THE INFLUENCE OF TEMPERATURE ON THE COMPLETION OF DIAPAUSE IN THE EGGS OF *GRYLLULUS COMMODUS* WALKER

By T. O. BROWNING*

[*Manuscript received October* 22, 1951]

Summary

The eggs of *Gryllulus commodus,* if incubated at high temperature (26.8°C.), soon after laying, did not develop and hatch promptly. Instead hatching was spread over a long period and many eggs died. If the eggs were given a period of exposure to low temperature (12.8°C.) before incubation at high temperature, prompt hatching occurred. This was due to diapause, which occurred at an early stage in the morphological development of the egg.

An experiment was done to measure the influence of various periods of exposure to high temperature followed by various periods of exposure to low temperature on the ability of the eggs to complete their diapause development.

It was found that a maximum period of about two days of high temperature treatment, followed by a minimum period of about 30 days low temperature treatment was most influential in promoting the completion of diapause.

About 20 per cent. of the eggs were found to be competent to develop without diapause when laid.

Preliminary high temperature treatment is considered to have induced the eggs to enter diapause more firmly than if no high temperature had been experienced before the exposure to low temperature. At the same time, high temperature permitted the completion of diapause, although under these conditions diapause development proceeded slowly and uncertainly.

It is shown that the processes responsible for hatching and melanin formation in the nymphs operate at low temperature but at a very slow rate.

I. INTRODUCTION

Diapause is a common phenomenon in the eggs of insects but great variability is found in its manifestation as between different species and even on occasions in the same species under varying circumstances. Among the eggs of any species there is always a distribution in the degree of intensity of diapause. For example, in species in which diapause disappears during an exposure to cold the intensity of diapause may be measured by the time required to be spent at low temperature. This may be very variable indeed and may be subject to influence by the environment, as will be shown later.

In some species all eggs enter diapause whilst in others the eggs are variable, some diapausing and some being competent to complete their development without interruption. In the latter type of species the proportions of diapausing and non-diapausing eggs are usually also very variable and dependent

* Waite Agricultural Research Institute, University of Adelaide.

on many factors, including the physiological state and genotype of the mother, her environment, and the environments of the eggs themselves.

In most species that have been studied it is found that an exposure to low temperature, if given at the appropriate stage in the egg's development, results in the egg becoming competent to complete its development promptly when placed at some appropriate incubation temperature. However, it is found that the range of temperatures to which the diapausing egg may respond is usually extensive and may overlap the range of temperatures at which morphogenesis proceeds. Diapause usually disappears most rapidly at some moderately low temperature and more slowly at temperatures much above or below the optimum. At higher temperatures particularly, abnormalities and death frequently result from long exposures.

These considerations have led to the abandoning of the idea of diapause as consisting of a developmental "block" that had to be "broken" before development could proceed and given rise to the concept of diapause as a physiological process, part of the normal development of the individual, that must be completed before the morphogenetic processes can proceed smoothly. The recent work on hormones in relation to diapause leaves little doubt that the processes concerned in the completion of diapause are those of organization and development. These processes have been termed "diapause development" (Andrewartha 1952) and it is in this sense that the term is used here. Andrewartha has recently elaborated the concepts set out above in an extensive review of diapause in the ecology of insects and has cited detailed examples and references.

The present paper deals with the results of an experiment on the influence of varying periods of exposure to low temperature following varying periods of exposure to high temperature on the ability of the eggs of *Gryllulus commodus* to complete their development and hatch when placed at an adequate incubation temperature.

II. Materials and Methods

(*a*) *The Eggs*

About 50 female crickets caught in the field at Penola, South Australia, were kept in a large cage with about an equal number of males, with grass and wheat grains for food. A tray of moist sand was kept in the cage and examined daily for eggs, and when it became apparent that eggs were being laid freely the crickets were transferred in their cage to a warmed (25-30°C.) glass-sided box in a glass-house. Trays of moist sand were placed in the cage and these were removed at daily intervals and new ones replaced.

The sand was sieved under water and the eggs removed, cleaned, and sorted, any discoloured, undersized, or damaged eggs were discarded, and 1200 eggs were counted out. This procedure was repeated on each of three consecutive days since it was not possible to obtain all the 3600 eggs required for the experiment (see Section II (*b*)) on one day.

Each lot of 1200 eggs was immediately counted out into lots of 25, and two such lots, together representing one replicate, were allotted at random to each

treatment. Replicates were split in this way since the containers (2-oz. pomade jars with a quarter-inch of plaster of Paris set in the bottom) were too small to accommodate 50 eggs easily. The plaster was thoroughly wetted and the jars placed in the appropriate incubators. This procedure was repeated each day for three days, giving three replicates of 50 eggs each for each treatment.

(*b*) *Experimental Procedure*

Eggs of *G. commodus* that have completed diapause hatch in about 13.1 days at 26.8°C., whilst at 12.8°C. no eggs had hatched even after exposure to this temperature for almost a year (Browning 1951). Further, it was known that diapause development was successfully completed after about a month at 12.8°C. Consequently 26.8°C. was chosen as the high temperature, and 12.8°C. as the low temperature to be used in the experiment.

The eggs were given a preliminary exposure of 0, 2, 6, or 12 days to 26.8°C. and this was followed by a period of 0, 5, 15, 30, 45, or 60 days of low temperature treatment, after which the eggs were returned to the thermostat at 26.8°C. for incubation. The 24 possible combinations of these treatments were used, with three replicates of 50 eggs each of each treatment, giving a total of 3600 eggs in all. After the preliminary treatment the eggs were incubated for 60 days, after which time the experiment was stopped.

The jars in the incubation thermostat were opened daily, and hatched nymphs were counted and removed. Each week the plaster in the jars was moistened with a few drops of water in order to keep the humidity within the jar as close to saturation as possible, since previous experience had shown this to be necessary for the survival of the eggs.

The treatments will be referred to by number hereafter for the sake of brevity. Thus 0-15 means no high temperature treatment, followed by 15 days of low temperature treatment, 12-45, 12 days of high temperature treatment followed by 45 days of low temperature, etc.

Since the temperature at which the eggs were incubated after treatment was the same as that used in the preliminary high temperature treatment, the four treatments 0-0, 2-0, 6-0, and 12-0 are all similar since none had any time at low temperature between exposures to high temperature. They are thus the controls and in effect went straight to incubation with no preliminary treatment.

It should be stated here that treatment 6-15, through an oversight, was left too long at low temperature. All the replicates were moved at once as soon as the error was discovered, after 16, 17, and 18 days respectively, and they thus had one, two, and three days too much low temperature treatment. However, this is not a great difference and would certainly be negligible in its effect (no significant difference could be detected between the replicates) and for this reason the results of this treatment have been included as if it had in fact been 6-15.

III. Results

The distributions of hatching of the eggs in each treatment during the 60-day period are shown in Figure 1 in the form of cumulative hatching totals plotted against time. The ordinates in the figures represent the sum of the three replicates in each treatment, the maximum possible being 150. The points are joined by straight lines with a line parallel to the abscissa drawn from the last recorded hatching to the 60-day ordinate. The height of this line on the ordinate represents the total number of eggs that hatched.

It can be seen from Figure 1 that the hatching distributions in the various treatments differ in three main ways, namely: (*a*) in the total number of eggs that hatched during the 60-day period of observation; (*b*) in the mean duration of the incubation period of eggs that hatched, and (*c*) in the variance of the distributions. These differences will be considered separately.

(*a*) *Total Number of Eggs that Hatched*

The mean total number of eggs that hatched in each treatment, expressed as a percentage of the total eggs used, is set out in Table 1, in angular scale (Fisher and Yates 1948) with the actual mean percentages shown in parentheses. Such transformation of the data was necessary before the ordinary methods of statistical analysis could be applied (Bartlett 1947). Least differences for significance in comparing means are set out below the table.

Table 1

MEAN PERCENTAGE OF EGGS THAT HATCHED, IN ANGULAR SCALE

H.T. = 26.8°C., L.T. = 12.8°C.

L.T. Treatment (days)	H.T. (days) 0	2	6	12	L.T. Treatment General Means
0	39.6° (40.7%)	42.7° (46.0%)	39.2° (40.0%)	44.6° (49.3%)	41.5° (44.0%)
5	56.4° (69.3%)	58.6° (72.7%)	45.8° (51.3%)	53.6° (64.0%)	53.6° (64.4%)
15	75.0° (92.7%)	63.5° (79.3%)	48.5° (56.0%)	63.0° (79.3%)	62.5° (76.8%)
30	74.3° (92.7%)	71.6° (90.0%)	62.1° (77.3%)	74.1° (92.0%)	70.5° (88.0%)
45	74.4° (92.7%)	78.7° (96.0%)	67.8° (85.3%)	59.9° (74.7%)	70.2° (87.2%)
60	76.1° (93.3%)	74.6° (92.7%)	70.6° (88.7%)	60.1° (74.7%)	70.4° (88.8%)
H.T. treatment general means	66.0° (80.2%)	64.9° (79.4%)	55.7° (66.4%)	59.2° (72.3%)	

Least differences for significance at $P = 0.01$ between: individual treatment means = 11.7°; high temperature treatment general means = 4.8°; low temperature treatment general means = 5.8°.

Table 1 shows that in treatments receiving no or two days preliminary high temperature treatment the total number of eggs that hatched was about the same at any particular level of low temperature treatment. An increase in the total hatch occurred in both treatments as the period of low temperature treatment was extended to about 15 or 30 days, further increase having

no significant influence on the total. The number of hatchings was reduced, however, when the preliminary exposure to 26.8°C. was increased to six days,

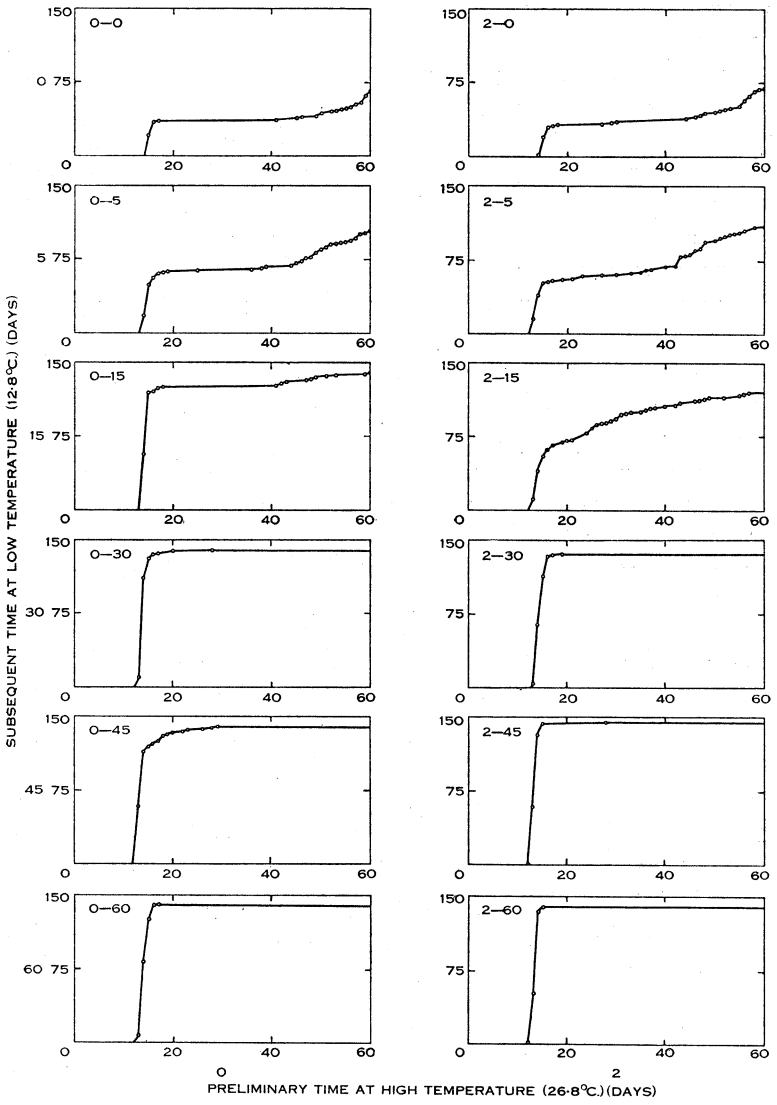

Fig. 1.—Cumulative frequency diagrams of hatching for all treatments, obtained by adding the results of all replicates of each treatment. In individual treatment graphs the ordinate represents the number of eggs hatched and the abscissa time in days.

but further increase to 12 days did not further decrease the number that hatched. In both the six-day and 12-day periods of preliminary high temperature treatment the number hatching increased as the subsequent period of

exposure to low temperature increased up to 30 days, but no significant increase occurred when this period was further extended.

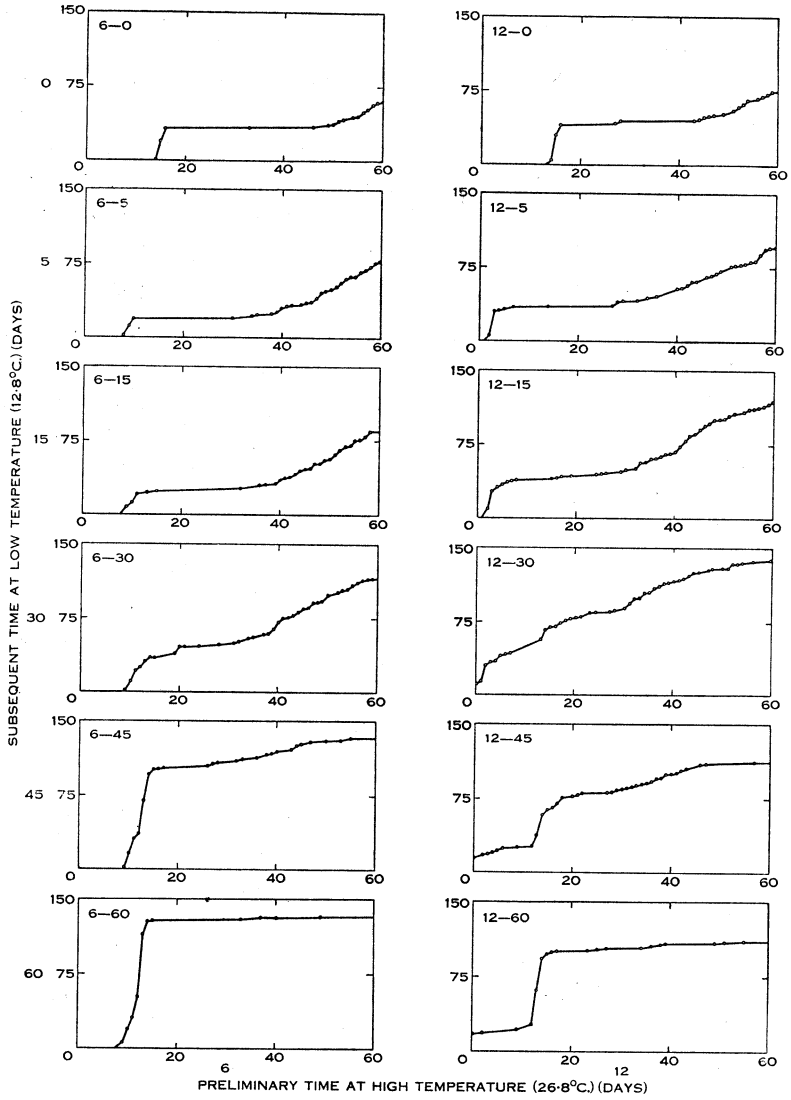

Fig. 1.—For explanation see opposite page.

In general, the preliminary period of treatment at high temperature had no influence on the total number of eggs that hatched unless it was extended beyond two days, whilst increase in the period of subsequent exposure to low temperature up to about 30 days resulted in an increase in the number hatching in all treatments. Further increase in the period of low temperature treatment had no significant influence. In general the number of eggs that hatched after experiencing a preliminary exposure to high temperature depended on

the duration of their subsequent exposure to low temperature; and the influence of low temperature in increasing the numbers that hatched depended on the duration of the preliminary exposure to high temperature. This is shown by a very highly significant variance ratio for the interaction between the high and low temperature treatments.

If the hatching of an egg is taken as the criterion that the embryo had successfully completed its diapause (or that diapause did not occur) then it is clear that extended periods of treatment at low temperature (30 days or more) were most influential in promoting diapause completion but that extension of the period of preliminary high temperature treatment greatly reduced the influence of the subsequent low temperature treatment.

(*b*) *Mean Duration of the Incubation Period*

In Table 2 are set out the means of the mean duration of the incubation period of those eggs that hatched following each treatment. It can be seen that increase in the duration of the preliminary high temperature treatment from nil to six days resulted in an increase in the duration of the incubation period, whilst further increase in high temperature treatment to 12 days was followed by a fall in the duration of the incubation period.

TABLE 2

MEAN DURATION OF INCUBATION PERIOD, IN DAYS

H.T. = 26.8°C., L.T. = 12.8°C.

L.T. Treatment (days)	H.T. (days) 0	2	6	12	L.T. Treatment General Means
0	33.5	35.0	33.4	30.8	33.2
5	28.3	28.5	39.2	30.5	31.6
15	17.6	22.2	40.7	29.0	27.4
30	13.8	14.2	32.0	20.3	20.5
45	14.0	13.2	18.2	18.7	16.3
60	14.0	13.2	12.6	13.4	13.3
H.T. treatment general means	20.2	21.5	29.3	23.8	

Least differences for significance at $P = 0.01$ between: individual treatment means = 7.6 days; high temperature treatment general means = 3.1 days; low temperature treatment general means = 3.8 days.

The duration of the incubation period became progressively shorter as the time spent at low temperature was increased from nil to 30 days in all cases except those receiving six days preliminary exposure to high temperature. Further increase in the duration of exposure to low temperature had no significant influence on the duration of the incubation period in treatments receiving nil or two days high temperature treatment whereas in treatments receiving six and 12 days high temperature treatment, increase in the duration of exposure to 12.8°C. up to 60 days continued to result in a decrease in the duration of the incubation period.

The very low values obtained for treatments 6-45, 6-60, 12-45, and 12-60 (Table 2) are due, in part, to the fact that a few eggs hatched very early after their return to high temperature for incubation (Fig. 1). These eggs must have completed a considerable part of their development during the long preliminary period of exposure to high temperature and then continued development, albeit very slowly, during the long periods spent at low temperature and so were almost ready to hatch on return to high temperature for incubation. A very small number of eggs in treatments 12-30, 12-45, and 12-60 were found to have hatched whilst at 12.8°C. This further assisted in reducing the mean duration of the incubation period following these treatments. Such eggs must have been virtually without diapause.

Eggs in which diapause had been successfully completed during the exposure to low temperature hatched promptly (after about 13 days) on return to high temperature for incubation and it can be seen from Figure 1 and Table 2 that the most influential treatments in reducing the average period required for incubation and so of promoting the successful completion of diapause were those in which nil or two days preliminary high temperature and 30 or more days low temperature treatment were given.

TABLE 3

MEAN LOG VARIANCE OF HATCHING DISTRIBUTION FOR EACH TREATMENT

H.T. = 26.8°C., L.T. = 12.8°C.

L.T. Treatment (days)	H.T. (days)				L.T. Treatment General Means
	0	2	6	12	
0	2.91 (406)	2.53 (343)	2.59 (388)	2.54 (350)	2.57 (372)
5	2.50 (319)	2.43 (272)	2.50 (334)	2.66 (465)	2.52 (348)
15	2.00 (108)	2.09 (130)	2.41 (275)	2.57 (376)	2.27 (222)
30	0.05 (2)	—0.21 (1)	2.45 (280)	2.43 (277)	1.18 (140)
45	0.77 (7)	—0.02 (2)	2.16 (147)	2.23 (186)	1.28 (85)
60	—0.68 (0)	—0.52 (0)	1.26 (20)	1.97 (95)	0.51 (29)
H.T. treatment general means	1.21 (140)	1.05 (125)	2.23 (257)	2.30 (292)	

Least differences for significance at $P = 0.01$ between: individual treatment means = 0.525; high temperature treatment general means = 0.208; low temperature treatment general means = 0.263.

(*c*) *Variance of the Distribution of Hatchings*

Figure 1 shows that in some treatments all the hatchings occurred during a few days and that in these cases the mean duration of the incubation period was also short, whilst in other cases hatchings occurred during a much longer period and often the mean duration of the incubation period was longer (cf. Table 2). Even in cases where the mean duration of the incubation period was short there were sometimes wide deviations from the mean among the individual hatchings. The variance of the hatching distribution during the 60 days of incubation was used as a measure of the variation in hatching time among the eggs in each treatment.

The variances were first transformed into logarithms and the analysis was performed on these. In Table 3 are set out the log variances, with the true variances in days^2 in parentheses.

It can be seen from Table 3 that in all treatments, except those receiving nil or two days preliminary high temperature treatment and 30 or more days subsequent low temperature treatment, the variances were very large, whilst in the latter treatments the variances were quite small. There was, however, a general tendency for the variances to be reduced as the period of low temperature treatment was extended.

Treatments in which most eggs hatched during a short period were those in which the diapause of the eggs had been successfully completed so that most eggs were ready to recommence their morphological development and hatch promptly on return to high temperature.

TABLE 4

DISTRIBUTION OF HATCHING OVER THE 60 DAYS OF INCUBATION* — *A*, WITH REGARD TO HIGH TEMPERATURE TREATMENT; *B*, WITH REGARD TO LOW TEMPERATURE TREATMENT

	Period (days)	0-7	8-11	12-20	21-40	41-60
	H.T.					
A	Treatment					
	0	0	0	634	12	81
	2	0	0	575	52	88
	6	0	104	246	63	190
	12	156	5	215	115	160
	Total	156	109	1670	242	519
	L.T.					
B	Treatment					
	0	0	0	139	8	122
	5	34	18	119	47	168
	15	37	6	219	66	133
	30	43	21	334	64	66
	45	24	31	401	46	26
	60	18	33	458	11	4
	Total	156	109	1670	242	519

* No attempt was made to analyse these results as they were very unwieldy from a statistical point of view and the differences seemed so great as to be self-evident. Also the replicates within each treatment agreed closely.

(*d*) *Distribution of Hatching*

Hatching was distributed very unevenly over the 60 days of observation when all eggs hatching during the experiment are considered. This is shown by the curves in Figure 1 but is demonstrated more explicitly in Table 4. In the table the 60-day observation period has been divided arbitrarily into five periods and the total number of eggs hatching during each period is shown with regard, in *A*, to the preliminary high temperature treatment and, in *B*, to the subsequent low temperature treatment.

All hatchings that occurred during the first seven days resulted from eggs that received 12 days high temperature treatment, and almost all the hatchings occurring during the following four days resulted from eggs that received six days high temperature treatment (Table 4*A*). In both these cases the subsequent low temperature treatment had very little influence on the number of eggs that hatched. These eggs must have completed a considerable part of their development during the treatment period and so must have been virtually without diapause (Table 4*B*).

The period from 12 to 20 days contained about three-fifths of the total number of eggs that hatched during the experiment and of these about three-quarters were due to treatments receiving nil or two days high temperature followed by 30 or more days low temperature. This period contains the modal period of incubation at 26.8°C. for eggs free from diapause (Browning 1952). Most of the hatchings occurring during the period from 21 to 40 days were due to treatments 12-15 and 12-30, the remainder being spread fairly evenly over all other treatments.

The final period was characterized by a marked rise in the total hatchings compared with the previous period, in fact it contributed about half the hatchings not occurring in the period from 12 to 20 days. Most of the total of 519 was contributed by the high temperature treatments 6 and 12 days which received 0, 5, or 15 days subsequent low temperature treatment. This was most probably due to the completion of diapause and subsequent development of these eggs even under conditions of continuous high temperature incubation. Diapause development required a much longer period and was much less certain to lead to the healthy hatching of the young nymph at high than at low temperature but nevertheless was successful in some cases.

(*e*) *Non-Diapause Eggs*

Figure 1 shows that in treatments in which no time was spent at low temperature (graphs 0-0, 2-0, 6-0, and 12-0), about 20 per cent. of the eggs hatched without obvious delay. The modal period of incubation of these eggs was between 14 and 15 days (see Table 4*B*, entries for no low temperature treatment). After this time few or no hatchings occurred until after about 45 days had elapsed. A comparison was made between the mean duration of the incubation period of all the eggs that hatched within the first 20 days in these four treatments (the treatments are in fact all similar: see Section II(*b*)) and the mean duration of the incubation period of all eggs that hatched following treatments 0-30, 0-45, and 0-60. The results are shown as a frequency distribution diagram in Figure 2. In Figure 2*A* the hatching distribution of all eggs from treatments 0-30, 0-45, and 0-60 have been divided by 3 to make them more easily comparable with those of Figure 2*B* which shows the distribution of hatching of all eggs from treatments 0-0, 2-0, 6-0, and 12-0 together. It is clear from the figure that hatching in eggs that hatched during the first 20 days of the experiment in treatments that received no low temperature treatment was distributed in a very similar manner to that in the treatments in which diapause had been completed at low temperature. The means

of the two distributions (13.9 days in Figure 2*A*; 14.8 days in Figure 2*B* shown by the broken lines), although significantly different, were very similar and lend weight to the conclusion that the early-hatching group of eggs in the control treatments developed virtually without diapause.

This conclusion is further substantiated by the fact that a group of eggs in treatments receiving 12 days preliminary high temperature treatment and some subsequent low temperature treatment hatched much sooner after return to incubation than any other eggs in the experiment (Table 4*A*). These eggs must also have been virtually without diapause since they were able to complete a substantial part of their development during their initial exposure to low temperature.

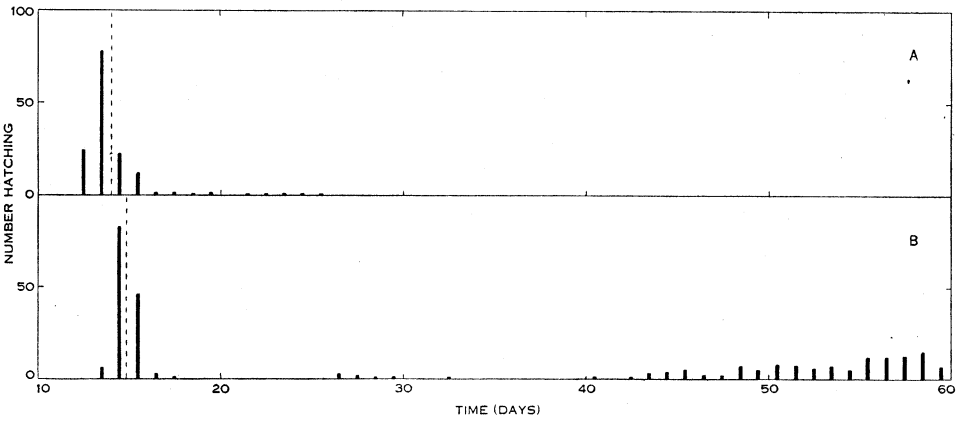

Fig. 2.—Frequency diagrams of the distribution of hatching of: *A*, all eggs from treatments with no preliminary high temperature treatment and 30 or more days low temperature treatment prior to incubation at 26.8°C. *B*, all eggs receiving no low temperature treatment and incubated from day of laying at 26.8°C. continuously for 60 days.

(*f*) *Pigmentation of Eggs and Hatching at Low Temperatures*

During the course of the experiment it was observed that certain eggs in treatments 12-30, 12-45, and 12-60, when removed from the low temperature cabinet, were quite black. It was also found that certain eggs in the above three treatments hatched whilst still at low temperature. Neither of these phenomena was observed in any other treatment.

Further consideration of these two observations will be deferred to Section IV.

IV. Discussion

In the experiment hatching was taken as the criterion of the successful completion of diapause. It is realized that this was perhaps an inadequate yardstick but it was considered to be the only one practicable in this case. Better criteria would have been the ability of young nymphs to feed and grow,*

* Dr. H. G. Andrewartha informs me that he was not able to induce nymphs of *Austroicetes cruciata* to feed when the embryonic diapause had been completed under experimental conditions, whereas nymphs hatched naturally in the field fed readily in captivity.

the ability of the eggs to produce fecund adults, or measurements could have been made of the oxygen consumption rate of the eggs and the time of completion of the diapause judged from this (Bodine 1932). None of these methods was practicable, however, and diapause was considered to have been completed if the egg hatched.

In a few instances nymphs were found to have broken out of the egg membranes but to have died before completing the first moult. Nevertheless, they were considered as having hatched. It seems from this that diapause may be completed under some circumstances but that development is in some way "unhealthy" or "abnormal." This is a subject that merits much more consideration than it has been given.

The experiment was stopped when each replicate, after treatment, had spent 60 days in the incubation cabinet. This also is a defect in the method but it was considered that the experiment could achieve its object even if eggs that would have hatched later than this were discarded.

It is usual to assume that the distribution of hatching of healthy eggs developing without diapause, when incubated at a particular adequate temperture, will be nearly normal about a particular mean and with a particular variance and that a high proportion of the eggs will hatch. It is known that in the eggs of *G. commodus*, in which the diapause was completed at low temperature, the mean duration of the incubation period at 26.8°C. is 13.1 days with a variance of 0.35 days^2 and that an average of 94 per cent. hatched (Browning 1952). Treatments producing results approaching these values then can be considered as having been influential in promoting the completion of diapause. For this reason, the three criteria chosen in the analyses of the results of the experiment were (1) total number of eggs that hatched during the 60-day period of observation, (2) mean duration of the incubation period, and (3) variance of the hatching distribution. A large value for the first and small values for the second and third indicate that diapause was at a minimum and that morphogenetic development was proceeding with a minimum of interruption.

It is evident from Figure 1 and Tables 1, 2, and 3 that the treatments in which the greatest numbers of eggs hatched in the shortest time and with the smallest "scatter" were those that received nil or two days of preliminary high temperature treatment and 30 or more days low temperature treatment prior to incubation. There was no increase in effectiveness as the low temperature treatment period was extended beyond 30 days nor was there much to choose between nil and two days preliminary high temperature treatment. Treatment 0-30 then exerted the maximum influence in the minimum treatment time.

Hatching in all treatments other than the six mentioned above showed some degree of departure from optimum conditions and so can be considered to have been less effective than these in promoting the completion of diapause in the eggs.

Figure 1 shows that with the exception of the above six treatments hatchings in all others occurred over a wide range in time. If hatching really means that diapause had been successfully completed, or did not occur, then this

"spread" of hatching must mean that there was a great variability in the intensity of diapause among the eggs. The progressive increase in the number of eggs that hatched promptly after return to incubation as the duration of the period spent at low temperature was increased leads to the same conclusion. Eggs in which the diapause was least intense were able to complete their diapause development in a very short time at low temperature whereas those in which diapause was more intense required a longer period at low temperature to allow of successful completion of their diapause development. Diapause having been completed at low temperature, eggs were able to make full use of the high temperature of incubation and resume their morphogenesis immediately.

Diapause was, however, completed by some eggs even when maintained at constant high temperature, since some eggs hatched under these conditions, but a much longer time was required than when diapause development proceeded at low temperature, and a much lower proportion of eggs were able to complete their development to the point of hatching. Andrewartha (1943) showed that certain low temperatures were more influential in promoting the completion of diapause development than others and the same is probably true of *Gryllulus*, 26.8°C. being near the upper end of the range of effective low temperatures. Work at present in progress confirms this.

Preliminary high temperature treatment for more than two days caused the eggs to enter diapause more firmly than they did if subject to little or no high temperature treatment prior to their treatment at low temperature. Such eggs required a much longer exposure to low temperature to permit them to complete their diapause development than eggs that had not been exposed to high temperature initially. This can be seen by comparing the hatching distributions of treatments 0-30 and 2-30 with those of 6-30, 6-45, and 6-60 (Fig. 1), when it is clear that the distribution of hatching following treatment 6-60 was very similar to those of treatments 0-30 and 2-30, whereas there was a progressive diminution in the number of eggs hatching and increase in the variance of the hatching distributions as the period spent at low temperature became shorter. This cannot have been due to any detrimental influence of the preliminary exposure to high temperature *per se*, but must rather have been due to the insufficiently long period of exposure to low temperature. But since 30 days was an adequate exposure to low temperature to enable diapause development to be completed following little or no preliminary high temperature treatment, it follows that the diapause in the eggs given a lengthy initial exposure to high temperature was more intense and so required a longer period for its completion.

High temperature then influenced the eggs of *Gryllulus* in two ways. Initially eggs were induced to enter diapause more firmly under the influence of high temperature than when no preliminary high temperature treatment was given, but diapause was completed in some cases at least whilst the eggs remained at high temperature. This double influence of high temperature is most satisfactorily explained on the assumption that the physiological processes concerned in the inception of diapause in the eggs and those occurring during

diapause (diapause development) have different temperature optima but that the ranges of temperature tolerable to each group of processes overlap to some extent. Very low temperatures (12.8°C.) then would be near the optimum for diapause development but would not be influential in increasing the intensity of diapause whereas high temperatures (26.8°C.) tend to increase the intensity of diapause but at the same time they permit the processes of diapause development to continue, though slowly and inefficiently.

It has been shown in Section III(*e*) that some 20 per cent. of the eggs laid under the conditions of this experiment were without a diapause stage in their development (Fig. 2), but the experiment gives no information about the conditions under which such eggs occur. It is not known, for example, whether the diapause-free eggs were laid by females laying only such eggs or whether most females laid some. Work at present in progress indicates that the temperature of incubation influences the percentage of eggs that enter diapause, and Simmonds (1948) has shown that in certain Hymenoptera the physiological state of the female is important in influencing the incidence of diapause in her progeny. Some such condition may have been operating in this case.

Diapause in the eggs of Orthoptera has been shown to occur at various stages in the morphological development of the embryo in different species. In *Austroicetes cruciata* the embryo is in a very early stage of development with little morphological differentiation when diapause occurs (Andrewartha 1943), whilst in *Melanoplus differentialis* diapause occurs at the stage when revolution round the posterior pole of the egg is about to begin (Slifer 1932). Again Moore (1948) and Salt (1949) have shown that in *Melanoplus bivattatus* and *M. mexicanus* diapause occurs at a stage when the embryo has almost completed its morphological development. In *G. commodus* it was found that, in eggs that had remained for about 20 days at 26.8°C. without evident signs of development, the embryos when fixed and stained were for the most part in the stage where katatrepsis had commenced but before the embryo had reached the stage where revolution occurs. This stage is reached by about the fourth day of incubation by eggs that had completed their diapause at low temperature with no preliminary high temperature treatment. This may account for the observation that two days preliminary high temperature treatment had no detrimental influence on the subsequent completion of diapause in the eggs whereas six days high temperature treatment considerably interfered with development at low temperature. It would seem that the egg was able to make full use of the low temperature period provided this occurred before embryonic development had proceeded beyond the point where diapause normally occurred, whereas if the low temperature treatment was given later than this diapause development proceeded less efficiently. It was notable that in eggs that had not hatched even after two months at constant high temperature, the embryos were frequently malformed. On two occasions embryos were found to have almost completed their development without having undergone revolution. Andrewartha (1943) reported similar "monsters" in *Austroicetes* that had been kept continuously at high temperature.

In Section III(*f*) it was noted that certain eggs in treatments 12-30, 12-45, and 12-60 were black on removal from low temperature. Dissections of these eggs showed that the embryos were fully developed and darkened, whereas in the young nymph darkening of the cuticle does not visibly begin at 26.8°C. until about an hour after hatching. It has been shown in the eggs of *Melanoplus differentialis* that tyrosinase activity rose steadily for the first 20 days of development and then remained fairly constant (Bodine and Boell 1935). The enzyme was almost entirely confined to the yolk and serosal cells until the time of yolk engulfment when activity appeared in the embryo. Dennell (1947) has shown that in the larva of *Sarcophaga falculata* tyrosine was present during the rise in tyrosinase activity but that the two were prevented from reacting by a low oxidation-reduction potential. At the time of pupation there was a marked rise in the oxidation-reduction potential followed by darkening and hardening of the cuticle. Probably some mechanism very akin to these occurs in the diapause-free eggs of *G. commodus* on about the twelfth day of incubation at high temperature. Subsequent low temperature treatment no doubt slowed down the rate of darkening and hardening of the cuticle without completely inhibiting it, with the result that at the end of about 30 days some of the embryos were black and hard. Hardening of the cuticle would then inhibit the action of the cephalic vesicle, which assists in hatching (Cappe de Baillon 1920).

It was also noted in Section III(*f*) that in the above three treatments some nymphs were found that had hatched whilst at low temperature. Slifer (1938) has shown that in *Melanoplus differentialis* hatching is accomplished by means of an enzyme (or complex of enzymes), secreted by the pleuropodia just before hatching is due to occur. These organs are also present on *Gryllulus* embryos and doubtless subserve the same function. It seems likely then that at about the time the eggs were moved to low temperature after 12 days at high temperature, the hatching enzyme was secreted by embryos that were in an advanced stage of development owing to the absence of a diapause stage and that the action of this enzyme, although retarded at low temperature, was not inhibited completely, with the result that after about 30 days some nymphs were able to hatch. All such nymphs were black, none had succeeded in escaping from the hatching membrane, and all were dead.

It may be said of both of the above phenomena that initiation need not have occurred at high temperature but rather after removal to low temperature. This may be so, but in view of the late stage of morphological development such embryos had reached after 12 days at high temperature it is likely that such processes would have come into operation by that time. In any case the principle remains unaltered that development, in the sense of the continuation of these two processes at least, was able to continue to completion at a slow but still perceptible rate at low temperature, whereas it seems likely that development in the sense of morphological differentiation was slowed to an extremely low rate at low temperature.

V. Acknowledgments

The author wishes to thank Mr. D. C. Swan, Head of the Department of Entomology, Waite Agricultural Research Institute, for his constant interest and encouragement during the course of this work. To Dr. H. G. Andrewartha, also of this Department, is owed a debt of gratitude for his kindly help and discussion on all phases of this work. Thanks are also due to Dr. E. A. Cornish, Section of Mathematical Statistics, C.S.I.R.O., for guidance in the statistical treatments used. Miss M. E. Morphett gave valuable technical assistance and prepared the figures.

VI. References

Andrewartha, H. G. (1943).—Diapause in the eggs of *Austroicetes cruciata* Sauss. (Acrididae) with particular reference to the influence of temperature on the elimination of diapause. *Bull Ent. Res.* **34**: 1-17.

Andrewartha, H. G. (1952).—Diapause and the ecology of insects. *Biol. Rev.* **27**(1) (in press).

Bartlett, M. S. (1947).—The use of transformations. *Biometrics* **3**: 39-52.

Bodine, J. H. (1932).—Hibernation and diapause in certain Orthoptera. II. Response to temperature during hibernation and diapause. *Physiol. Zoöl.* **5**: 538-48.

Bodine, J. H., and Boell, E. J. (1935).—Enzymes in ontogenesis (Orthoptera). I. Tyrosinase. *J. Cell. Comp. Physiol.* **6**: 263-75.

Browning, T. O. (1952).—The influence of temperature on the rate of development of insects, with special reference to the eggs of *Gryllulus commodus* Walker. *Aust. J. Sci. Res.* B **5**: 96-111.

Cappe de Baillon, P. (1920).—Contributions anatomiques et physiologiques à l'étude de la reproduction chez les locustiens et les grilloniens. II. La ponte et l'éclosion chez les grilloniens. Conclusions générales. *Cellule* **32**: 1-193.

Dennell, R. (1947).—A study of an insect cuticle: the formation of the puparium of *Sarcophaga falculata* Pan. (Dipt.). *Proc. Roy. Soc.* B **134**: 79-110.

Fisher, R. A., and Yates, F. (1948).—"Statistical Tables for Biological, Agricultural and Medical Research." (Edinburgh.)

Moore, H. W. (1948).—Variations in fall embryological development in three grasshopper species. *Canad. Ent.* **80**: 83-8.

Salt, W. R. (1949).—A key to the embryological development of *Melanoplus bivittatus* (Say), *M.m. mexicanus* (Sauss.) and *M. packardii* Scudder. *Canad. J. Res.* D **27**: 233-5.

Simmonds, F. J. (1948).—The influence of maternal physiology on the incidence of diapause. *Philos. Trans.* **233**: 384-414.

Slifer, E. H. (1932).—Insect development. IV. External morphology of grasshopper embryos of known age and with a known temperature history. *J. Morph.* **53**: 1-21.

Slifer, E. H. (1938).—A cytological study of the pleuropodia of *Melanoplus differentialis* (Orthoptera, Acrididae) which furnishes new evidence that they produce the hatching enzyme. *J. Morph.* **63**: 181-205.