BIOLOGY

A Rapid Method for examining Diapause Embryos of *Acheta commodus* W.

IN the course of current work on the diapause of eggs of the common field cricket *A. commodus* it became necessary to determine the exact stage of embryonic development at which the arrest of development occurs.

Brookes[1] has described a method by which she traced the morphological development of non-diapause embryos of *Acheta*. The method involves fixing, staining and sometimes sectioning the eggs, and may take several weeks to complete.

In the present diapause studies, numerous observations are necessary to determine the onset and duration of diapause at different temperatures, so the long delay in the method used by Brookes would severely limit the scope of the work.

The desirability of using whole eggs rather than sections is evident. Slifer[2] found that by simply immersing eggs of *Melanoplus differentialis* in water the stages during katatrepsis can be observed under a dissecting microscope. However, diapause in *A. commodus* occurs during anatrepsis, the embryo is small and immersed in the yolk, and is not visible by this method.

The following treatment enables large numbers of eggs to be rapidly fixed and cleared in such a way that good optical differentiation of the embryo is provided without the use of stains (Fig. 1).

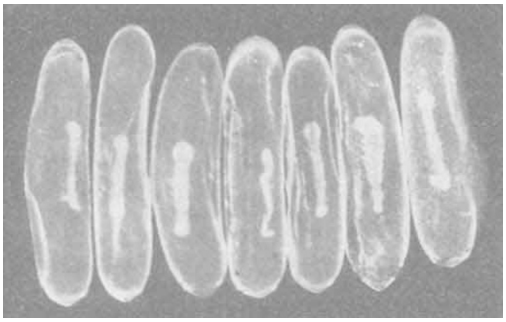

Fig. 1. Embryos of *A. commodus*, showing both diapause and early post-diapause stages of development. (× *c.* 12)

The eggs are placed in a watch box and soaked in water for about ½ hr. The water is then drained off and replaced with the following mixture for 20–30 min. at 34° C.: glacial acetic, 2 parts; chloroform, 2 parts; absolute alcohol, 1 part. They are then transferred to a mixture of glycerol, 1 part, and alcohol (70 per cent), 1 part.

The first mixture will be recognized as having the same ingredients, in different proportions, as Carnoy's fixative. However, the fixative mixture is not effective. The time necessary in this solution varies with different batches of eggs according to their history, particularly the temperature at which they entered diapause. A preliminary trial for the timing is therefore desirable.

The time in the second solution is not critical. Clarity will be improved by standing at room temperature overnight and the improvement will continue for several days, but the stage of development can be identified within the first hour.

After the treatment the embryos are visible to the naked eye, but, for details of their morphology, the examination is made under a dissecting microscope. A black background and incident light are essential. Careful attention should be paid to the angle of incidence of the light.

I am indebted to Miss J. E. Kenyon for help in developing the method.

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[1] Brookes, H. M., *Trans. Roy. Soc. South Aust.*, **75**, 150 (1952).
[2] Slifer, E. H., *Science*, **107**, 152 (1948).

Influence of Larval Population Density on Fluctuation in Mosquito Numbers

RECENT ecological studies on culicine mosquitoes in and around a village in south-western Nigeria[1] revealed that the numbers of *Aedes aegypti* fluctuated throughout the year in a manner which could not be correlated with the observed external environmental factors. During a preliminary survey it was established that this species was restricted to breeding within the village area in clay water-pots of the type used for collecting and storing water, but that it was absent from natural habitats in the surrounding rain forest. The fact that it showed a typically domestic breeding pattern facilitated a study of the population dynamics throughout the year, and the method used for obtaining an index of breeding intensity was as follows. A number of clay pots were placed in the village and sampled weekly, the pre-adults (larvæ and pupæ) being removed for counting while the pots were cleaned and refilled with fresh water. The number of pre-adults found weekly was taken as an index of the changes in density of the breeding population within the village.

To study this numerical fluctuation of *A. aegypti* the value N was used as a population index for the village, it being equal to $n/(e)(w)$, where n is the total number of pre-adults taken per month, e is the number of experimental pots sampled per month and w is the number of weekly samplings per month. During the first four months of the year, N increased from 24·3 to 135·0, this being followed by a sharp decline in May (52·6), a slight increase in June (71·2) and a continued decline until October (21·3). There was then a further build-up, reaching a peak in December (71·2). It will be seen (Fig. 1) that these fluctuations were not related to the observed environmental factors, either directly or with a suitable time-lag to allow for the climate to be influencing the egg-laying behaviour of the female. It was thought that these fluctuations may have been dependent on density, related to the numbers of larvæ and pupæ found in the pots. Each pot may be considered as an isolated environment containing a unispecific population subject to intraspecific competition, such that any increase in numbers beyond a certain point would result in an increased pre-adult mortality due primarily to starvation.

An examination of this postulate was carried out in the laboratory in the following manner. A series of cultures was maintained, each containing different numbers of larvæ but in otherwise standard conditions of food supply and water volume. To ensure constant food supply, a solution of larval food was made up having the following constituents: wholemeal flour (46 per cent), ground oats (40 per cent), fish meal (8 per cent), dried skimmed milk (3 per cent), cod-liver oil (1 per cent), dried yeast