

THE INFLUENCE OF TEMPERATURE ON THE
INTENSITY OF DIAPAUSE IN THE EGGS
OF THE EMMA FIELD CRICKET
(Orthoptera : Gryllidae)*

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During the course of studies on the geographic variation in certain adaptive characters of the Emma field cricket (= *Gryllulus mitratus* in former publications), it was necessary to establish a standardized method by which the diapause characters of local strains might be compared. Series of tests have consequently been carried out in order to know reactions of the diapause eggs to external conditions—especially temperature. As a result of these tests, some information of physiological interest has been obtained. One of the findings is the effect of temperature operating in early stages of the embryonic development on the subsequent intensity of diapause. Such an aspect of diapause phenomenon has hitherto scarcely been explored in crickets, except Hogan (1960) occasionally referred to this in his study on the Australian species, *Acheta commodus*.

An account of this particular effect of temperature on the embryonic diapause of the Emma field cricket is the object of the present article. No effort has, however, been made to approach the physiological mechanisms involved because of the subsidiary nature of this work.

Material and Method

This work was initiated at Tsu while the author was at the Mie University, with eggs which were deposited by adults of the 1956-year brood collected in the field. Most of the data were obtained after he changed his post to the Hirosaki University, using the Hirosaki strain of the same species. Laboratory stocks of this strain were maintained throughout the year by rearing nymphs and adults at about 28°C in darkness and by exposing eggs in every generation to 10°C for longer than four months before incubation at the high temperature. The latter procedure not only ensures a simultaneous hatching of most individuals on incubation, but also excludes a possibility of selection of diapause characters during successive laboratory generations. The eggs of this field cricket are, however, able to resume development at constant high temperatures.

In every test, young adults were reared for a few weeks in order to mature them, and then they were allowed to lay their eggs in dishes of moist sand for 48 hours. Eggs laid in the dishes were sieved out from sand in water, and placed on a piece of filter paper covering moist absorbent cotton in petri dishes. They were then subjected to test conditions, about 100 eggs being allotted to each treatment.

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As stated elsewhere (Masaki, 1960), diapause in the eggs of the Emma field cricket begins, at least morphologically, at the so-called dumb-bell shaped stage. As the embryo approaches this stage, the egg takes up water if it is kept in moist surroundings, and as a result conspicuously swells out. The completion of the pre-diapause development can, therefore, be judged from the external appearance without killing the egg. In the experiments described below, batches of eggs of up to 48-hour age were exposed to different temperatures until they completed water uptake, and then each batch was further divided into different temperature treatments. This simple procedure was adopted to detect any effect of the preliminary temperature treatment on the subsequent intensity of diapause. The duration of the egg stage was taken as a measure to represent the relative intensity of diapause, because the rate of post-diapause development seemed to be scarcely influenced by the previous temperature history of the eggs so that any change in the length of egg stage could be ascribed to the variable duration of diapause.

Before describing the results, it should be noted that the clear-cut demarcation was impossible between the pre-diapause and diapause stages by the above method, since the process of moisture absorption possibly extends into early stages of diapause (cf. Hogan, 1960). Moreover, there were considerable variations among eggs in the speed of water uptake. In the present study, the completion of water uptake was arbitrarily represented by the completion of water uptake of all eggs in a given sample. It was then inevitable that at the time of interchange of temperature any batch would comprise of eggs of different physiological states, i. e., some eggs would be just initiating diapause but others would have been already in diapause a few days ago. From these reasons, the effects of temperature during the pre-diapause and early diapause stages could not be estimated separately. This is an important defect of this work, rendering it impossible to determine the precise stage of the embryo sensitive to the particular action of temperature.

Results

The effect of preliminary temperature treatment on the hatching time at constant temperatures

Evidence of the carry-over effect of temperature operating in initial stages of the embryonic life on the subsequent length of the egg stage was first observed in experiments conducted at Tsu. The eggs of the Tsu strain of the Emma field cricket took about 20, 11 and 8 days to complete water absorption at 20, 25 and 30°C, respectively. After the completion of water uptake at each of these temperatures, eggs were further divided into three batches and again subjected to temperatures of 20, 25 and 30°C, respectively, until hatching.

As set out in Table 1, the duration of the egg stage varied noticeably, being influenced by the treatments. The general tendency was that the lower the temperature of the first exposure, the shorter was the length of the egg stage at each temperature of the second exposure. In addition to this, the higher the temperature of the second exposure, the less remarkable was this carry-over effect of the first exposure. Thus the duration of the egg stage increased by about 50, 30 and 20 days at the subsequent temperatures of 20, 25 and 30°C, respectively, when the temperature in the first exposure was raised from 20 to 30°C.

Table 1. *The effect of preliminary temperature treatment on the duration of egg stage in the Emma field cricket.*

Strain	First exposure		Second exposure		Total length of egg stage		No. of viable eggs	Significant difference	
	Temp. (°C)	Duration in days	Temp. (°C)	Duration in days	Mean	S. D.		t	P
Tsu	20	20	20	136.9	156.9	27.3	154	6.34	<0.01
	25	11	"	168.9	179.9	35.8	153	3.37	<0.01
	30	8	"	185.0	193.0	31.1	153		
	20	20	25	79.8	99.8	13.1	131	2.17	<0.05
	25	11	"	92.3	103.3	12.7	125	6.80	<0.01
	30	8	"	108.5	116.5	17.5	121		
	20	20	30	67.9	87.9	15.5	116	5.29	<0.01
	25	11	"	87.7	98.7	15.2	110	1.15	>0.05
	30	8	"	88.3	96.3	13.4	84		
Hirosaki	20	15	25	55.6	70.6	7.3	118	11.75	<0.01
	30	9	"	75.0	84.0	8.6	74		

A subsidiary test was made with eggs of the Hirosaki strain, the results of which are given at the bottom in Table 1. The eggs of this northern race hatched in a much shorter time than did the southern race (cf. Masaki, 1961), but they responded to temperature in the same way. The higher temperature in the preliminary treatment delayed the hatching time at 25°C by about 20 days as compared with the low one.

From these results, there leaves little doubt that the temperature during the pre-diapause and/or early diapause stages affects either the duration of diapause or the rate of post-diapause development, or both.

It will further be noted that within the limits of available temperatures the response of eggs to temperature in the second exposure was just reversed from that in the first exposure. Namely, eggs from each previous treatment hatched earlier when they were removed and kept at a higher temperature than at a low. The egg stage was consequently the shortest when the lowest temperature in the first exposure was followed by the highest one in the second exposure. It was conversely the longest when eggs were first exposed to the highest temperature and then removed to the lowest one. The over-all length of the egg stage was thus determined by the interaction of temperatures in the two consecutive exposures.

The effect of preliminary temperature treatment on the response of diapause eggs to cold exposure

In the above experiments, the eggs underwent the two different physiological stages, diapause and post-diapause development, during the second exposure. In order to know the nature of the carry-over effect of the first exposure, it is essential to clarify which of these two stages is affected.

Now, the eggs of the Emma field cricket are able to terminate diapause at temperatures both below and above the lower threshold for development (between 10 and 15°C in the Hirosaki strain). The answer for this question may therefore be given by evaluating the period of time taken at a cold temperature for the break-

ing of diapause in those eggs which had initially been kept at different temperatures, respectively. If the strength of diapause would be affected, the median effective duration of cold exposure for the termination of diapause would be expected to vary depending on the previous temperature.

Two series of freshly laid egg-batches were subjected to 20°C for 15 days and to 30°C for 9 days, respectively, during which they were allowed to absorb sufficient moisture. Each series was then exposed to about 6°C — a temperature well below the developmental zero. After various durations of this exposure, egg batches from both series were incubated at 25°C and the numbers of nymphs that hatched out within three weeks of incubation were counted. These numbers approximately represented the proportion of eggs which resumed their development without an appreciable delay after the cold exposure.

Table 2. *The effect of preliminary temperature treatment on the subsequent response of diapause eggs of the Emma field cricket to cold exposure.*

Exposure to 6°C in weeks	Hatching at 25°C of eggs kept at 30°C for 9 days before cold exposure			Hatching at 25°C of eggs kept at 20°C for 15 days before cold exposure		
	No. of prompt hatch	No. of delayed hatch	Percentage prompt hatch	No. of prompt hatch	No. of delayed hatch	Percentage prompt hatch
0	0	74	0	0	118	0
3	0	63	0	4	121	3.2
6	13	58	18.3	86	31	73.5
8	31	38	44.9	123	6	95.3
10	55	21	70.5	112	1	99.1
11	47	17	73.4			
12	54	12	81.8			

The results are summarized in Table 2. In both series, the proportion of prompt hatch at 25°C increased with the increase in the length of cold exposure. There was, however, a striking difference between the two series. The eggs of the 20°C series needed a much smaller amount of cold treatment than did those of the 30°C series to become free of diapause. From the straight lines obtained by probit transformation of the data, the median effective duration of the treatment was estimated at about 60 days in the latter series while it was at about 40 days in the former (Fig. 1). On the other hand, there was no detectable difference between them in the time taken for hatching at 25°C of those eggs having completed the substantial part of diapause during the cold exposure.

It can be concluded from these results that the temperature before cold exposure affects the intensity of diapause but not the rate of post-diapause development.

The effect of high temperature treatment in different stages on the intensity of diapause

A second problem to be dealt with is the stage of development at which the eggs are most sensitive to this diapause-intensifying action of high temperature. To clear up this point, eggs were first subjected to 30°C for different periods ranging from 0 to 42 days, then transferred to 20°C, and held at this temperature until hatching. The mean durations of the egg stage in these tests are given in Table 3. The table reveals that the intensification of diapause — as judged from

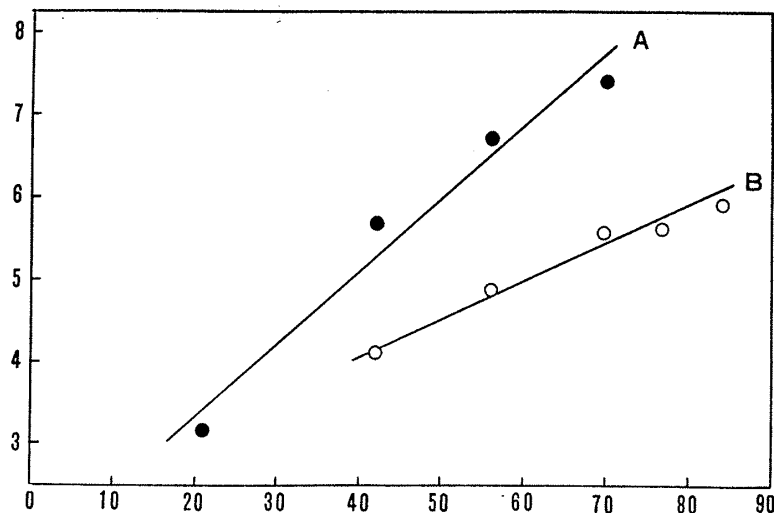


Fig. 1. The response to cold exposure (6°C) of eggs of the Emma field cricket kept previously at 20°C (A) and 30°C (B), respectively. Ordinate: probit of percentage hatching within three weeks' incubation at 25°C after the cold exposure; abscissa: the duration of cold exposure in days.

the over-all duration of the egg stage—became more conspicuous in proportion to the extending duration of the heat treatment until it reached its maximum by 9 or 14 days' treatment. A further increase in the amount of heat exposure resulted in the shortening of hatching time. This is probably due to the fact that the diapause development is accelerated by high temperatures in its later stages.

Observations at Tsu indicate that the embryo reaches the diapause stage about a week after being laid at 30°C, though there is a considerable variation in the rate of development among individuals. If the rate of pre-diapause development

Table 3. *The effect of high temperature treatment of different durations on the hatching time of the Emma field cricket; the treatment was applied from the beginning of the egg stage, after which the eggs were kept at 20°C.*

Exposure to 30°C in days	Length of egg stage in days		No. of viable eggs	Significant difference	
	Mean	S. D.		t	P
0	118.2	15.4	99	3.87	<0.01
2	125.7	11.4	97	1.52	>0.05
4	122.7	15.3	85	2.84	<0.05
6	128.4	11.8	96	1.91	>0.05
9	132.1	13.7	76	0.14	>0.05
14	132.4	14.0	100	0.91	>0.05
21	130.5	15.9	109	2.47	<0.05
28	124.7	16.6	84	3.55	<0.01
35	115.0	19.7	97	2.13	<0.05
42	108.5	22.0	90		

Table 4. *The effect of high temperature treatment (30°C) lasting for one week in different stages of development on the duration of the egg stage of the Emma field cricket; the eggs were kept at 20°C before and after the treatment.*

Age in days of the eggs subjected to treatment	Length of egg stage in days		No. of viable eggs	Significant difference	
	Mean	S. D.		t	P
4	131.2	12.0	91	2.32	<0.05
8	126.7	12.5	71	2.71	<0.01
12	121.2	16.7	76	2.64	<0.01
16	114.4	15.3	79	1.55	>0.05
20	117.9	13.8	89		

is not remarkably different in the Hirosaki strain, it appears that the diapause-intensifying action of high temperature is effected within the relatively short period, covering the late pre-diapause and early diapause stages. As the eggs go further into diapause, they seem to become unresponsive to this particular effect of temperature. A more precise determination of the sensitive stage was not practical from the present data, partly due to defects in the adopted method of experiment.

A similar conclusion was reached by another series of tests. The eggs in this series were first kept at 20°C for different periods ranging from 4 to 20 days, after which they were exposed to 30°C for one week and again returned to 20°C. As summarized in Table 4, the results show that the length of the egg stage was gradually shortened as the eggs were subjected to the high temperature in successively later stages. The effect of the high temperature lengthening diapause was no longer recognizable when eggs had been previously kept at the low temperature for longer than two weeks—the time sufficient for the eggs to reach the diapause stage at this temperature.

Discussion

The data given above would seem to suggest that the temperature during certain stages of the process inducing or establishing the state of diapause influences the future intensity of diapause. Such an effect is probably exerted on the diapause mechanism without disturbing the reaction which triggers the induction process of diapause, since diapause in the eggs of the Emma field cricket occurs obligatorily. This means that the “intensification” and the “induction” of diapause are different phenomena. On the other hand, it would seem difficult to admit the intensification as a physiological process distinct from “diapause development”, if one assumes that diapause development may take place both before and after the actual onset of diapause. The latter view was emphasized by Browning (1952) and Lees (1955).

Browning demonstrated that the eggs of *Acheta commodus* were able to hatch promptly at a high temperature when they had been exposed to 13°C for a certain period of time in the pre-diapause stage. This he attributed to the occurrence of diapause development before diapause actually sets in. According to this theory, the intensification (or, conversely, weakening) of diapause could hardly be realized as such. And the present results would be interpreted as showing the influence

of temperature on the rate of diapause development in the pre-diapause and early diapause stages. That is, freshly laid eggs would undergo different amounts of diapause development at different temperatures within a given time; accordingly, when they reach the morphological stage of diapause, their residual amounts of diapause development would vary with temperature at which they had previously been exposed. The "intensity" of diapause would mean nothing else but this residual amount of diapause. There is, however, evidence opposing this view.

Recently, Hogan (1960) repeated and extended the study on diapause in *Acheta commodus*. He showed that an exposure to cold itself during the pre-diapause stage does not avert diapause at all, and hence suggested that transference to a higher temperature would provide a necessary stimulus to make previously chilled eggs free from diapause. From this, he inclined to think that the averting of diapause prior to the diapause stage involves a unique physiological reaction differing from the normal completion of diapause. The diapause-weakening action of low temperature, which has been observed in the Emma field cricket, presumably differs from such a diapause-averting action, since the weakening of diapause by a low temperature was effected without subsequent transference to a higher temperature. For example, the eggs which were kept at 20°C throughout the whole period hatched out in a shorter time than did those that had previously been exposed to 30°C for a brief period and then removed to 20°C.

From these arguments, it seems reasonable to think that there occur variations in the intensity of diapause under the influence of temperature just before or after the onset of diapause. Hogan (1960) must have been aware of this phenomenon in *Acheta commodus* when he wrote, "There is a possibility that the intensity of diapause may be affected by the temperature at which onset takes place". In discussing the diapause of this cricket, he further stated, "the stronger diapause during the warm weather of early autumn means that eggs laid at this time are less likely to complete diapause development and hatch out at the wrong time". Although pertinent data were not given in his paper, these statements certainly imply that the influence of temperature on the strength of diapause is much the same in the Australian species as in the Emma field cricket.

The survival value of this physiological response is evident, as pointed out by Hogan in the above quotations. In our locality we hear the Emma field cricket singing from the end of July to the middle of October. During this long reproductive season, temperature usually attains its annual maximum at the beginning and declines towards the end. It follows that the diapause would tend to be weaker as eggs are laid later in the season. The relatively intense diapause occurring in the early season enables eggs to remain in the resistant state until winter, while the weaker diapause in the later season would suffice to prevent premature hatching. Moreover, an intense diapause—if it occurs in late autumn—would not be advantageous, since it would not be completed before the next growing season.

There is, however, a simpler way to prevent the wrong timing of hatching date. If a long period of cold is indispensable to overcome diapause, there is almost absolutely no hazard of untimely hatch before winter. A number of univoltine insects indeed take this way as exemplified by *Lymantria dispar*, the eggs of which

are laid in early summer but persist in diapause as long as warm weathers prevail. The trouble for the Emma field cricket, on the other hand, is that the eggs readily terminate diapause even at high temperatures favouring rapid development. Such a character of diapause might be linked to the whole physiological make-up and the evolutionary history of the species. Under these circumstances, at any rate, the variable intensity of diapause might be an efficient way of adjusting the life cycle to the seasonal changes of the environment. At the same time, it would confer on the species a higher chance to withstand the geographic gradient of climatic conditions, since a longer diapause is preferable in warmer areas and a weaker diapause in cooler areas. In reality, this phenotypical adjustment is superimposed by a genetic adaptation. Thus there have been found adaptive variations in the intensity of diapause among local populations of this field cricket (Masaki, 1961).

Summary

Series of experiments have been carried out with eggs of the Emma field cricket (= *Gryllulus mitratus* in former publications) in order to analyse the effects of temperature in early stages of development on the intensity of diapause, which supervenes at around the time the eggs absorb water.

In the first series, batches of pre-diapause eggs were first exposed to different temperatures, respectively, and after the completion of water uptake each batch was further divided and transferred to temperatures of the second exposure for hatching. The results show that the higher the temperature in the first exposure, the longer was the hatching time at each temperature of the second exposure.

When pre-diapause eggs had initially been kept at different temperatures and, soon after the onset of diapause, were subjected to cold exposure (6°C), the median effective duration of this treatment for the rupture of diapause was strikingly shorter in those eggs first kept at a low temperature (20°C) than in those kept at a high (30°C). From this it was concluded that the preliminary temperature treatment affects the intensity of diapause.

In further experiments, eggs were first exposed to a high temperature for different periods, or for the same period (one week) in different stages, and kept at a low temperature in the remaining stages. From the record of their hatching time, it was suggested that the eggs were responsive to the diapause-intensifying action of high temperature in the late pre-diapause and early diapause stages.

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