THE ONSET AND DURATION OF DIAPAUSE IN EGGS OF *ACHETA COMMODUS* (WALK.) (ORTHOPTERA)

By T. W. Hogan*

[*Manuscript received July* 9, 1959]

Summary

The stage of morphogenesis at which diapause supervenes in eggs of the common field cricket *A. commodus* was critically determined, and found to be the 46-hr stage in terms of the series described by Brookes (1952). The examination revealed a difference in morphology between diapause and non-diapause embryos.

The onset of diapause is governed by temperature. Low temperatures induce diapause, whereas high temperatures tend to avert it. The change-over in response occurs at about 23°C below which all viable eggs enter diapause. Although exposure to 12·8°C for several weeks during pre-diapause enables development without delay at an incubation temperature of 26°C or higher, eggs held constantly at 12·8°C entered diapause.

The capacity of high temperature to avert diapause was used as a means of determining the time of exposure to a diapause-inducing temperature necessary to ensure onset of diapause in 90 per cent. of the viable embryos and also the stage of development of the embryo most sensitive to the effect of temperature. These experiments indicated that conditioning for diapause occurred several days before the actual onset, and that the maximum sensitivity was just prior to this stage.

The effect of constant temperature on the duration of diapause was measured at 23·3, 26·7, and 29·4°C. Diapause was terminated effectively at each temperature, with the minimum duration at 29·4°C. Preliminary exposure to 12·8°C for 21 days in the control treatment led to uniform, instead of extended, hatching.

A comparison of the effect of low temperature on pre-diapause and diapause eggs showed that those in diapause required considerably longer treatment for the elimination of diapause. From this, and other evidence, it is concluded that prevention of the onset of diapause by preliminary exposure to low-temperature treatment may not be the same process as termination of diapause, and that until more is known the term "diapause development" should be reserved for the latter.

I. Introduction

Diapause is one means by which certain species of insects are able to survive in environments that are periodically unfavourable to their development. The subject is of interest to the economic entomologist in that a knowledge of its mechanism may enable it to be manipulated by artificial means and its protective value to the insect destroyed.

The variations of diapause and the conditions under which it occurs are innumerable. The immense amount of literature on the subject has been reviewed a number of times, more recently by Andrewartha (1952) and Lees (1955). Accordingly only references having specific application to the work in this paper will be mentioned here.

* Plant Research Laboratory, Department of Agriculture, Burnley, Vic.

There is now considerable evidence that embryonic and postembryonic diapause differ in several important respects, although this does not remove the possibility that the final cause of the arrest may be the same. Williams (1952) has shown that in the pupal diapause of the giant silkworm *Platysamia cecropia* (L.) the arrest of development is associated with an endocrine failure. The secretion of the growth and moulting hormone from the prothoracic gland is suppressed by the brain in response to external stimuli.

Such a mechanism cannot be the means by which development is brought to a halt in the embryo prior to the existence of the brain and endocrine system. Nevertheless, the endocrine system of the parent is involved. Fukuda (1952) has shown that in the silkworm *Bombyx mori* (L.) the brain controls the release of a hormone from the suboesophageal ganglion of the parent female. Hasegawa (1957) has isolated the active principle which, when injected into pupae of non-diapause stock, produced moths which laid diapause eggs. Morohoshi (1959) has shown that voltinism in *Bombyx* is determined by a balance between the hormones from the corpora allata and suboesophageal ganglion. In *Bombyx* and other examples of embryonic diapause, the embryo develops to a definite stage of morphogenesis before the arrest occurs. The critical stage varies with the species concerned (Lees 1955). The study of embryonic diapause has a distinct advantage in that once oviposition has occurred there is no complication by reactions from the brain. *Acheta commodus* (Walk.), the test insect in the studies described hereunder, has the additional advantage that the onset of its embryonic diapause can be affected by the environment of the egg.

In *Acheta* temperature is the important environmental factor controlling the onset and duration of diapause. Browning (1952*a*, 1952*b*) has studied certain aspects of the effect of temperature on the diapause of this insect, mainly in relation to the pre-diapause stages. He found that pre-diapause eggs exposed to low temperature for several weeks did not enter diapause when incubated at a temperature of 26°C or higher. This he ascribed to the completion of diapause development before the onset of diapause. The most favourable temperature for this purpose was approximately 13°C.

The present paper is concerned with the stage of morphogenesis at which diapause supervenes, and with the effect of different levels of temperature on both the onset and duration of diapause.

II. Materials and Methods

The field cricket has only one generation per annum in southern Victoria and egg laying extends for about six weeks during the autumn. Continuity of supplies of eggs for experimental work was maintained by rearing crickets in constant-temperature cabinets. This method was developed in 1953 in connection with laboratory insecticide tests against this pasture pest.

The immature stages were reared at about 30°C, in order to promote rapid growth, and transferred at maturity to a cabinet kept at 26·7°C for oviposition.

Comparisons were made between the diapause behaviour of eggs from crickets collected in the field and those reared in the laboratory, and no greater variation

was noted than between different batches of crickets from the field. As a further check the cultures were renewed annually by replacements from the field in order to avoid the possibility of selection. The temperature at which the culture is reared does not appear to affect the diapause behaviour of the eggs. A small proportion of eggs that did not develop was present in both the field and culture samples.

Eggs for experimental work were obtained by placing trays of moist sand in the cages for several hours during which time the eggs were laid singly at a depth of $\frac{1}{2}$–$\frac{3}{4}$ in. in the sand. The trays were then removed and the eggs sieved out under water. The swirling water during this procedure ensures a good mixture of eggs from the various females. The eggs were then tipped on to moist blotting-paper and counted into tubes. Large supplies of eggs can be obtained within a few hours by withholding the oviposition trays for several days beforehand. This does not affect the subsequent development of the eggs.

After a considerable number of tests of various containers for holding the eggs, air-tight plastic tubes 1 by 1 in. were found to be the best. At the bottom of each tube was placed a disk of blotting-paper, standard in size, weight, and moisture content. The tubes were left for 24 hr, and then examined for any signs of a change in the moistness of the paper. Any tube in which this occurred was replaced, on the assumption that it was not air-tight. After the eggs had been counted into the tubes, these were put into a double-walled plastic box having a layer of moistened plaster of paris across the base. This served the double purpose of maintaining high humidity in the box and provided additional heat capacity to damp out temperature fluctuations. These precautions were necessary in order to avoid variations in the moisture level from either evaporation or condensation. Such differences can affect the rate of development which, in turn, can alter the number of eggs hatching within the prescribed time.

The eggs were transferred into the tubes by means of a camel-hair brush and, as far as practicable, all were placed flat so as to make equal contact with the moist surface.

Comparisons between treatments were made on the basis of the percentage of eggs reaching the "eyespot" stage (Plate 1, Fig. 2(*h*)) in a time considered equivalent to that taken by eggs that develop without evidence of delay. These will be referred to as diapause-free. Eyespot counts were preferred to hatching counts as some cannibalism can occur after hatching. All stages at eyespot were included in the count, which was made as soon as the first eggs reached the most advanced eyespot stage. In this way an automatic correction was obtained for differences in the rate of development at each temperature, since the latter affects the number of days over which development can be considered diapause-free. After counting, the eggs were held until hatching was complete so that they could be examined for any evidence of abnormality attributable to particular treatments.

At the conclusion of the period of experimental observation, more detailed information on the stage of development reached by the embryo was obtained in certain of the tests. This was done by immersing the eggs in water and examining them under a microscope, by which means it was possible to record them as non-viable, pre-diapause, diapause, or the particular stage of post-diapause development.

III. Results

(*a*) *Stage of Embryonic Development at which Diapause Supervenes*

There is no record of any accurate determination of the stage of embryonic development at which diapause supervenes in the eggs of *Acheta*. Andrewartha (1952) included *Acheta* as an example of the group in which "onset occurs while the embryo is at an early stage, usually before segmentation is complete." Browning (1952*a*) suggests that it is about the fourth-day stage of development. Lees (1955), presumably quoting from Browning, gives the $3\frac{1}{2}$-day stage. In each case the stage of development referred to is that reached by the modal number of embryos when they are held for the time stated, at a temperature of 25·2°C, as described by Brookes (1952). These eggs were previously exposed to low temperature, and, therefore, not subject to diapause.

In this experiment, and those following, the stage of development of the embryo is referred to by a number that enables it to be identified in the series described by Brookes. Thus "stage 48" corresponds to the morphogenetic development listed by Brookes as having been reached after 48 hr at 25·2°C. The time unit has been omitted as it does not apply to other temperatures.

The morphogenesis of large numbers of embryos was traced with relative ease by direct examination of the embryo, after clearing the eggs by a method previously described (Hogan 1959). The technique for clearing is equally effective for diapause or non-diapause eggs but prior to water uptake by the eggs clearing is more rapid, and lower temperatures should be used.

The characteristics of the embryos on entry into diapause were observed at 23·3, 26·7, and 29·4°C. A total of 150 eggs was held at each temperature and a sample of 20 eggs taken from each immediately before, during, and after the onset of diapause. Further samples were taken at intervals appropriate to the temperature. The timing of the sampling was based on preliminary examinations which showed that the onset of diapause occurred prior to water uptake. Supplementary observations on the comparative morphology of diapause and non-diapause embryos were made on a further series of eggs, after the main test had been concluded.

Considerable variation occurred in the rate of development of embryos at each temperature. A small proportion of the eggs showed no development after oviposition, presumably being infertile or non-viable. At each temperature the arrest of development took place at the stage shown in Plate 1, Figure 1, estimated to be about 2 hr younger than the 48-hr embryo illustrated by Brookes. It will be referred to as stage 46. After some time, depending on the temperature, the accumulation of embryos included stages 46–48 (Fig. 1). It was concluded, therefore, that diapause takes place at the stage 46, and that in the process of resuming normal development, the embryo gradually drifts through to stage 48. There was no indication of any further delay once the embryo had developed beyond stage 48.

Embryos in diapause were observed to be smaller and more compact than non-diapause embryos in the same stage of morphogenesis, the difference being greatest at the lower temperatures. In Plate 1, Figure 1, typical diapause embryos

are illustrated, while Plate 1, Figure 4, shows a diapause and non-diapause embryo. The proportion of larger embryos present at each temperature agrees with the expected proportion of non-diapause eggs (see Section III(*b*)), but the difference in size is less marked at the high temperatures.

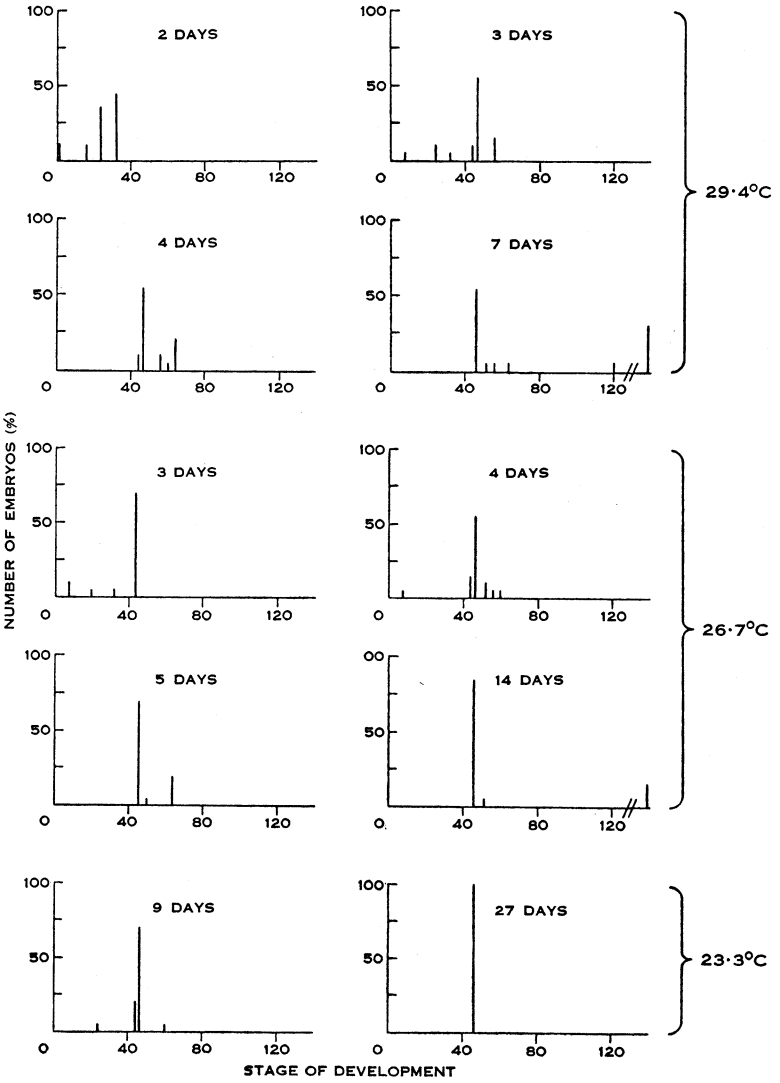


Fig. 1.—Stage of development reached by embryos after different periods at three temperatures. Note accumulation in stages 46–48.

In the course of development the eggs of *Acheta* absorb moisture almost equal to their own weight (Browning 1953). In diapause eggs, water uptake commences just as the embryo reaches the diapause stage, and is completed after the arrest of development takes place. In non-diapause embryos, on the other hand, develop-

ment continues during water uptake. After 4–4½ days (25·2°C scale) a clear space appears at the posterior end of the egg and this remains up to the end of the 6th day, after which the egg revolves. This clear space also appears in the diapause egg after a similar time, but the embryo is at a different stage of morphogenetic development, viz. stage 46 (Plate 1, Figs. 6 and 7).

(*b*) *Effect of Temperature on the Inception of Diapause*

It has been pointed out by Lees (1955) that as a rule high temperatures tend to avert diapause while low temperatures favour the "arrest of growth." However, the capacity of low temperature to remove diapause after onset is so well known that its importance in favouring the inception of diapause is apt to be overlooked. Andrewartha (1952) has remarked on this dual role.

TABLE 1

PERCENTAGE OF EGGS HATCHING WITHOUT EVIDENCE OF DIAPAUSE WHEN INCUBATED AT THE TEMPERATURES INDICATED

Temperature (°C)	23·3	26·7	29·4	34·0	Control
Hatching (%)	0	16·0	64·0	83·3	91·3

Browning (1952*b*) noted that in *Acheta* as the incubation temperature was raised an increasing percentage of eggs developed without interruption. These observations have been confirmed and extended.

Six replicates of 25 eggs per treatment were held at temperatures of 23·3, 26·7, 29·4, and 34°C. Controls were held for 21 days at 12·8°C prior to incubation at 26·7°C. The other conditions of the experiment were as described in Section II.

From Table 1 it will be seen that there was no diapause-free hatching at 23·3°C, but that some prompt hatching occurred at all temperatures above this and that the percentage hatching increased as the temperature increased. The maximum increase was between 26·7 and 29·4°C when the hatching increased from 16 to 64 per cent. (Fig. 2). The form of the curve between these readings is based on subsidiary tests made after the main experiment. The non-diapause hatching at 34°C was 83·3 per cent. compared with 91·3 per cent. for the control. This difference was not statistically significant, whereas the difference between other treatments were highly significant.

Thus at high temperatures there is a strong tendency for diapause to be averted. This tendency diminishes with the lowering of temperature until at about 23°C it disappears. It would appear that 23·3°C is near the threshold of this effect, since an occasional egg develops without diapause at this level, whereas none have been recorded at the lower temperatures. The actual proportion of eggs from which diapause is averted by high temperature will depend on the original strength of diapause in the eggs. This, as has been pointed out by Browning (1952*b*), depends on factors, at present unknown, in the environment of the parent generation of crickets.

Browning also found that eggs held at low temperatures for several weeks shortly after oviposition and then incubated at 26°C, or higher, invariably developed without interruption. This would suggest that in *Acheta* eggs, contrary to the general rule,

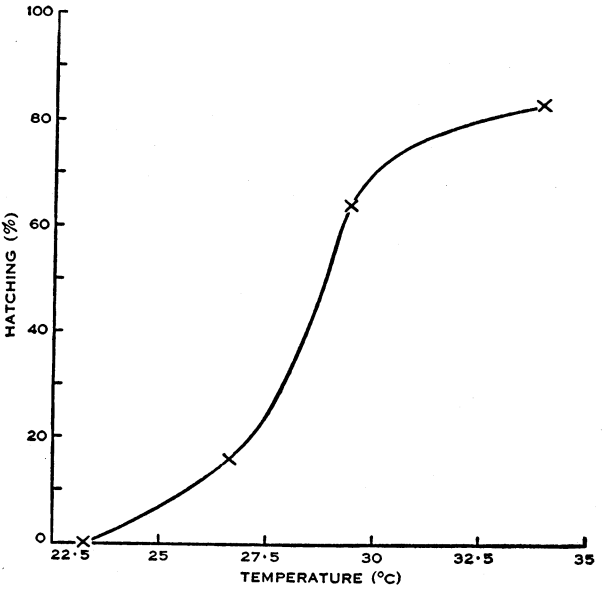


Fig. 2.—Effect of temperature on the induction of diapause. The proportion of eggs from which diapause is averted rises sharply at about 26°C.

diapause is averted by low temperatures. This, however, is not the case, as is shown by the following experiment in which a further series of eggs were held at 12·8, 14·8, 17·5, 21, and 29·4°C. All other conditions of the experiments were as in the first test.

TABLE 2

PERCENTAGE OF NON-DIAPAUSE EGGS AT THE LOWER TEMPERATURES

Temperature (°C)	12·8	14·8	17·5	21·0	29·4
Non-diapause eggs (%)	0	0	0	0	75·0

The results of these experiments (Tables 1 and 2) show that at the lower temperatures all the eggs entered dispause. Observations on parallel samples of eggs, kept for that purpose and examined by the clearing method mentioned previously, showed that at 12·8°C development proceeded normally until the diapause stage was reached after which no further morphogenetic development took place. Whether they eventually resume post-diapause development or not, if held for a very prolonged period at 12·8°C, is not known. In further observations eggs which had entered diapause at 23·3°C and had been held at that temperature for

a further 3 months were cleared and examined, but morphogenesis had not been resumed.

The anomaly then arises that while low temperatures favour the onset of diapause, exposure to them during the pre-diapause stages accentuates the effect of high temperatures in promoting development without interruption. Browning (1952*b*) attributes this to the occurrence of "diapause development" during the preliminary exposure at low temperatures. This term was introduced by Andrewartha (1952) to describe the processes which occur during diapause and which must be completed before the embryo is competent to resume morphogenesis. Whether it is appropriate to apply the same term to processes which result in the complete elimination of diapause from the life history will be discussed later.

(*c*) *Sensitivity of Different Stages of Embryonic Development to a Diapause Temperature*

From Section III(*b*) it is clear that at a temperature of 23·3°C, or lower, diapause occurs in all eggs. It is not known, however, what period of exposure to such a temperature is required to cause the onset of diapause. Nor is it known whether conditioning for diapause occurs at a particular stage of pre-diapause development, over a number of stages, or in the latter event, whether some stages show greater sensitivity than others. One reason for investigating such influences is to enable proper standardization of the experiments on other aspects of diapause. If, for instance, the conditions existing over the first 24 hr after oviposition affect diapause behaviour, then this should be known and taken into account in the design of the experiments.

In other species of insects a considerable amount of information is available on the exact stage of the life cycle at which diapause supervenes, but there is relatively little on the stage at which conditioning for diapause occurs. Way and Hopkins (1950) investigated the larval diapause of *Diataraxia oleracea* (L.) to determine the stage at which photoperiod was operative in inducing diapause. Masaki (1957) found that the proportion of larvae of *Barathra brassicae* (L.) entering diapause was proportional to the length of the exposure to a diapause-inducing temperature. Other examples of larval and pupal responses are quoted by Lees (1955).

In *Acheta* the ability of high temperature to avert diapause in a high proportion of the eggs provides a means of measuring the requirements of the embryo for diapause induction and the responsiveness of the various pre-diapause stages of the embryo to the temperature of the environment.

In the following experiments the eggs were held at a temperature of 29·4°C, which averts diapause in a high proportion of the eggs, and a diapause-inducing temperature of 23·3°C substituted at the stages and intervals indicated in Figure 3. The interchange of temperatures was carried out over a period equivalent to 8 days at 23·3°C from the time of oviposition. This time was sufficient to ensure that all viable eggs would reach the diapause stage.* This period was divided into eight

* At 23·3°C the 46-hr stage in the terminology of Brookes is reached only after about 6 days incubation. This discrepancy is due partly to the lower temperature and partly to the fact that 2–3 days incubation at 25·2°C are required before the embryo reaches the "0 hour" stage described by Brookes (1952).

stages, each corresponding to one day's development at this temperature. Since the rate of development at 29·4°C is twice that at 23·3°C (Hogan, unpublished data 1958), it is a simple matter to substitute one temperature for another so that the same amount of embryonic development is completed at each temperature. One day at 29·4°C, for example, will enable the same amount of development as 2 days at 23·3°C. By holding a control group of eggs at a constant temperature of 29·4°C the effect of exposure to 23·3°C at different periods during pre-diapause development could be measured.

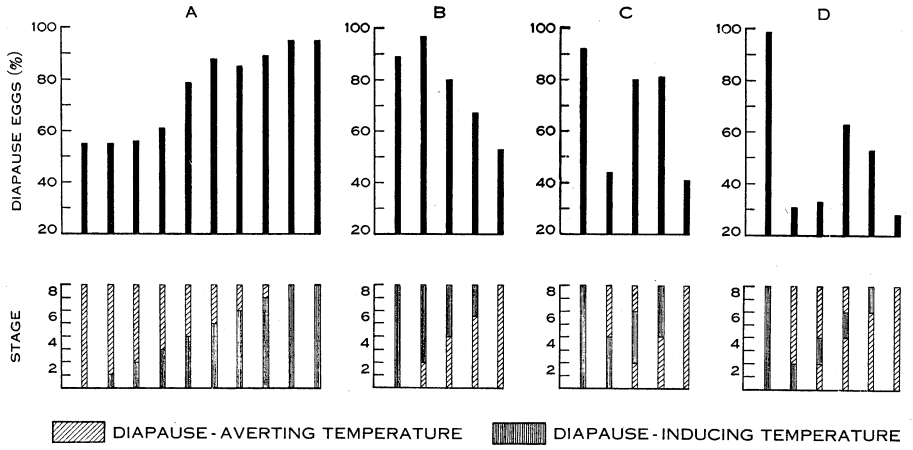


Fig. 3.—Diapause induction in pre-diapause embryos subjected to various temperature regimes as indicated.

In the first test, cumulative one-day periods at the diapause-inducing temperature, 23·3°C, were added from the time of oviposition up to a maximum of 8 days. Figure 3*A* shows that no effect was obtained until the third day, after which there was a rapid rise in the proportion of eggs entering diapause. This continued till the fifth day and was only slightly higher by the eighth day.

The lack of response over the first 2 days could be due to either insensitivity at this stage of development, or a too short exposure. The treatments were, therefore, reversed so that the shorter periods of exposure to the diapause-inducing temperature took place just prior to the diapause stage (Fig. 3*B*). Increments equivalent to 2 days at 23·3°C were used so that there were then only four subdivisions. The responses obtained show some effect from the 2 days treatment just prior to diapause, but to achieve the maximum effect a period of 6 days was necessary.

Both aspects were investigated by two further tests. These were carried out by interposing four successive 2-day intervals at 23·3°C in one case, and three 4-day intervals in the other. Figure 3*D* shows that for the 2-day exposures the fifth- and sixth-day interval was the most responsive, but that evidently the total time of exposure was too short to induce diapause in all eggs. It is apparent again from this test that this time of exposure has little influence when applied to the early stages of embryonic development.

The 4-day interval tests (Fig. 3*C*) indicated that this period of time was highly effective in inducing diapause, particularly when applied so as to include the fifth and sixth days. When these stages were included, approximately 90 per cent. of the full effect was obtained.

Thus over the first 2 days after oviposition the embryos are relatively insensitive to the diapause temperature except insofar as this may add to the effect of treatment applied later. The fifth and sixth days are the most responsive, but all stages after the first 2 days respond appreciably. In Section III(*a*) it was found that in the majority of the eggs the morphology of the embryo changes about the sixth day at 23·3°C to show the characteristics of either a diapause or a non-diapause embryo. Hence the most sensitive stage is judged to be just prior to stage 46, probably between stages 40 and 44. The response obtained on the seventh and eighth days can be accounted for partly by the variation in the rate of development, since some of the embryos would still be in the stage corresponding to the fifth or sixth day.

These experiments indicate that all but the earliest stages of pre-diapause development give some response to a diapause-inducing temperature, and that there is a period of maximum sensitivity near the fifth and sixth days of development under the conditions described, during which the embryo may or may not enter diapause according to environmental conditions at the time. To cause diapause in 90 per cent. of the viable eggs a minimum period of exposure of slightly more than half the pre-diapause development time is required, and this should coincide with the most sensitive stages, viz. that reached after 5–6 days after oviposition when held at a temperature of 23·3°C.

(*d*) *Effect of Low Temperature on Pre-diapause and Diapause Eggs*

It is known (Browning 1952*a*) that if eggs of *Acheta* are held at 13°C for several weeks and then transferred to a suitable incubation temperature, they will continue development without evidence of diapause. Browning refers to this as completion of diapause development. The completion of diapause development in this way would be most unusual. The only other comparable case appears to be *Melanoplus bivittatus* (Say). In this insect, eggs which do not reach the diapause stage by winter-time continue development without diapause in the following spring. This is referred to simply as "diapause averted" by Church and Salt (1952).

In the present series of experiments it has been shown (Section III(*b*)) that eggs held at 13°C enter diapause and are still in this condition after a further 3 months. This would suggest that low temperature does not terminate diapause and also provides clear evidence that the low-temperature treatment of pre-diapause eggs does not eliminate diapause. However, the data on this subject can be interpreted to mean that diapause development does proceed, since the reduction in the proportion of eggs entering diapause after exposure to low temperature for a limited period (15–30 days) is most readily explained on the assumption that diapause development has proceded, but has not been completed.

Further evidence has been sought by comparing the responses from the pre-diapause eggs with those from diapause eggs given the same low-temperature treatment.

For this purpose 1200 eggs, up to 16 hr old, were divided into two groups, one of which was transferred immediately to low temperature, and the other to 23·3°C for 14 days in order to induce diapause. Each group was subdivided into three lots of six tubes and held for 30, 45, and 60 days at 12·8°C followed by incubation of one-half of the tubes at 23·3°C and the other half at 26·7°C. Controls had no preliminary low temperature treatment.

There was a marked difference between the pre-diapause and diapause eggs with respect to the period of low temperature required to remove, or prevent, diapause (Table 3). The effect was masked at an incubation temperature of 26·7°C because the hatching at this temperature was high for all treatments. It is clear from the hatching at 23·3°C, however, that once diapause supervenes, the length of exposure to low temperature necessary to eliminate diapause was at least twice that required to prevent its inception. Of the treatments for 30, 45, and 60 days at low temperature, only the 60-day treatment of the diapause eggs gave hatching comparable to that obtained after 30 days at low temperature of the pre-diapause eggs.

TABLE 3

PERCENTAGE OF EGGS HATCHING FREE OF DIAPAUSE FOLLOWING THE TEMPERATURE REGIMES APPLIED TO PRE-DIAPAUSE AND DIAPAUSE EGGS

Time at 12·8°C (days)	Control		30		45		60	
Incubation temperature (°C)	23·3	26·7	23·3	26·7	23·3	26·7	23·3	26·7
Pre-diapause eggs (%)	—	28	88	84	84	88	85	92
Diapause eggs (%)	4	8	16	80	56	92	80	92

The difference is even more marked when it is considered that after 30 days at low temperature only 16 per cent. of the diapause eggs resumed development, while 88 per cent. of the pre-diapause eggs developed without evidence of delay.

(e) *Effect of Constant Temperature on the Elimination of Diapause*

In the majority of the insect species investigated the elimination of diapause, when it occurs, is governed wholly, or in part, by temperature. The striking feature of the temperature requirements, both for different species and for embryonic or post-embryonic stages, is their comparative uniformity. Andrewartha (1952) has observed that, with few exceptions, completion of diapause development takes place most efficiently in the temperature range between 0°C and the threshold of development of the insect.

Acheta differs from most other insects in that diapause development can proceed at normal developmental temperatures. Browning (1952*a*) showed that eggs held at 26·7°C will eventually emerge from diapause. The optimum temperature for elimination of diapause from eggs in diapause was not determined, but he found *c.* 13°C to be the most efficient temperature for the treatment of pre-diapause eggs.

Since temperatures in the region of 30°C are considerably more effective than 26·7°C in averting diapause, it was decided to compare the effectiveness of 29·4°C with that of moderate and low temperatures in eliminating diapause.

There is a possibility that the intensity of diapause may be affected by the temperature at which onset takes place. Hence, if the eggs are held at a constant temperature from the time of oviposition to hatching, a comparison of the effectiveness of each temperature in eliminating diapause will not be valid, since the strength of diapause may have been affected by the inception temperature.

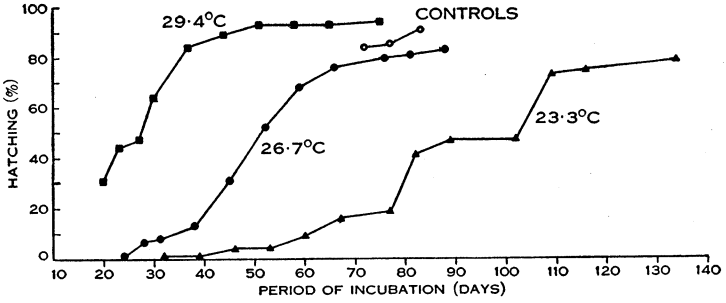


Fig. 4.—Cumulative frequency of eggs hatching at different constant temperatures after being induced to enter diapause at 23·3°C. The controls were kept at 12·8°C for 21 days and were then incubated at 26·7°C.

In this experiment, therefore, diapause was induced in the eggs by holding them for 10 days at 23·3°C. These were then divided into four treatments, viz. incubation at 12·8, 23·3, 26·7, and 29·4°C. The control treatment was held for 21 days at 12·8°C and was incubated at 26·7°C. Observations were made on the eggs at intervals governed by their rate of development. The counts for comparison of treatments were made at the eyespot stages, but all eggs were held till hatching, to enable detection of any abnormalities which might occur from the effect of high temperature on diapause eggs.

Development was resumed and hatching completed at all temperatures except 12·8°C in the times shown in Figure 4. At each temperature the time taken to complete development varied considerably between individual eggs. At 23·3°C it ranged from *c.* 30 to 129 days; 50 per cent. had hatched after 86 days and the peak of emergence was from 77 to 104 days, during which 55 per cent. of the eggs hatched. The 50 per cent. level appears to be the most suitable parameter for the comparison between treatments.

At the higher temperatures the completion of morphogenesis was accelerated so that at 26·7°C 50 per cent. of the diapause eggs hatched after 46 days, and at 29·4°C after 26 days. At 29·4°C the percentage hatching, 94·7 per cent., was equal to that from the control treatment, i.e. with preliminary low temperature. No abnormalities in hatching were observed.

It can be said, therefore, that the termination of diapause is efficient at high temperature. Moderate temperatures are less effective.

Low-temperature treatment is in a separate category. At a constant temperature of 12·8°C eggs developed until they reached the diapause stage. Morphogenesis then ceased. It was not resumed during the 3 months that these eggs were held after entering diapause.*

Thus, diapause was terminated at all temperatures above 12·8°C, but most readily at the higher temperatures. To obtain uniform hatching in the minimum time, the double treatment consisting of a preliminary low-temperature treatment followed by incubation at a high temperature is the most effective.

IV. Discussion

From the foregoing experiments it is clear that the temperature of the environment affects the diapause behaviour of *A. commodus* eggs in a number of interacting ways. The tendency to enter diapause, the intensity with which it is induced, and the time taken for its elimination, all vary with the temperature regime. The reactions also suggest that while *A. commodus* is well adapted to the environment of southern Victoria, it may also possess a capacity to develop a multivoltine life cycle in a warmer climate.

Under the conditions normally prevailing in southern Victoria the life cycle is uni-voltine. Since the eggs are laid in the soil at a depth of about $\frac{3}{4}$ in., and the pasture growth adds further protection from the effects of direct sunlight, the temperatures they experience are more uniform than the air temperature and approximate to the mean air temperature. Early in autumn the mean air temperature is about 19°C and gradually declines to about 14°C. These temperatures are within the range at which all eggs enter diapause (Section III(*b*)) and, therefore, diapause would be expected to be obligate under field conditions. Samples of eggs, totalling some hundreds, taken from the field during June and cleared for examination of the embryo have confirmed these expectations (Hogan, unpublished data 1958).

As the temperatures decline with the approach of winter, the diapause is weakened. Thus, the stronger diapause during the warmer weather of early autumn means that the eggs laid at this time are less likely to complete diapause development and hatch out at the wrong time.

The function of diapause under Victorian conditions appears to be not only to ensure overwintering in the egg stage, but also the prevention of hatching during the spring. Wet, cool conditions, such as normally occur at intervals in spring, appears, from laboratory observations, to be inimical to development. Diapause causes hatching to be delayed until early summer when high temperatures and drier conditions normally occur.

The reactions of *Acheta* eggs to high temperatures appear to give some support to the ideas of Salt (1947) in relation to larvae of *Cephus cinctus* (Nort) that both low and high temperatures are required for the complete elimination of diapause. *Acheta* shows a difference from *Cephus* in that high temperature alone is quite effective in terminating diapause. However, when both low and high temperatures are used,

* In more recent work, diapause eggs held at 14·4°C commenced to emerge from diapause after about 100 days.

the higher the temperature at which they are incubated the shorter the time they need at low temperature to enable morphogenesis to be resumed. This suggests that the transfer to high temperature provides a stimulus which overcomes the residual diapause in the egg, or, alternatively, that high temperature may be an actual requirement for the complete elimination of diapause, as suggested by Salt.

Further evidence on this would be provided by the length of time taken for the embryo to resume morphogenesis after transfer to high temperature. Data on this is being obtained.

There have been indications in the course of the foregoing investigation that the onset of diapause can be affected by moisture conditions, but so far it has not been possible to obtain experimental proof of this. Moisture has been shown to be a factor in the diapause of *Melanoplus differentialis* (Thos.) by Bucklin (1953) and by Slifer (1958), but in *Melanoplus* it is the entry of moisture into the egg via the hydropyles that controls diapause. In *A. commodus* water enters both the diapause and non-diapause egg at approximately the same time after oviposition. It is the availability of moisture to the embryo that is in question.

Lees (1952) excludes *Acheta* from those species in which hydration of the egg might be "insufficient to permit active embryonic growth", and includes it as a species in which "the active control of diapause must be exercised through some agency other than water." He bases this on a statement, attributed to Browning, that the egg enters diapause in a fully hydrated condition. It is now known that diapause ensues before hydration of the egg, so that this reasoning no longer applies.

The other reason that Lees gives for dissociating diapause and the moisture content of the egg in this insect is that, if the freshly laid eggs are chilled for an adequate period, "diapause development" is completed before the embryo has grown to the morphological stage. "All the water required for healthy post-diapause growth . . . is taken in subsequently".

There is reason to doubt the validity of this evidence also. Browning (1952*a*) used the term "completion of diapause development" to describe the prevention of onset of diapause in eggs that were given appropriate low-temperature treatment shortly after oviposition, and were then incubated at a higher temperature.

Whatever processes may lead to the termination of diapause can reasonably be described as "diapause development", and in this paper the term has been used in this sense.

However, there is no clear evidence that the failure to enter diapause after treatment of the pre-diapause eggs results from the same processes that cause the termination of diapause, even although low temperature is effective in each case. For one thing, it has been shown (Section III(*d*)) that it takes considerably longer to terminate diapause than it does to prevent the onset of diapause.

Moreover, the physical differences between diapause and non-diapause eggs must result from reactions set in train at an earlier stage of embryonic development. The failure to trigger these reactions is not the same as their removal after onset, even although the destruction of a diapausing agent may be involved in each case.

Until more is known of the processes that affect the onset of diapause and those that terminate diapause, it would seem undesirable to make the assumption that they are exactly the same by the use of the term "diapause development" to cover both phenomena.

Since the intensity of diapause seems to be affected by the temperature at which the onset of diapause takes place, the question of the factors affecting strength of diapause are of special interest and are being investigated.

V. Acknowledgments

The author is indebted to Miss J. Kenyon for her able assistance in carrying out the tests, to Mr. R. Jardine, for the statistical aspects of the work, to Mr. M. P. Gellert for the preparation of the figures, and to Dr. F. H. Drummond for constructive criticism in the preparation of the manuscript.

VI. References

Andrewartha, H. G. (1952).—Diapause in relation to the ecology of insects. *Biol. Rev.* **27**: 50–107.

Brookes, Helen M. (1952).—The morphological development of the embryo of *Gryllulus commodus* Walker (Orthoptera: Gryllidae). *Trans. Roy. Soc. S. Aust.* **75**: 150–9.

Browning, T. O. (1952*a*).—The influence of temperature on the completion of diapause in the eggs of *Gryllulus commodus* Walker. *Aust. J. Sci. Res.* B **5**: 112–27.

Browning, T. O. (1952*b*).—On the rate of completion of diapause development at constant temperatures in the eggs of *Gryllulus commodus* Walker. *Aust. J. Sci. Res.* B **5**: 344–53.

Browning, T. O. (1953).—The influence of temperature and moisture on the uptake and loss of water in the eggs of *Gryllulus commodus* Walker (Orthoptera, Gryllidae). *J. Exp. Biol.* **30**: 104–15.

Bucklin, D. H. (1953).—Termination of diapause in grasshopper embryos cultured *in vitro*. *Anat. Rec.* **117**: 539.

Church, N. S., and Salt, R. W. (1952).—Some effects of temperature on development and diapause in eggs of *Melanoplus bivittatus* (Say) (Orthoptera: Acrididae). *Canad. J. Zool.* **30**: 173.

Fukuda, S. (1952).—Function of the pupal brain and suboesophageal ganglion in the production of non-diapause and diapause eggs in the silkworm. *Annot. Zool. Jap.* **25**: 149–55.

Hasegawa, K. (1957).—The diapause hormone of the silkworm *Bombyx mori*. *Nature* **179**: 1300–1.

Hogan, T. W. (1959).—A rapid method for examining diapause embryos of *Acheta commodus* W. *Nature* **183**: 269.

Lees, A. D. (1955).—"The Physiology of Diapause in Arthropods." (Cambridge Univ. Press.)

Masaki, S. (1957).—Larval-sensitive stage for the action of external factors controlling the occurrence of diapause in the cabbage moth pupa, *Barathra brassicae* Linné. *J. Fac. Agric., Hokkaido* **50**: 197–210.

Morohoshi, S. (1959).—Hormonal studies on the diapause and non-diapause eggs of the silkworm *Bombyx mori* L. *J. Insect Physiol.* **3**: 28–40.

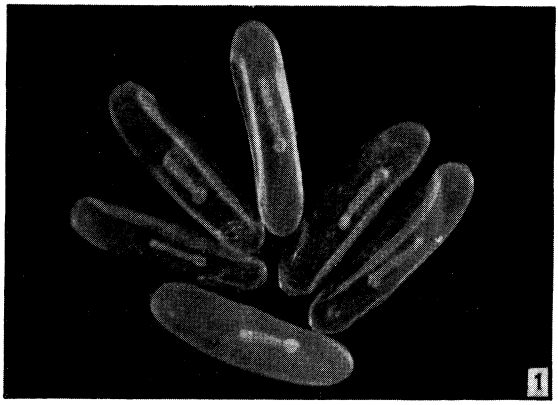
Salt, R. W. (1947).—Some effects of temperature on the production and elimination of diapause in the wheat stem sawfly *Cephus cinctus* Nort. *Canad. J. Res.* D **25**: 66–86.

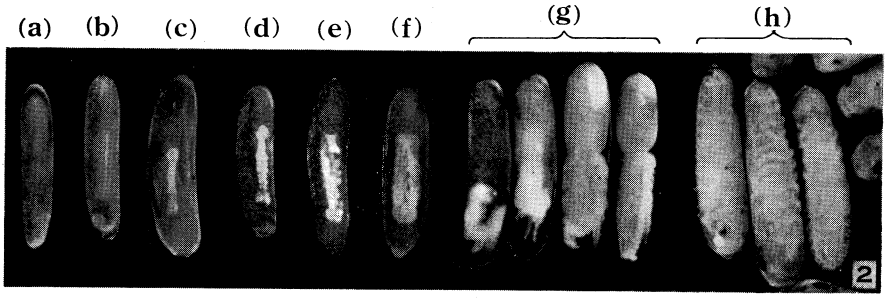
Slifer, E. H. (1958).—Diapause in the eggs of *Melanoplus differentialis* (Orthoptera: Acrididae). *J. Exp. Zool.* **138**: 259–82.

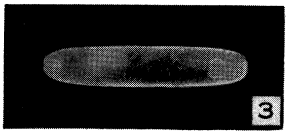
Way, M. J., and Hopkins, B. A. (1950).—The influence of photoperiod and temperature on the induction of diapause in *Diataraxia oleracea* L. (Lepidoptera). *J. Exp. Biol.* **27**: 365–76.

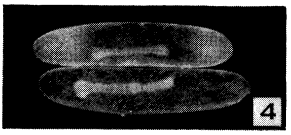
Williams, C. M. (1952).—Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the *Cecropia* silkworm. *Biol. Bull., Wood's Hole* **103**: 120–38.

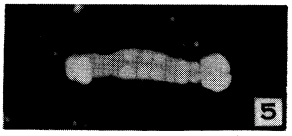
ONSET AND DURATION OF DIAPAUSE IN ACHETA COMMODUS

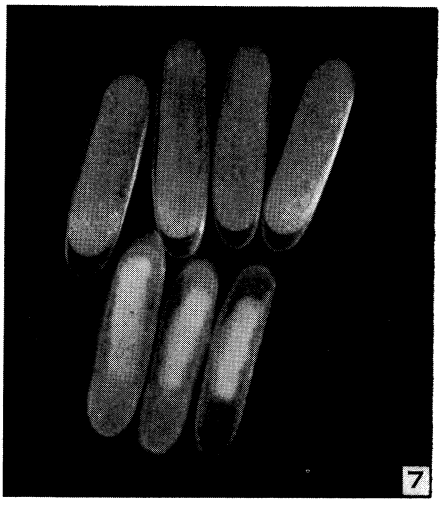












Explanation of Plate 1

Fig. 1.—Diapause embryos.

Fig. 2.—Stages of development of the embryo. In terms of Brookes' series they represent (*a*) 8 hr; (*b*) 24 hr; (*c*) 46 hr; (*d*) 56 hr; (*e*) 64 hr; (*f*) 4½ days; (*g*) rotation of embyro; (*h*) "eyespot".

Fig. 3.—Frontal view of the 24-hr stage. ×9.

Fig. 4.—Diapause (upper) and non-diapause (lower) embryo at the same stage of morphogenesis. ×9.

Fig. 5.—An embryo (48 hr) dissected from the egg to show details of its structure. ×18.

Figs. 6 and 7.—Diapause (Fig. 6) and non-diapause (Fig. 7) eggs after the same period of incubation. The clear space at the posterior end of the eggs has developed to the same extent but when cleared (lower parts of Figures 6 and 7) the stage of morphogenesis is very different: stage 48 (Fig. 6), stage 108 (Fig. 7).