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FIELD AND LABORATORY OBSERVATIONS IN SABAH, EAST MALAYSIA ON THE PROPORTION OF ANOPHELES BALABACENSIS BALABACENSIS EGGS HATCHING AFTER HOLDING IN A HUMID ATMOSPHERE

by

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1. INTRODUCTION

Anopheles balabacensis balabacensis is one of the most important vectors of malaria in South-East Asia, and the main vector of malaria in Sabah, East Malaysia. It is a mosquito of the rain forest and its fringe. Its efficiency as a vector of malaria in this region is well known. Interest in this species has been stimulated by the fact that the presence of Plasmodium falciparum strains resistant to all or most synthetic antimalarials appeared in areas where this species is the sole or principal vector (Sandosham et al., 1963). The failure to interrupt malaria transmission in many areas where this species is a vector has also stimulated more fundamental research on its bionomics.

Sabah is highly mountainous and the land could be described as a tangled mass of forested hills and ridges occupying the major part of the country. The climate varies markedly from one valley to the other. Two monsoon rainy seasons occur, but many areas in the interior that are in the rain shadows of hill ranges have frequent dry spells lasting up to two to three months.

The breeding places of A. b. balabacensis are normally to be found in shade under the vegetative cover, amongst secondary vegetation near villages bordering the jungle, or within the jungle itself. The favourite types of breeding places are buffalo hoof prints, buffalo wallows in partially dried streambeds, temporary pools on the margins of stream banks after a dry spell, seepages, blocked earth drains in rubber plantations and in wheel ruts.

Breeding places may also be found in dense primary jungle far from villages near water holes where wild animals come to drink. Adults have been collected biting man in the evening at these locations in the jungle.

2. OBSERVATIONS AND RESULTS

Careful observations were made of known breeding places in one of our study areas (Papar) to see when first stage larvae appeared after dry potential breeding places were filled up with rain water. In nearly all these previously dry breeding places observed, first stage larvae appeared on the third or fourth day after the rain had filled them. In the laboratory observations on duration in water before hatching of A. b. balabacensis eggs gave an average period of between 2.5-3 days, which tallied with our field observations.

The eggs of A. b. balabacensis in natural breeding places, as in the laboratory, strand easily. They could be seen when floating freely on the water surface, forming irregular patterns, either linear edge to edge, triangular edge to edge, flower-like whorls or haphazard arrangements.

In the laboratory it was noticed that first instar larvae were often found in the breeding trays that had third and fourth instar larvae, although all the eggs placed in that particular tray came from the same female and were of the same age. It was later found that these larvae were a late brood from stranded eggs which hatched when fresh water was introduced to compensate for evaporation. Davidson (personal communication) reported that A. b. balabacensis eggs sent to him from Sabah gave a good hatch five days after despatch. The eggs were sent in plastic soap boxes that had moist filter paper placed inside in layers between egg batches.

Cheong and Santa Maria (unpublished document)[1] reported that A. b. balabacensis eggs could hatch after a period of slow drying in a moist chamber. On Day 0 they found an 83.6% hatch and on Day 15 a 9% hatch.

The rainfall in Sabah is unpredictable. Breeding places may dry up as quickly as they are filled with rain water. It is possible that newly laid eggs are stranded for days or weeks in such breeding places before the next shower. It would be an excellent way to tide over this unfavourable period if the eggs could withstand some degree of temporary desiccation.

In an experiment to check whether stranded eggs are found in known breeding places when drying up, 17 mud samples were collected from breeding places in Lingan Papar from which free water had disappeared. The samples were introduced into beakers and water added. The mud was stirred with a glass rod so that any free floating matter that was trapped could come to the surface. The samples were left overnight. In three out of the 17 samples tested, a total of seven Anopheline eggs could be seen floating at the edge of the water line. The eggs were carefully transferred with a camel-hair brush to finger-bowls containing water as used for laboratory rearing. Six out of the seven eggs hatched within three days and went through all the aquatic stages to emerge as two female and four male A. b. balabacensis.

In these earlier studies it was apparent that A. b. balabacensis eggs from mud in drying breeding places could hatch when water was added to stranded eggs. To further check the actual number of days that eggs remain viable when kept in a humid atmosphere, and the percentage hatchability, we devised the following experiment.

Both halves of plastic petri dishes were spread with a 1/4-inch (0.6 cm) layer of cotton-wool; over this layer two layers of filter paper were placed in each half. The cotton-wool and filter paper were moistened with distilled water so that the absorbent material soaked up enough water, without run-off when the dish was tilted.

A known number of eggs on 1/2-inch (1.2 cm) diameter filter paper discs were placed on the bottom half of the petri dish. The top half was now put in place and sealed tight to the bottom half with cellotape. Twenty-three such sets were made, containing a total of 10 900 eggs. The petri dishes containing eggs were kept at room temperature (average, 27°C). The eggs were from the F4 generation of the colony (Lingan Papar strain, Sabah) and from wild females from Lingan.

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[1] The effect of slow drying of Anopheles balabacensis balabacensis eggs on hatching. Cheong, W. H. & Santa Maria, M. E., presented at Seminar and laboratory meeting, Singapore, 1968.

One of the 23 petri dishes was opened each day and the contents transferred to trays containing tap-water. Daily counts of the numbers hatching were then made over several days. There were no delays in hatching beyond the third day following transfer, except in the "Day 8" batch when a few eggs hatched after the third day in the water.

Hatches of over 50% were noted from Day 0 to Day 5, while later the percentage hatch progressively decreased except on Day 8 when slightly over 50% hatched. Viable eggs were found up to the 27th day (see Table 1).

It was noticed that eggs laid by wild-caught females sometimes did not hatch for over seven days, even in water. While checking these eggs in the laboratory for embryonation it was found that when slight pressure was applied on the coverslip the operculum "popped out" and live larvae were seen creeping out of the artificially hatched eggs.

3. DISCUSSION AND CONCLUSIONS

Many vector insect species are known to produce eggs that can withstand desiccation but these reviewed by Bates (1949) are mainly found in the aedine and culicine groups. Although with anophelines, Holstein (1954) obtained hatching up to 16 days after holding A. gambiae eggs in damp clay and Breeland et al. (1970) found A. albimanus eggs would hatch up to 15 days after being held in saturated mud, it seems rather unique that the egg of A. b. balabacensis could remain viable out of free water for a period of nearly a month.

A. b. balabacensis is a mosquito of the tropical rain forest where the rainfall is unpredictable. The breeding places are always shallow and temporary and are normally under shade. The forest floor is very humid due to slow evaporation from the spongy moisture-absorbent humous material and accumulation of dead leaves and vegetation. Hence although breeding places lose their free water, the high humidity probably keeps the eggs viable for fairly long periods till the next rains come. These eggs were found towards the end of the rainy season and hence may possibly have been specially adapted to survive the oncoming dry period.

The success of A. b. balabacensis, as a malaria vector so widely spread throughout the forests of South-East Asia, may be related to its adaptability to the unpredictable climatic conditions and to the ecosystem of the rain forest. Survival of this species in the egg stage during dry spells may be a means to tide over the dry season when breeding places containing water are scarce.

TABLE 1. THE PERCENTAGE HATCH OF A. B. BALABACENSIS EGGS KEPT IN HUMID ATMOSPHERE AND WETTED AT VARIOUS INTERVALS OF TIME

Number of eggs	Day of wetting	Number of larvae	% Hatch
200	0	172	86%
200	1	167	83.5%
500	2	396	79.2%
500	3	327	65.4%
500	4	297	59.4%
500	5	263	52.6%
500	6	218	43.6%
500	7	222	44.4%
500	8	260	52%
500	9	215	43%
500	10	187	37.4%
500	11	163	32.6%
500	12	170	34%
500	13	57	11.4%
500	14	63	12.6%
500	15	66	13.2%
500	16	71	14.2%
500	17	14	2.8%
500	18	17	3.4%
500	19	8	1.6%
500	20	2	0.4%
500	27	3	0.6%
500	28	0	0%

RESUME

Des études ont été faites à Sabah (Malaisie orientale) sur l'éclosion d'oeufs d'Anopheles balabacensis balabacensis, moustique qui vit dans la forêt équatoriale et sur sa lisière dans l'Asie du Sud-Est, où il est l'un des principaux vecteurs du paludisme.

En remuant dans de l'eau des échantillons de terre prélevés à l'état sec dans des endroits connus pour servir de gîtes larvaires, on a pu mettre en évidence la présence d'oeufs de A. b. balabacensis qui ont éclos et donné des adultes en laboratoire. Dans un lot de 10 900 oeufs conservés en atmosphère humide et introduits à différents intervalles dans des plateaux d'élevage larvaire, on a observé des éclosions jusqu'au 27ème jour.

Il ressort de ces observations que, comme certains culicinés, Anopheles b. balabacensis peut survivre à des périodes de sécheresse à l'état d'oeuf.

SUMMARY

Mud samples from known Anopheles balabacensis balabacensis breeding places which were drying up revealed, when stirred with water, A. b. balabacensis eggs that hatched and produced adults in the laboratory. Ten thousand nine hundred eggs kept in a humid atmosphere, when introduced into larval trays at different intervals, gave hatches up to the 27th day. It appears from these observations that Anopheles b. balabacensis can survive dry spells in the egg stage like some culicines and aedines.

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