *A. balabacensis* has been demonstrated to be an important malaria vector, particularly in forested hill regions (Scanlon and Sandhinand, 1965). The increasing interest in this species as a malaria vector indicated the desirability of establishing a laboratory colony, and this was undertaken at the U. S. Army Medical Component-SEATO in 1963.

METHODS AND MATERIALS. Female *A. balabacensis* were brought to the insectary in Bangkok from a study area at Khao Mai Khaeo, Cholburi Province, southeast of Bangkok over a period of several months in late 1963 and 1964. The stock from which the colony was finally established was captured in February 1964. Extreme care was exercised in handling these wild adults, as a significant number were believed to be infective for malaria. Despite these precautions one of the technicians was bitten while removing an oviposition dish and contracted a chloroquin-resistant malaria infection. Females were permitted to feed on laboratory animals and then allowed to oviposit on filter paper discs kept in contact with moist cotton pads. The females were housed in two-cubic foot cages in an insectary maintained at 26° to 28° C., with a relative humidity of 60 to 80 percent. The insectary was illuminated for 13 hours a day with a combination of natural daylight and fluorescent lighting.

Eggs were removed from the filter paper discs to enamel trays containing 2,500 ml. of seasoned tap water, over a layer of sterilized stream sand. Water in the trays was aerated gently, using a standard aquarium pump. The larvae were fed finely ground dog biscuit added in small quantities several times a day to the water surface. Ordinary paper drinking straws, formed in circles, were placed on the water surface to prevent stranding of the eggs and later to provide refuge for the larvae. Pupae were removed from the larval trays with a wide-mouthed pipette and placed in bowls of water in the colony cages for adult emergence. The colony cages contained cotton pads soaked in multiple vitamin syrup diluted to 40 percent with water or to 5 percent with canned apple juice. One day after emergence the adults were separated by sex into groups of 20 to 30 mosquitoes in drinking cups with mesh covers. While in the cups the adults were continuously furnished the multi-vitamin mixture. The following day the females were released

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into a cage and permitted to feed on a guinea pig after a period of starvation lasting from 12 to 24 hours. The males were retained in paper cups. Soon after feeding the females were mated with males using the system of forced insemination described by Yang *et al.* (1963) for *A. maculatus* Theobald, 1901. Inseminated females were maintained in paper drinking cups with moist filter paper on the bottom and mesh covers until oviposition occurred.

RESULTS AND DISCUSSION. The colony was established from the progeny of 128 females captured in February 1964. Sixty-three of these females deposited 3,871 eggs (61.4 eggs/female), a somewhat lower average than seen in later laboratory generations. Detailed observations were made on oviposition by the females of the 22nd laboratory generation. At that time the minimum number of eggs per female was 37, the maximum was 488 and the mean was 163. A fairly large proportion of the 22nd generation females underwent more than one oviposition cycle, and the results of the multiple ovipositions are summarized in Table 1. The number of eggs per female fell off markedly after the third cycle. The combination of this factor and the natural decrease in surviving females resulted in the accumulation of approximately 97 percent of the egg total by the end of the third cycle. Efficient management would probably be best served by limiting producing females to one or two cycles once the colony is firmly established. Inseminated females survived from 7 to 40 days in the insectary.

TABLE 1.—Oviposition by Inseminated Female *Anopheles balabacensis*—Twenty-Second Laboratory Generation.

Oviposition cycle	Number of females	Percent of total	Number of eggs	Eggs per female
First	111	100.0	11,305	101.4
Second	58	52.2	5,020	86.5
Third	13	11.7	1,082	83.4
Fourth	7	6.3	402	57.4
Fifth	3	2.7	202	40.4
Sixth	1	0.9	60	60.0

Eggs oviposited in water containers hatched in approximately 48 to 72 hours. More uniform hatching could be obtained by allowing the females to oviposit on moist filter paper and accumulating the papers for up to 5 days. Eggs hatched rapidly and synchronously under such conditions, particularly when the eggs were 72 hours old. The larval stage lasted for 1 to 3 weeks, depending on the insectary temperature. Use of the sand substrate in the rearing pans seemed to be helpful but not essential for larval development. A marked effect was noted, however, when attempts were made to dispense with aeration of the water in the larval pans. The hatching and survival of the early instars were adversely affected by lack of aeration, while the older instars showed little or no difference. Larvae fed equally well on a suspension of powdered dog biscuit in water added to the pans with a pipette, or on the dry food sprinkled on the surface. Pupation took place almost entirely at night, as did emergence of the adults.

Adult survival was promoted by allowing them ready access to the multiple vitamin syrup, and the males in particular succumbed rapidly unless they were taken from the emergence cages and placed in cups in close proximity to the nutritive fluids. Females did not feed well on vertebrate hosts unless starved for a period of over 12 hours, but when so treated they fed rapidly at any time of the day in subdued light.

Wild caught females took blood readily from guinea pigs, but the first laboratory generation females fed poorly on guinea pigs or other laboratory animals, including chicks. They were also somewhat reluctant to feed on man, the preferred host in nature. After several generations the females again fed readily on guinea pigs, and no further difficulty has been encountered.

Several attempts were made to establish a sub-colony by natural mating in cages

of various sizes, but no consistent success has been achieved. On one occasion 700 females and an equal number of males were placed in a cage one cubic meter in capacity and provided with guinea pigs as a source of blood. Three generations were obtained, in decreasing numbers, but no fourth generation was obtained. Work is continuing on this problem. However, as noted by Yang *et al.* (1963) it is quite feasible to obtain sufficient numbers of adults for experimental purposes by the forced insemination technique if necessary.

The Thailand colony has now been established for over thirty generations. A thriving sub-colony has been established at the Walter Reed Army Institute of Research (WRAIR), using the same techniques as reported here. An additional attempt was made at WRAIR to establish a colony using field-collected female *A. balabacensis* collected at Sabah, Malaysia by Dr. F. Y. Cheng, WHO entomologist at Jesselton. This strain has proved to be extremely reluctant to feed on man or laboratory animals under any circumstances, and it does not appear likely to become well established. However, Miss E. Wall (1963) established a colony of *A. balabacensis* at the London School of Tropical Medicine several years ago using eggs shipped from Jesselton. The colony was allowed to die out due to lack of demand, but it appears that some strains from Sabah are amenable to colonization. This matter deserves further attention, since colonies from areas other than Thailand may be useful for comparative malaria transmission studies.

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References Cited

COLLESS, D. H. 1956. The *Anopheles leucosphyrus* group. Roy. Ent. Soc. London, Trans. 108(3):37–116.

———. 1957. Further notes on the systematics of the *Anopheles leucosphyrus* group. Roy. Ent. Soc. London, Proc. 26(7–8):131–139.

MCARTHUR, J. 1950. Malaria and its vectors in Borneo. Ind. J. Malar. 4(1):1–90.

———. 1951. The importance of *Anopheles leucosphyrus*. Trans. Roy. Ent. Soc. Trop. Med. Hyg. 44(6):683–693.

OW YANG, C. K., STA. MARIA, F. L., and WHARTON, R. H. 1963. Maintenance of a laboratory colony of *Anopheles maculatus* Theobald by artificial mating. Mosquito News 23(1):34–35.

SANDOSHAM, A. A., WHARTON, R. H., EYLES, D. E., WARREN, M. and CHEONG, W. H. 1963. Malaria in Perlis. Med. Jour. Malaya 18(1): 46–51.

SCANLON, J. E., and SANDHINAND, U. 1965. The distribution and biology of *Anopheles balabacensis* in Thailand. Jour. Med. Ent. 2(1):61–69.