ENTOMOLOGY

Hatching Pattern of *Aedes aegypti* Eggs

DURING their work on the effect of ultrasonic waves on the hatching of *Aedes aegypti* eggs Quraishi *et al.*[1] found an interesting pattern of hatching in the controls. *Aedes aegypti* eggs laid overnight were kept in water in Petri dishes and the percentage of larvae hatching out each day was recorded for 20 days. On the first day 2·5 per cent of the eggs hatched out; on the second day this percentage was 13, the highest recorded for any single day. The percentage of eggs hatching out gradually decreased after the second day, until after the 13th day, when there was an upward trend in hatching, and a small peak was discernible between the 14th and 16th days. There was a sudden fall in the number of eggs hatching out after the 16th day and by the 20th day only 62·5 per cent of the eggs hatched out. Thus 37·5 per cent of apparently normal eggs were left unhatched. We therefore decided to follow the hatching pattern of *Aedes aegypti* eggs for 50 days.

Fresh and well-formed eggs laid during the previous night were selected early in the morning and were divided into batches of 50 each and kept in Petri dishes, 13·5 cm in diameter, containing 75 ml. tap water. A few mg of yeast in tablet form was added to each dish and the dishes were kept in incubators at 88° ± 2° F. They were taken out for half an hour every 24 h for observation. The first, second, and subsequent days were counted as 24, 48, etc., hours after the eggs had been selected; since at the time of selection the age of these eggs was already 0–16 h, the time lapse will be number of days plus 0–16 h. Fifty replicates were done and the data are thus based on 2,500 eggs.

The pattern of hatching is shown in Fig. 1. Four peaks in the pattern of hatching are easily discernible; the first peak, which is also the highest, appears on the second day and is followed by a much smaller peak between the 14th and 16th days. Very few larvae hatch out after the 16th day, though some larvae come out each day. An upward trend in hatching is again discernible between the 20th and 23rd days representing a third peak which is smaller than the second. Between the 23rd and 43rd days only a few eggs hatch out, not more than 0·2 per cent of the total on any one day. On the 44th and 45th days, however, 0·2 per cent and 0·76 per cent of the eggs hatch out, representing the fourth and smallest peak.

After the completion of this work in Karachi, Pakistan, I worked in Tehran, Iran, and maintained another colony of *Aedes aegypti* from a local strain obtained from the Institute of Parasitology and Malariology, University of Tehran. Although only a few observations were made, a similar pattern of hatching with four peaks was observed. The last peak in this case was also observed on the 45th day.

The occurrence of four hatching cycles, each succeeding one smaller than the previous one, in the eggs of an insect the larvae and pupae of which are entirely aquatic, is evidently an adaptation to meet the exigencies of a situation where the first few showers may not provide enough water for the completion of the entire aquatic cycle.

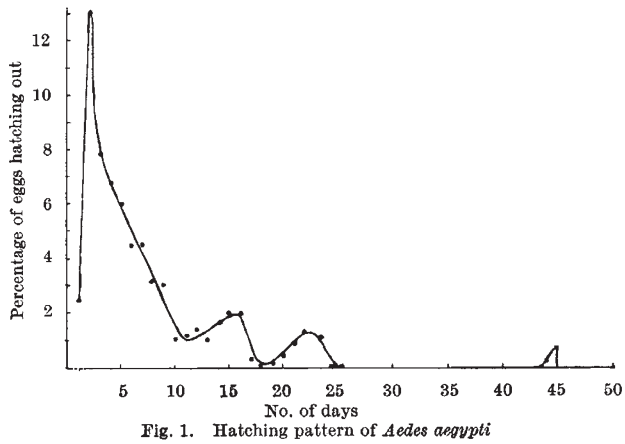

Fig. 1. Hatching pattern of *Aedes aegypti*

This work was done while I was working as senior scientist in the Pakistan Council of Scientific and Industrial Research and in the CENTO Institute of Nuclear Science, Tehran. I thank Dr. S. Siddiqui, Dr. I. H. Usmani and Dr. M. L. Smith for advice, and Mr. Wahajuddin Roomi and Mr. A. B. M. Lutful Kabir for their help. Dr. Ch. M. H. Mofidi provided the eggs of *Aedes aegypti* in Tehran.

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[1] Quraishi, M. Sayeed, Osmani, M. H., and Ahmad, S. Hilal, *J. Econ. Ent.*, **56**, 668 (1963).

Rearing *Pieris brassicae* L. Larvae on a Semi-synthetic Diet

A METHOD for breeding *Pieris brassicae* L., the large white butterfly, all the year round has been developed[1,2], and the larvae have proved to be convenient insects to use for insecticidal tests and other investigations. However, in severe winters cabbage and kale leaves are killed by frost and it becomes difficult to provide food for large numbers of larvae. One possibility, namely using the heart leaves of cabbage from clamps[3], has not proved to be satisfactory. Furthermore, for investigations on pathogens, it is desirable to have a food which can be sterilized.

Recently Ignoffo[4] described a medium for the mass rearing of *Trichoplusia ni* Hübner, the cabbage looper, which is a modification of that developed by Vanderzant and Reiser[5,6]. We have slightly modified this medium and have shown it to be satisfactory for rearing *P. brassicae*:

Formula of medium

(*a*) Distilled water	110 ml.
Potassium hydroxide 4 molar	1·8 ml.
Casein (light white soluble)	12·6 g
(*b*) Sucrose	12·6 g
Wheat germ ('Bemax')	10·8 g
Cabbage (dried powder)	5·4 g
Salt mixture	3·6 g
Whatman, chromedia cellulose powder, *CF*11 grade	1·8 g
(*c*) Choline chloride (10% soln.)	3·6 ml.
Methyl parahydroxy-benzoate (15% in 95% EtOH)	3·6 ml.
Formaldehyde soln. (10% w/v soln.)	1·5 ml.
Vitamin stock	0·8 ml.
(*d*) Distilled water	200 ml.
Agar (fine Japanese powder)	9 g
(*e*) *l*-Ascorbic acid	1·5 g
Aureomycin (veterinary grade)	0·8 g

The composition of the salt mixture (in g) is[7]: $CaCO_3$, 120; K_2HPO_4, 129; $CaHPO_4 \cdot 2H_2O$, 30; $MgSO_4 \cdot 7H_2O$, 40·8; NaCl, 67; $FeC_6H_5O_7 \cdot 6H_2O$, 11; KI, 0·32; $MnSO_4 \cdot 4H_2O$, 2·0; $ZnCl_2$, 0·10; $CuSO_4 \cdot 5H_2O$, 0·12.

The composition of the vitamin stock (in mg) is: nicotinic acid 600; calcium pantothenate, 600; riboflavine (B_1), 300; aneurine hydrochloride (B_2), 150; pyridoxine hydrochloride (B_6), 150; folic acid, 150; D-biotin, 12; cyanocobalamine (B_{12}), 1·2; 100 ml. water.

The dried cotton leaf in the original medium (about 1·4 per cent wt.) was replaced by dried cabbage leaf powder. This was prepared by drying cabbage leaves in thin layers in a ventilated oven at 105° C for 15–20 min. The leaves can then be ground by hand with a roller and sieved through a 60-mesh sieve, but it is much quicker, of course, to have them ground in a small Christy-Norris mill fitted with a 0·5-mm mesh screen. The veterinary grade aureomycin soluble powder (Cyanamid of Great Britain) contained 25 g chlortetracycline hydrochloride per pound.

The ingredients listed in (*a*) are placed in a blender with a capacity of 800 ml. and thoroughly mixed together. The mixed solids (*b*) are then added with further blending. The solutions (*c*) are next added, separately, while the blender is running. Meanwhile, the agar solution (*d*) has been prepared in a water bath. It is cooled to 70° C and added to the mixture. Finally, the ingredients (*e*) are added and the whole medium is thoroughly blended.