ON THE RATE OF COMPLETION OF DIAPAUSE DEVELOPMENT AT CONSTANT TEMPERATURES IN THE EGGS OF *GRYLLULUS COMMODUS* WALKER

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[*Manuscript received December* 24, 1951]

Summary

The median duration of low-temperature treatment required by the eggs of *G. commodus* in order that they may develop and hatch without interruption due to diapause was least at 12.7°C., slightly longer or about the same at 15.9°C., and considerably longer at 10.3°C. At 8.5°C. it was not found possible to obtain 50 per cent. of the eggs free from diapause.

The temperature at which the eggs were incubated following exposure to low temperature greatly affected the influence of the low-temperature treatment. At 26.5°C. the median exposure to each low temperature necessary for the completion of diapause development was considerably shorter than that necessary when the eggs were incubated at 20.9°C., whilst when 29.9°C. was used as the incubation temperature, the median effective exposure to low temperature was still further reduced.

At 20.9°C. no eggs were found to complete morphogenesis and hatch without interruption when not first exposed to low temperature, whereas at 26.5°C. an average of 1 per cent. of the eggs did so and at 29.9°C. 26 per cent. of the eggs were found to hatch promptly.

The total numbers of eggs that hatched regardless of whether they experienced diapause or not were about the same at the two higher incubation temperatures but the totals hatching at 20.9°C. were significantly reduced.

Following low-temperature treatment the total numbers of eggs that hatched at all the incubation temperatures were greater the longer the exposure to low temperature they had received.

I. INTRODUCTION

The processes that go on during diapause resemble ordinary morphogenetic processes in that they exhibit a trend in the rate at which they are completed at different constant temperatures (Andrewartha 1944). For this reason they have been termed *diapause development* (Andrewartha 1952). The details of these processes are not known but in certain species it has been shown that they culminate in the elaboration of a hormone that evokes competence to develop (Williams 1948).

If eggs of *Gryllulus commodus* be incubated from the time they are laid at some temperature within their developmental range, most of them will develop for a few days and then enter diapause (Browning, unpublished data). But if newly laid eggs are first exposed to an adequate low temperature for an

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adequate period and then incubated, most of them will develop continuously and hatch promptly without the intervention of any diapause (Browning 1952*b*). In this *G. commodus* is unusual for with most species in which diapause occurs low-temperature treatment is most influential *after* the insect has entered diapause (Andrewartha 1943; Burdick 1937; Parker 1930). However, Salt (1952) indicates that in *Melanoplus bivittatus* eggs diapause development may be completed before the eggs have reached the morphogenetic stage at which diapause usually supervenes, so that development may continue through this stage without interruption. There is no reason to believe that the processes that occur in these two species during exposure to cold, resulting in their competence to continue their development without interruption, differ fundamentally from those occurring in other species when exposed to cold after diapause has become manifest. In what follows these processes will be referred to as "diapause development" even though the egg has not actually entered diapause at the time when they are going on.

Three other properties of *G. commodus* tend to complicate the investigation of diapause in this species:

(*a*) The proportion of eggs entering diapause depends upon the environment in which the female was living before she laid the eggs. For example, crickets collected from the same place in successive years laid eggs that differed in the proportion entering diapause when incubated at the same temperature.

(*b*) When batches of newly laid eggs were incubated at a series of temperatures within their developmental range, the proportion of the eggs that entered diapause varied with the temperature. A higher proportion of the eggs entered diapause when the temperature was low and relatively more developed without interruption at the higher temperatures. In this the eggs of *G. commodus* resemble the larvae of *Loxostege* (Steinberg and Kamensky 1936) or *Heliothis* (Ditman, Weiland, and Guill 1940).

(*c*) Because the proportion of eggs that exhibit diapause is dependent upon the temperature at which the eggs are incubated it is not feasible to specify the influence of low temperature on the completion of diapause independently of the temperature at which the eggs were subsequently incubated. This is perhaps most easily visualized if one considers that the egg incubated at a relatively low temperature has a powerful tendency to enter diapause and therefore may escape diapause only if it has previously been exposed for a long period to low temperature, during which time diapause development has been carried to an extreme. On the other hand, the egg incubated at a relatively high temperature has a weaker tendency towards diapause and may escape diapause even though it had experienced only a short exposure to low temperature, during which diapause development may not have been carried very far.

Thus a sample of eggs obtained in any one year will have a certain average propensity for diapause, depending upon the environment of the parents, and this may be modified by the temperature at which the eggs are incubated. However, if the eggs are stored at certain low temperatures the tendency

towards diapause may be eliminated from more or fewer of the eggs, depending on the duration of the low-temperature treatment and the temperature at which the eggs are incubated.

The aim of the experiment reported in this paper was to determine the range of temperatures over which diapause development could be completed successfully by the eggs of *G. commodus* and to determine which temperatures within this range were more favourable and which less favourable for its completion. At the same time the experiment aimed at determining whether the influence of low-temperature treatment was modified by the subsequent incubation temperature and at assessing the degree of interaction between the two influences.

II. Experimental Design and Methods

The experiment involves the estimation of the mean time required to be spent by the eggs of *G. commodus* at each of a series of low temperatures, in order that they may develop and hatch promptly when subsequently placed at some convenient temperature within their developmental range. This may conveniently and precisely be done by making use of the method of probit analysis (Finney 1947) to estimate the "median effective dose" (or E.D. 50), of low-temperature treatment, which in this case is the duration of exposure to a particular low temperature necessary to promote the completion of diapause in 50 per cent. of the individuals in a random sample of eggs.

The experiment was therefore designed to estimate the median effective exposure to low temperature in a sample of eggs and this was done at each of four different temperatures: 8.5°, 10.3°, 12.7°, 15.9°C. Five random samples of 150 newly laid eggs each were placed at each low temperature and removed to another thermostat for incubation after varying periods of exposure to cold. Four samples of 50 eggs were placed at the incubation temperature with no preliminary low-temperature treatment, to serve as controls.

At 8.5° and 15.9°C. the samples of 150 eggs were treated for either 10, 20, 25, 30, or 40 days; at 10.5°C. treatment was continued for 5, 10, 15, 20, and 30 days; whilst at 12.7°C. treatments of 5, 9, 13, 16, and 21 days were given. These particular durations were chosen because it was anticipated that they would result in a range of effectiveness on either side of the median, as measured by the percentage of the eggs that hatched promptly. It became evident that these were not the best durations to have chosen, largely because the lowest temperature was not so influential and the highest more influential than was anticipated.

When each lot of 150 eggs had been at low temperature for the appropriate time, it was subdivided into three random groups of 50 eggs each. One of these was incubated at 20.9°C., one at 26.5°C., and the third at 29.9°C. This was done because previous experience had shown that the temperature at which the eggs were incubated influenced the proportion that hatched with no appreciable interruption due to diapause.

When setting up the experiment 3600 eggs were obtained, within 24 hours of having been laid, from field-caught crickets by the method previously described (Browning 1952*b*). These were placed in random groups of 25 eggs each on damp plaster in small jars. Two jars were allotted to each treatment.

Following low-temperature treatment, the eggs remained at the incubation temperatures for varying periods. At 20.9°C. daily observations of hatchings were continued for 90 days, at 26.5°C. for 75 days, and at 29.9°C. for 60 days. This was done in an attempt to enable the same proportion, at each temperature, of all the eggs that were competent to hatch, to do so within the time allotted. When the period of incubation was complete the number of eggs in each jar that appeared alive and healthy was recorded.

Hatching in most treatments was spread unevenly over most of the period of incubation and in order to decide which eggs had developed with no appreciable delay due to diapause, reference was made to the distribution of hatching of diapause-free eggs from which the results given by Browning (1952*a*) were calculated. In this way it was decided that at 20.9°C. eggs that hatched between the 30th and 38th days of incubation inclusive were those in which diapause was complete or did not occur. Similarly, the limits at 26.5°C. were taken between 14 and 19 days inclusive, and at 29.9°C. between 10 and 14 days inclusive.

III. Results

The solid black rectangles in Figure 1 represent the numbers of eggs that hatched within the time required by diapause-free eggs at each of the incubation temperatures and it can be seen that an increased duration of exposure to low temperature in general resulted in an increase in the number of eggs that developed and hatched promptly. From these results the median effective duration of exposure to each low temperature was calculated for each incubation temperature, making allowance for the numbers of eggs that developed free from diapause among the controls. Where it was not possible to carry out a rigorous calculation, an estimate of the E.D. 50 was made by eye. This was necessary either because the duration of low-temperature treatment had not been long enough to promote diapause completion in at least 50 per cent. of one of the samples (e.g. 12.7°C. followed by 20.9°C.) or because the numbers of diapause-free eggs in the samples of an array were too erratic to enable a precise calculation to be made (e.g. 15.9°C. followed by 29.9°C.) or, in one case (15.9°C. followed by 26.5°C.) because following the shortest duration of low-temperature treatment more than 50 per cent. of the eggs hatched promptly. In some cases no estimate of the median effective duration of exposure to low temperature was possible because at the duration producing the greatest number of diapause-free eggs less than 50 per cent. hatched promptly, whilst further increase in the duration of low-temperature treatment resulted in a decrease in the numbers of diapause-free eggs. This occurred at all the incubation temperatures following exposure to 8.5°C. In these cases it seems that no duration of exposure to low temperature would result in 50 per cent. of

the eggs hatching promptly and so the median effective duration of low-temperature treatment becomes meaningless. Similarly there is no reason to suppose that an E.D. 50 existed for the eggs treated at 10.3°C. and incubated at 20.9°C. (Fig. 1).

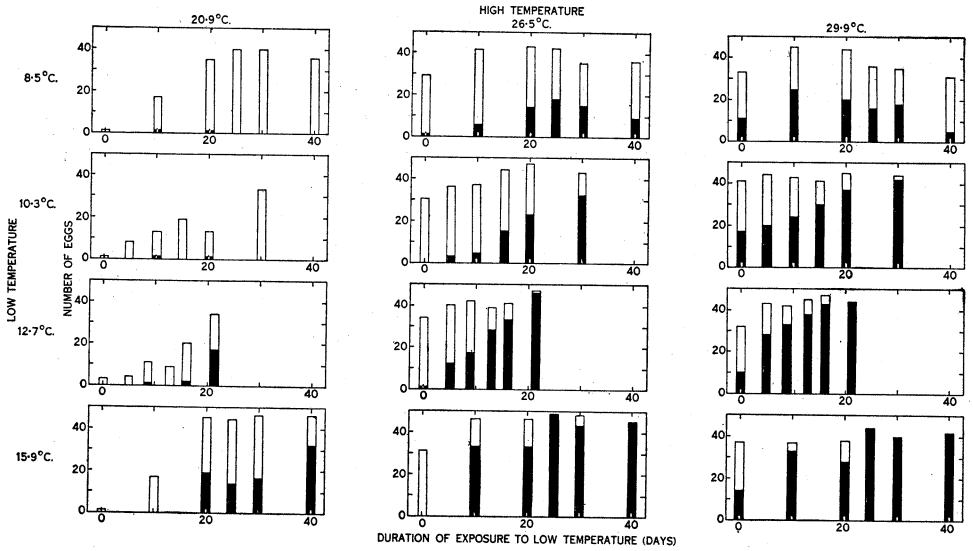

Fig. 1.—Histograms showing the total numbers of eggs that hatched in each treatment during the experiment (hollow rectangles) and the numbers of eggs in each treatment that hatched in the time required by diapause-free eggs at each particular incubation temperature (solid rectangles).

In Table 1 are set out the median durations of exposure to low temperature necessary to promote uninterrupted and prompt development of the eggs at each incubation temperature. These values may be converted to reciprocals and multiplied by 100 to give the percentage of diapause development completed per day, which is the most convenient form in which rates of morphogenetic development at constant temperatures may be represented (Davidson 1944). The results thus obtained are represented graphically in Figure 2. From the figure and Table 1 it can be seen that the rate of completion of diapause development at low temperature was dependent on the temperature used, being very slow at 8.5°C., increasing up to 12.7°C., and then becoming either less rapid or only slightly more rapid at 15.9°C. Such a trend in the rate of completion of diapause at low temperature has been demonstrated by Andrewartha (1943, 1944) and is implicit in a great deal of the work on the influence of temperature on the completion of diapause (e.g. Parker 1930).

From Table 1 and Figure 2 it can be seen that the influence exerted by any particular low temperature in inducing eggs to hatch without delay when subsequently incubated was dependent upon the temperature at which the eggs were incubated, being greatest at the highest of the incubation temperatures used. Indeed, under the conditions of the experiment, treatment at the

two lowest temperatures had no measurable influence on the completion of diapause when the eggs were incubated at 20.9°C. and at both 12.7° and 15.9°C. treatment had to be extended before any appreciable influence was discernible when the eggs were incubated at 20.9°C. When either of the other incubation temperatures was used all the low temperatures had a marked influence on reducing the proportion of eggs that entered diapause.

Temperature influenced the proportion of eggs among the controls (eggs that received no preliminary low-temperature treatment) that hatched without appreciable delay due to diapause (Fig. 1). Of the eggs placed immediately at 20.9°C., none was found to hatch without diapause; at 26.5°C. an average of 1 per cent. hatched promptly, whilst at 29.9°C. an average of 26 per cent. of the eggs hatched promptly. This response is similar to that of the larvae of *Loxostege* in which a greater proportion develop without diapause when reared at higher temperatures than at lower (Steinberg and Kamensky 1936).

TABLE 1

MEDIAN DURATION OF EXPOSURE TO EACH LOW TEMPERATURE (DAYS) NECESSARY TO PROMOTE PROMPT DEVELOPMENT AT EACH INCUBATION TEMPERATURE

Low Temperature (°C.)	Incubation Temperature		
	20.9°C.	26.5°C.	29.9°C.
8.5	—	—	—
10.3	—	22.9	14.5
12.7	23.4*	11.2	7.0
15.9	32.3*	10.5*	8.9*

A dash indicates that under these conditions an E.D. 50 is probably non-existent.

* E.D. 50 estimated by eye. Significant differences between E.D. 50's were estimated from the logarithms of the E.D. 50's and their standard deviations. Among the calculated E.D. 50's, 22.9 is significantly greater than 11.2 and 7.0 whilst both 11.2 and 14.5 are significantly greater than 7.0.

The incidence of eggs initially free from diapause seems to be influenced in some way by the environment of the parents during their active life, for in an experiment with the eggs of crickets caught in 1950 in the same area as those used in the present work (which were from the 1951 generation) and treated similarly in the laboratory, the following results were obtained: At 25.1°C., 5 per cent. of the eggs hatched with no appreciable delay due to diapause; at 27.0°C., 16 per cent. of the eggs hatched promptly; and at 29.0°C., 18 per cent., the difference between the first of these percentages and the other two being highly significant. Similarly in 1949, using crickets from the same area and similarly treated, an average of 23 per cent. of the eggs were found to be free from diapause when incubated at 26.8°C. (Browning 1952*b*).

In Figure 1 the total numbers of eggs that hatched during the period of observation are represented by the height of the hollow rectangles and it can be seen that, in general, increase in the duration of preliminary low-temperature treatment resulted in an increase in the numbers of eggs that hatched. At

8.5°C., however, prolonged low-temperature treatment resulted in a decrease in the number of eggs that hatched. However, when the numbers of apparently healthy eggs remaining in the jars at the end of the experiment were taken into account, there was no evidence of a significant increase in mortality among the eggs following the longer exposures to low temperature.

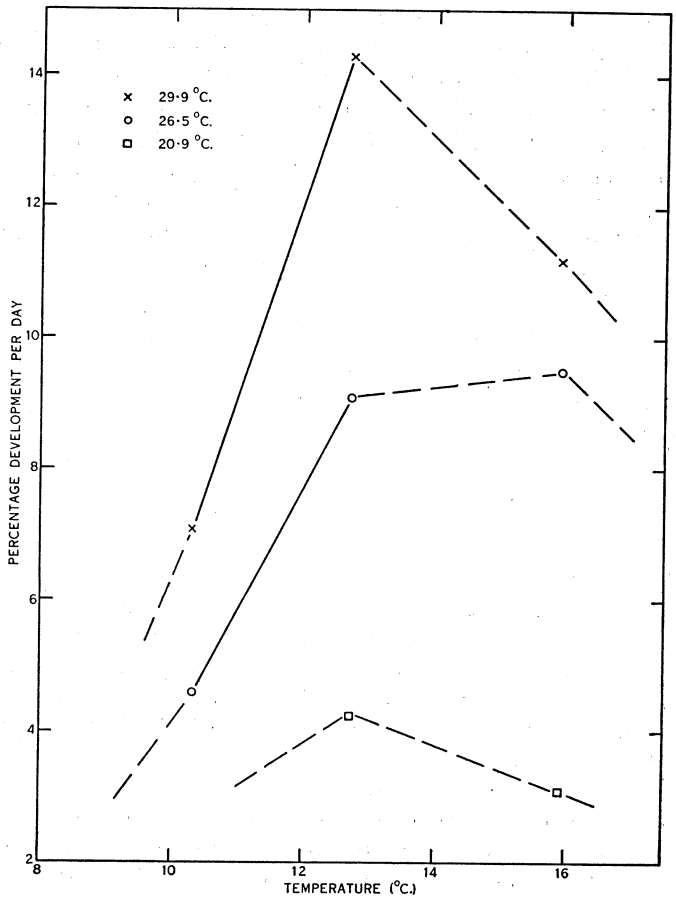

Fig. 2.—Freehand curves showing the trend in rate of completion of diapause development with change in temperature at each of the incubation temperatures. Continuous lines join points whose E.D. 50's were calculated and broken lines indicate points whose E.D. 50's were estimated by eye.

In Table 2 are shown the mean numbers of eggs that hatched during the whole period of observation following each temperature treatment, but without regard to the various durations of low-temperature treatment. The means were calculated from the totals in the two replicate sets of jars; since there were five durations at each low temperature, there were five jars in each set, giving a total of 125 eggs in each. In general, the numbers of eggs that

hatched at 26.5° and 29.9°C. were similar but these differed significantly from the numbers that hatched when the incubation temperature was 20.9°C. The interaction between the high-temperature and low-temperature treatments was highly significant as exemplified by the significant differences between the numbers that hatched at 20.9°C. following treatment at 8.5° and 15.9°C. on the one hand and 10.3° and 12.7°C. on the other.

TABLE 2
MEAN NUMBERS OF EGGS THAT HATCHED FROM 125 EGGS AT EACH INCUBATION TEMPERATURE FOLLOWING EXPOSURE TO EACH LOW TEMPERATURE

Incubation Temperature (°C.)	Low Temperature			
	8.5°C.	10.3°C.	12.7°C.	15.9°C.
20.9	84.5	43.5	40.5	99.5
26.5	113.5	118.5	121.5	132.5
29.9	112.0	129.0	126.5	119.0

A difference of 15.7 between any two means is significant, with a probability of 1 per cent.

IV. DISCUSSION

The curves of Figure 2 show a progressive change with temperature in the rate at which diapause development was completed in the eggs of *G. commodus,* similar to that demonstrated in the eggs of *Austroicetes* (Andrewartha 1944). Such a trend is very similar to that demonstrable for most developmental processes, although the range of temperature is lower than that usual for other kinds of development. These results lend support to the concept that the completion of diapause is essentially a developmental process (Andrewartha 1952).

The inception of diapause in eggs that had experienced no preliminary low-temperature treatment was influenced by the temperature at which the eggs were incubated, more eggs hatching promptly at the highest than at the lower incubation temperatures. The same influence was probably exerted by the incubation temperature on eggs that had experienced low-temperature treatment, as evidenced by the influence of any particular duration of exposure to any low temperature on the subsequent development of the eggs being modified by the incubation temperature at which they were placed. For example, 30 days exposure to 10.3°C. resulted in 84 per cent. of the eggs hatching without interruption when incubated at 29.9°C., whereas following the same low-temperature treatment 64 per cent. hatched promptly at 26.5°C. and none at 20.9°C. In each case the average proportion of diapause development that had been completed by the eggs during treatment at low temperature would not differ significantly; yet more eggs hatched at the higher temperatures than at the lowest. In explanation of this, it would seem that an egg that is to be incubated at a particular temperature requires to have completed a particular amount of diapause development; this amount is greater if it is to be incubated at a lower temperature than that required if the incubation temperature is to be higher.

That the increased numbers of eggs that hatched during the whole period of observation following increased durations of low-temperature treatment (Fig. 1) was due to the influence of low temperature on the progress of diapause development in the eggs is exemplified by the low-temperature treatments that were unsuccessful in inducing eggs to hatch promptly at the incubation temperatures. Increase in the duration of exposure to 8.5°C. made little or no difference to the numbers of eggs that completed their diapause development to the point where they were competent to hatch without delay when incubated at 20.9°C. However, as the period of treatment at 8.5°C. was extended there was a progressive decrease in the mean time required for the completion of morphogenesis by eggs that did hatch at 20.9°C. (Table 3), and at the same time an increasing number of eggs hatched. This suggests that the influence of low-temperature treatment, although insufficient to enable diapause development to reach the point where morphogenesis could proceed uninterrupted at 20.9°C., was nevertheless sufficient to enable some eggs to complete the remainder of their diapause development concurrently with morphogenesis when placed at 20.9°C. and that the proportion of eggs competent to do this increased as the duration of treatment at low temperature increased.

TABLE 3

INCREASE IN PERCENTAGE HATCH AT 20.9°C. AND DECREASE IN MEAN TIME REQUIRED TO HATCH FOLLOWING INCREASED DURATION OF TREATMENT AT 8.5°C.

	Duration of Exposure to 8.5°C. (days)					
	0	10	20	25	30	40
Percentage hatching	2	34	70	80	80	72
Mean time required to develop and hatch (days)	84	74.5	64.8	65.0	60.4	54.4

V. ACKNOWLEDGMENTS

The author is indebted to Dr. H. G. Andrewartha for his helpful advice and criticism and to Miss M. E. Morphett and Miss C. M. Hill for technical assistance.

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