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Preliminary studies on blood meal interval of *Aedes polynesiensis* in the field[1)]

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Abstract: Preliminary studies on the interval between two successive blood meals of *Aedes* (*Stegomyia*) *polynesiensis*, the main vector of subperiodic bancroftian filariasis in the South Pacific, were undertaken by two methods: one, by mark-and-release experiments in Fiji, and the other, by the size of developing filarial larvae in the mosquitos from Tuvalu. The results obtained by both methods were similar, and the average blood meal interval in the field was estimated to be three or four days under the temperature range 25–31°C. The latter method is considered to be useful for estimation of blood meal interval of vector mosquitos in endemic areas of filariasis.

INTRODUCTION

Aedes (*Stegomyia*) *polynesiensis* is the main vector of subperiodic bancroftian filariasis in the South Pacific. They bite humans in the daytime, with two peak activities of early morning and late afternoon.

The interval between two successive blood meals of a mosquito species is one of the essential variables of transmission dynamics of mosquito-borne diseases, since daily mortality of mosquitos is usually estimated from two variables, the parous rate and the blood meal interval, or gonotrophic cycle, of the mosquitos.

A number of field studies were undertaken on blood meal interval, or gonotrophic cycle, of *Aedes aegypti* (Sheppard *et al.*, 1969; McClelland and Conway, 1971; Pant and Yasuno, 1973; Conway *et al.*, 1974). No reports of such field studies, however, have been made so far on *Ae. polynesiensis*, except laboratory observations by Ingram (1954) and Jachowski (1954).

Presented herewith are preliminary studies on the blood meal interval of *Ae. polynesiensis*; one by mark-and-release experiments in Fiji, and the other, by the size of developing filarial larvae in field-caught mosquitos from Tuvalu (formerly the Ellice Islands).

STUDIES BY MARK-AND-RELEASE EXPERIMENTS

1. Materials and methods

Preliminary mark-and-release experiments were carried out from December 1973 to January 1974 in the backyard of the anthor's residence in Suva, Fiji, which was located close to a swamp and heavily populated with *Ae. polynesiensis*. The capture and recapture were made in a limited area of about 10 m², and the release was made in the centre at the same square. Very few human blood sources were available for mosquitos in the area, except the human bait of the present experiments.

A number of *Ae. polynesiensis* which intended to bite human bait were collected by the author with a sucking tube and transferred to a mosquito cage. The author's left hand and forearm were kept in the cage for one to one-and-one-half hours so

1) This study was undertaken when the author was assigned to the WHO Intercountry Filariasis Advisory Team, stationed in Suva, Fiji.

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that the mosquitos could feed. Then they were anaesthetized lightly by chloroform and only fully-fed mosquitos (partially-fed mosquitos too in the last experiment) were used for the marking procedure.

Three dye solutions were used for marking, *i. e.* methylene blue, metanil yellow and crecyl violet. Dye solution (2%) was prepared by dissolving the dye in the mixture of ethyl alcohol and water (1 : 1). The solution was sprayed onto the anaesthetized mosquitos by means of a small perfume atomizer. The marked mosquitos were allowed to fly out naturally from a paper cup at the release spot. The approximate time for each procedure was as follows: collection: 06.30–08.30 h; feeding: 08.30–10.00 h; marking: 10.00–11.00 h; and release: 11.30 h.

In the first series of experiments, marking and release were made on three consecutive mornings, each morning with a different colour. In the second series, on the first morning, marking was made with blue colour on fully-fed mosquitos, and on the second morning, with yellow colour on fully-fed mosquitos and purple colour on partially-fed mosquitos. In the first series of experiments, recapture on human bait was made every morning at 07.00 h for about two hours, from the day following the last release until the eleventh day. In the second series, in addition to recapture in the morning, a recapture was also made in the afternoon of the day of the release of yellow- and purple-marked mosquitos. During the recapture of mosquitos, care was taken to collect only the mosquitos which started feeding on human bait. Recaptured mosquitos were spread on a sketch-paper. A small amount of a manifesting agent consisting of ethyl alcohol, glycerine and chloroform (3 : 3 : 1), was dropped on each mosquito which was then squashed with forceps. By this procedure, the marking was easily identified.

It was confirmed by a cage test that a few mosquitos sprayed with the dye solution died, mostly without recovering from the anaesthesia, but most of the recovered mosquitos survived for 13 days, with a similar mortality to the unmarked control.

During the period of the experiments, the weather was fine almost every day, except for occasional rain showers during the second series; the mean daily maximum and minimum temperature was 31.7°C and 25.4°C, respectively.

2. Results

The total number of fully-fed mosquitos released after marking was 449, of which 40 (8.9%) were recaptured; and of 64 partially-fed mosquitos released, 30 (46.9%) were recaptured. In the first series of experiments, 1,301 mosquitos were captured during an 11-day survey; and in the second series, 1,409 were captured during a 13-day survey.

The results of the experiments with fully-fed mosquitos are shown in Table 1 and Fig. 1. In the figure, the first peak is ob-

Table 1 Number of mosquitos released and of marked mosquitos recaptured (Fully-fed mosquitos only)

Series and Exp. No.	No. of mosquitos released	No. of marked mosquitos recaptured (Days after release)												
		1	2	3	4	5	6	7	8	9	10	11	12	13
I-(1)	116			7	1				2					
(2)	105		2		4		2	1			1			
(3)	76				4		1	2	1			1		
II-(1)	98			1	4	1								
(2)	54			2	2			1						
Total	449	0	2	10	15	1	3	4	3	0	1	1	0	0

Note: The frame in the table shows the period within which recapture and identification is possible.

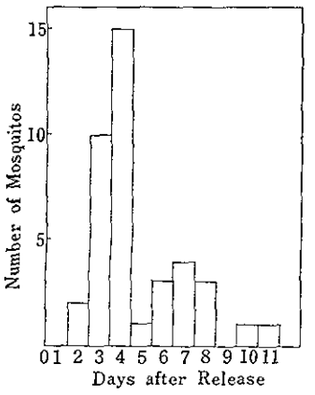

Fig. 1 The distribution of 40 recaptured *Ae. polynesiensis* by the number of days between capture and recapture

served three to four days after release; and the second peak six to eight days after release. It is reasonable to assume that the mosquitos forming the second peak have finished one blood meal in the field before recapture. Therefore, the interval of two consecutive blood meals is estimated to be three to four days.

In the experiments with partially-fed mosquitos, 64 mosquitos were released. In the afternoon of the day of release, 26 or 40.6 % were recaptured, about six hours after a partial blood meal. At about 24 hours after the partial blood meal, three mosquitos were recaptured; and about 48 hours after the meal, one was recaptured. Therefore, it can be concluded that a good part of the mosquito population which fed once but not to repletion could easily visit hosts again as early as six hours after the partial blood meal.

Studies by Size of Developing Filarial Larvae in Mosquitos

1. Materials and methods

An experimental infection of subperiodic *Wuchereria bancrofti* with *Ae. polynesiensis* was undertaken during the period February to March 1974, in Nukulaelae Island of Tuvalu, where filariasis was endemic. Females of *Ae. polynesiensis* collected on human bait in the bush of an inhabited islet, were fed on two volunteers with respectively 360 and 122 microfilariae per 60 cmm of peripheral blood.

The experimentally-infected mosquitos were caged in paper cups at room temperature, but with higher humidity. Every day until day 13, a few live mosquitos in the cups were killed with chloroform, and kept in a mixture of 70% ethyl alcohol and glycerine (19 : 1) for at least one hour. After staining for two days with 0.05% Azur-II solution, they were dissected under a dissecting microscope. The length and width of the developing larvae were measured with a micrometer attached to a compound microscope.

The mean daily maximum and minimum temperature during the experiment was 31.2°C and 26.0°C, respectively.

Dissection of field-caught mosquitos. Females of *Ae. polynesiensis* were collected on human bait in six islands of Tuvalu, *i. e.* Nui, Niutao, Nanumea, Nanumanga, Nukulaelae and Nukufetau, mainly by the local filariasis control team, during the period November 1972 to May 1974. Immediately after collection, the mosquitos were blown into the mixture of alcohol and glycerine, and were kept in the solution until staining. Staining, dissection, identification and measurement of the developing larvae were done by the author using the same procedure as in the experimental infection studies.

2. Results

Size of developing larvae in the experimentally-infected mosquitos. The mean length and width of developing larvae are shown in Table 2 and Fig. 2, on each day from day one to day seven, and on each two days from day eight to day 13. On a day-size graph, the mean length of the larvae forms a parabola-like curve, as shown in Fig. 2.

On day one, the larvae were thin and long, then they decreased in length, and, at the same time, increased in width. The shortest stage was between day three and day four. Thereafter, both length and width increased until around day seven. After day eight, the length still increased, but the width remained the same. On day 12, the first infective larva appeared.

There was not much difference in the length of larvae, between day one and day

Table 2 Mean length and width of developing larvae of *W. bancrofti* in experimentally-infected *Ae. polynesiensis*

Day	No. of larvae measured	Mean length in micron	Mean width in micron
1	28	256	5.2
2	100	168	6.9
3	105	126	11.6
4	48	120	17.6
5	39	164	17.3
6	30	218	21.8
7	37	260	26.1
8- 9	80	380	26.3
10-11	72	521	28.0
12-13	33	637	26.7

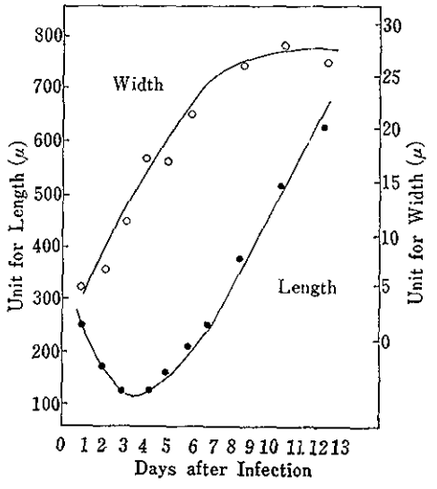

Fig. 2 Mean length and width of developing filarial larvae
A calibration curve for estimating duration of infection

seven, day two and day five, and day three and day four. However, the width increased continuously and markedly from day one to day seven. Therefore, it is easy to differentiate the larvae of day one from day seven, those of day two from day five, and those of day three from day four.

The relationship mentioned above makes it possible to estimate the duration of filarial infection in field-caught mosquitos, if the larvae found are in the developing stages.

Estimate of blood meal interval of field-caught Ae. polynesiensis. Forty-five *Ae, polynesiensis* with developing filarial larvae which were collected from six islands of Tuvalu, were used for the current studies. Estimate of duration of filarial infection was made by applying the mean length and width of the larvae in each mosquito to the calibration curve shown in Fig. 2. Since all the mosquitos were collected on human bait when they intended to take a blood meal, the duration of infection in each mosquito could be equal to n times (n=1, 2, 3.....) of the interval of two successive blood meals.

In Fig. 3 and Table 3, four peaks are demonstrated : the first one on day three to day four ; second, day six to day seven ; third, day nine to day 10 ; and fourth, day 11 to day 12, although the last two peaks were low. From the first and second peaks, the mean interval of two successive blood meals can be estimated at three to four days.

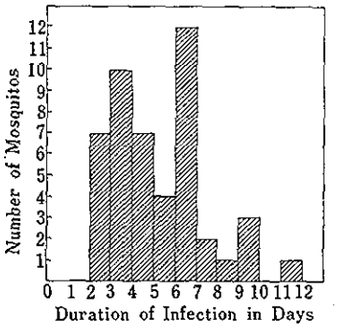

Fig. 3 Duration of filariasis infection in field-caught *Aedes polynesiensis* assessed by size of developing larvae

During these studies, double infection was observed in two mosquitos ; one from Nui, and the other from Nukufetau. In the former mosquito, the duration of infection was estimated at 6.1 days for the first infection and 2.7 days for the second. In the latter mosquito, the duration was 9.1 days for the first infection, and 6.7 days for the second.

DISCUSSION

The blood meal interval of *Ae. polynesiensis* was estimated at about three to four days, both by mark-and-release experiments and by the size of developing filarial larvae. The interval assessed by the two different meth-

Table 3 Estimate of duration of filarial infection in field-caught *Ae. polynesiensis* by size of developing larvae

Duration of infection in days		No. of mosquitos						
Range	Median	Nui	Niutao	Nanumea	Nanumanga	Nukulaelae	Nukufetau	Total
1- 2	1.5	0	0	0	0	0	0	0
2- 3	2.5	7	0	0	0	0	0	7
3- 4	3.5	5	3	0	0	0	2	10
4- 5	4.5	2	0	0	0	1	4	7
5- 6	5.5	1	0	0	1	0	2	4
6- 7	6.5	8	0	1	0	0	3	12
7- 8	7.5	1	0	0	0	0	1	2
8- 9	8.5	0	0	0	0	0	1	1
9-10	9.5	1	0	0	0	1	1	3
10-11	10.5	0	0	0	0	0	0	0
11-12	11.5	1	0	0	0	0	0	1
Total		26	3	1	1	2	14	47

Note: One mosquito each from Nui and Nukufetau Islands harboured larvae of two batches of different size (see text). In the above table, two different batches in a mosquito are shown as if two mosquitos.

ods in the different places coincided well with each other.

Ingram (1954) and Jachowski (1954) reported the interval between two blood meals of *Ae. polynesiensis* by individual rearing in the laboratory. Ingram (1954) mentioned that the average interval from blood meal to oviposition is about four days, and the interval from oviposition to the next blood meal is about three days, consequently the blood meal interval is about seven days, at a temperature of 21.2–32.7°C. Jachowski (1954) reported that the interval between two blood meals is 7.3–7.6 days at a temperature of 26.7–28°C.

These reported intervals agree well with each other, but curiously enough, they are much longer than, or about double, the estimates in the field during the present experiments. It is presumed that, under artificial and restricted laboratory conditions, it sometimes takes a long time from oviposition to blood meal, even if a blood source is made available continuously, as was the case of the above studies; while in the field, mosquitos would take a blood meal almost immediately after oviposition.

The method of estimating blood meal intervals of host mosquitos by the size of developing filarial larvae could be applied to any other mosquito vectors of filariasis, provided that mosquitos are collected during their biting activities, and that the temperature fluctuation between different seasons or between different localities is reasonably small, as was the case of the present studies in Tuvalu. Duke (1968) reported similar studies on onchocerciasis vectors, *i.e.* the age distribution of *Simulium damnosum* by stages of *Onchocerca volvulus* in the vector flies.

The methods for estimating daily survival rates of vector mosquitos of filariasis were devised by Laurence (1963) from the ratio of the number of mosquitos with infective larvae to the total number of infected mosquitos, and by van Dijk (1966) from the number of mosquitos with the first, second, and third stage larvae in an endemic area.

Althougn the present studies were preliminary and on a small scale, future well planned experiments involving observations on ovarian developmental stages and the presence of partial blood meals of recaptured mosquitos could give conclusive results on whether *Ae. polynesiensis* takes repeated

small blood meals subsequent to the first oviposition. Further, a sample of mosquitos from which those fed and marked for release should be dissected for parity in order to determine the age structure of the population to be studied.

The incubation period of subperiodic *W. bancrofti* in *Ae. polynesiensis* was reported to be about 12 days by Iyengar (1965), and from 11 to 17 days with an average of 14 days by Byrd and Amant (1959). A period of at least 12 days was also confirmed in the present studies. Based on the incubation period of the filarial larvae and the blood meal interval of *Ae. polynesiensis*, it can be concluded that *Ae. polynesiensis* coming to bite humans with infective larvae should have finished at least three blood meals, or that they should be at least three-parous mosquitos.

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摘　要

Aedes polynesiensis の野外における吸血間隔について——予報的研究

南太平洋において，亜周期性バンクロフト糸状虫症の主要媒介種である *Aedes* (*Stegomyia*) *polynesiensis* の吸血間隔について研究を行なった．1つは色素でマークした蚊を放し，再捕獲する方法により，他は，野外から吸血時に採集した蚊の体内におけるフィラリア仔虫の大きさによるものである．この両方法によって推定された蚊の吸血間隔はほぼ同様であり，25～31°C の温度環境下で3～4日と推定された．

なお，後の方法，つまり蚊の体内のフィラリア仔虫の大きさから吸血間隔を推定する方法は，はじめての試みであり，フィラリアの流行地域ではどこでも応用できる有効な吸血間隔推定法と考えられる．