

THERMAL RELATIONS OF DIAPAUSE IN THE EGGS OF CERTAIN CRICKETS (ORTHOPTERA : GRYLLIDAE)*

Sinzo MASAKI
Laboratory of Entomology

INTRODUCTION

Diapause in an insect might ecologically be defined by the regimes of environmental factors responsible for its onset and termination. In the latter process temperature has been known to take most important part. The conception of diapause development, which has been set forward by ANDREWARTHA (1952) and now generally accepted, is indeed based on the analogy of the thermal relations of this physiological process to that of morphological development. The specific character of diapause can therefore be represented by the optimum, limit and coefficient of temperature for its completion. The pattern of the response illustrated by these measures would vary between different species and even between local populations of the same species (ANDREWARTHA, 1952; LEES, 1955).

The present work has dealt with the results of a study on the embryonic diapause in several species of common crickets. The primary purpose was to find out a species suitable for tackling the problem of local variation in diapause. In the results of this comparative study, certain interesting features of diapause in the crickets could be found, which will be stated below. Accounts of local variation of diapause in one of these crickets will be published elsewhere.

MATERIAL AND METHOD

In the experiments eggs of the following species of crickets were used: the Emma field-cricket (*Gryllulus* sp.), *Scapsipedus aspersus* WALKER, the Tambo-okame cricket (*Loxoblemus* sp.), *Pteronemobius fascipes* WALKER, *P. taprobanensis* WALKER, and *P. ohmachi* SHIRAKI. The two species referred to above by the common names are problematical ones and their situations more or less resemble that of the field crickets of North America (ALEXANDER, 1957). The colloquial names used here have been proposed by OHMACHI and MATSUURA (1950, 1951) in describing very closely related species of crickets.

All these crickets enter diapause in the egg stage. The first three species are strictly univoltine throughout their range in Japan, while the last three smaller species are bivoltine and their diapause is probably facultative at least in most, if not all, parts of their habitat. At Hirosaki, near the northern tip of Honshu, these

*Contribution No. 33 from the Laboratory of Entomology, Faculty of Agriculture,
Hirosaki University.

Pteronemobius seem to produce a partial second generation in a year.

Several pairs of adults of each species were captured on the university campus at Hirosaki in the middle of September, 1958. They were reared in jars in the laboratory by the method described by GHOURI and MCFARLANE (1958). About a week later a tray of moist sand was introduced into each jar for oviposition and after a day the eggs were sieved out under water. They were washed, counted and divided into batches, and kept in a small Petri-dish on a piece of blotting paper covering a thin layer of moist absorbent cotton. The batches of eggs were then subjected to experimental conditions. These procedures were repeated every day until the necessary number of eggs were allotted to each treatment.

The treatments comprised of two series. In the first trial the eggs were constantly kept at temperatures above the threshold for growth, namely, 20, 25 and 30°C. This was done, since previous experience shows that the length of diapause can easily be measured under these constant conditions without any exposure to cold. The second test was carried out in order to estimate the approximate time during hibernation at which the cricket eggs become competent to hatch. For this purpose, eggs were kept under the floor of the laboratory from the beginning of October (i.e., soon after oviposition), where the weekly mean of temperature gradually fell from 13°C in the first week of October to about 6°C at the beginning of December as shown below:

Week	1	2	3	4	5	6	7	8
Temperature	13.0	13.1	12.0	10.7	9.2	9.2	6.1	6.4°C

The daily fluctuation was usually within 3°C. At intervals of two or three weeks eggs were removed and divided into three batches, which were incubated at 20, 25 and 30°C, respectively. The three species of *Pteronemobius* and *Loxoblemus* were included in this experiment, though eggs of *P. ohmachii* were incubated only at 30°C after cold exposure.

The eggs were daily observed when they were at the high temperatures, and the number of nymphs that hatched was recorded. The observation was usually continued until there were no surviving eggs, but in the case of *P. ohmachii* the eggs were discarded after ten months of observation. At the time of observation spoiled eggs were removed if they were found, and the blotting paper on which the eggs were kept was renewed when it became filthy. In order to keep moisture chlorinated tap water was dropped to the cotton from time to time, since the eggs of all species under observation require to absorb water at a certain stage of development, after which they are still susceptible to desiccation (cf. BROWNING, 1953).

RESULTS

Hatching at constant temperatures

When the eggs of the crickets were constantly kept at temperatures above the threshold for growth, most of them could complete diapause and many nymphs subsequently hatched out over considerably long periods, except in *P. ohmachii* the

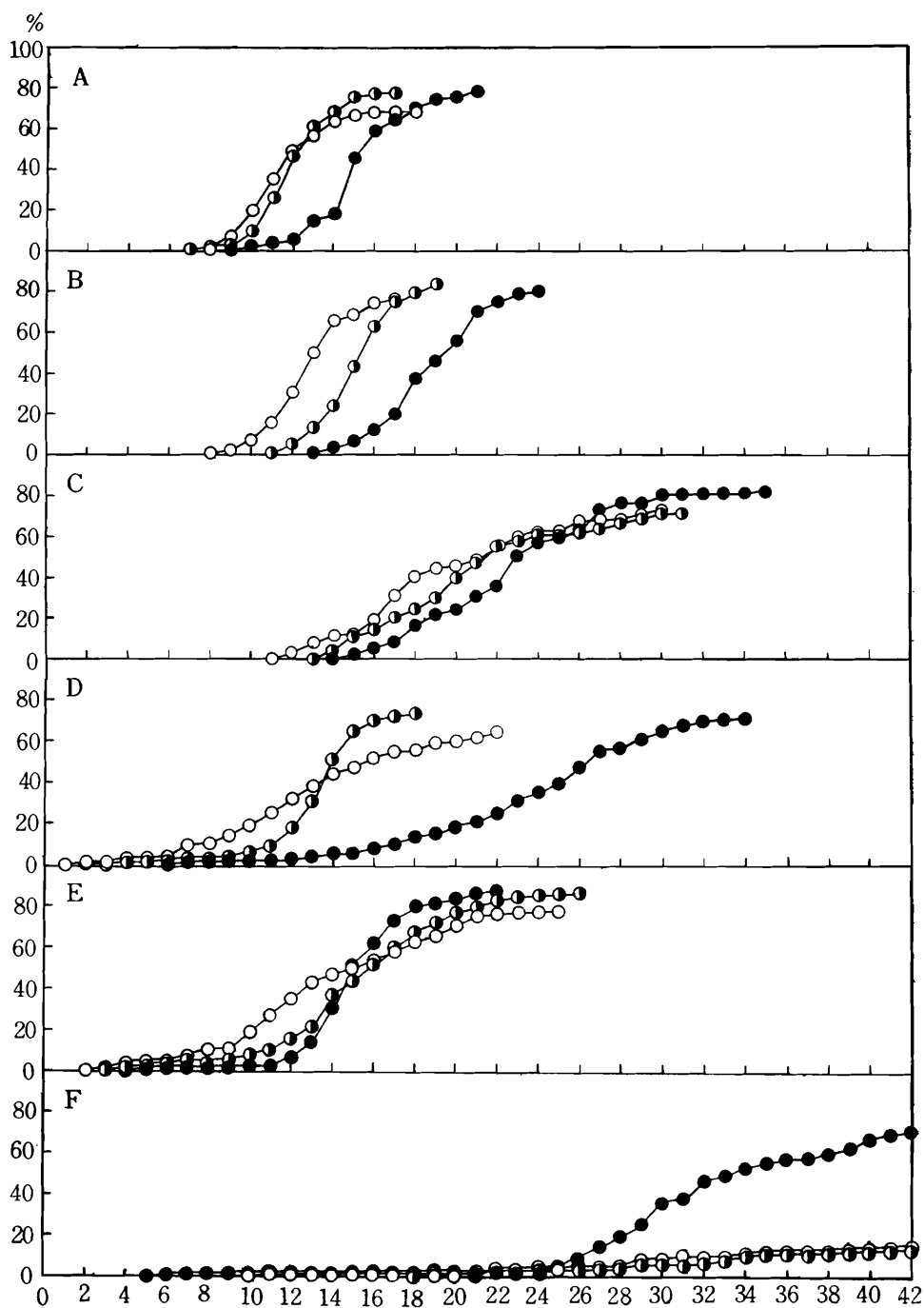


FIG. 1. Hatching curves of six species of crickets at 20° (closed circle), 25° (half closed) and 30°C (open). A, the Emma field-cricket, *Gryllulus* sp.; B, *Scapsipedus aspersus*; C, the Tambo-okame cricket, *Loxoblemus* sp.; D, *Pteronemobius fascipes*; E, *P. taprobanensis*; F, *P. ohmachi*. Ordinate, cumulated percentage of eggs that had hatched; abscissa, time in weeks after deposition.

eggs of which suffered heavy mortality before resuming development. The mode of hatching at 20, 25 and 30°C is illustrated by the curves showing the weekly total of nymphs that had hatched (fig. 1). These curves are generally sigmoid in shape, showing no great deviation from normal distribution. The details of the curves are, however, considerably different in different species at the same temperature, and at different temperatures in the same species. The position, slope and height of the curves are thus all subject to variation according to species and treatment. These features can be represented by the mean length of egg stage, its variation and the percentage survival as summarized in table 1.

Length of diapausing egg stage

Reference to table 1 reveals that the length of egg stage varied from one species to another and also that different species respond to temperature in different ways. Of all the six species examined, *P. ohmachii* underwent the longest period of egg stage at all temperatures tested; only a few nymphs hatched at 25 or 30°C throughout the observation of ten months, while over two-thirds did so at 20°C. In the other species there was a general tendency that the higher the temperature at which the eggs were kept, the shorter was the duration of the egg stage. This accelerating effect of high temperature was most remarkable in *P. fascipes* which hatched after about three months at 25 or 30°C but after as long as five months at 20°C. Contrary, in *P. taprobanensis* the egg stage was only two weeks shorter at 30°C than at 25°C, and no further delay was observed at 20°C. In the remaining three species the egg stage was gradually shortened by rising temperature, and this trend was a little more conspicuous in *S. aspersus* than in *Gryllulus* or *Loxoblemus*.

As a matter of course the egg stage of the crickets comprises both diapausing and morphogenetic stages. The results stated above were brought about by the sum total of the influences of temperature on the two distinct phases of development. It is necessary to analyse each effect of temperature on each phase in order to elucidate the ecological sequence of events involved in the egg stage. This analysis will be deferred to the last section.

Variability in the length of egg stage

The duration of diapausing egg stage was highly variable as seen from the large value of its standard deviation (table 1). It ranged from about 10 to over 30 per cent of the mean, and temperature seems to be responsible for this variation. Thus in all crickets examined variability in the length of egg stage was generally larger at 30°C than at the lower temperatures. The nymphs of *Gryllulus*, *S. aspersus* and *P. fascipes* hatched over a shortest period at 25°C and the spread of hatching time again a little increased at 20°C. In *P. taprobanensis* the variability decreased regularly as temperature fell from 30 to 20°C. A similar result was observed, though less conspicuously, in *Loxoblemus*. In *P. ohmachii* the coefficient of variation for the length of egg stage could not be calculated, because hatching was not complete before the end of experiment. However, the distribution of hatching time represented by the hatching curves in figure 1 spreads over a very wide range of days, which suggests the great variation in the length of egg stage of this species under these conditions.

TABLE 1. *The duration of the diapausing egg stage and mortality in several species of crickets at constant temperatures*

Species	Temperature (°C)	Length of egg stage (days)			Mortality (%)	Number of eggs
		Mean	S. D.	C (%)		
Emma field cricket	20	104.6	15.6	14.9	22.0	200
	25	82.4	11.4	13.8	24.0	200
	30	79.5	12.8	16.1	31.5	200
<i>Scapsipedus aspersus</i>	20	130.3	16.1	12.4	20.0	100
	25	105.0	11.8	11.2	17.0	100
	30	87.4	12.2	14.0	25.0	100
Tambo-okame cricket	20	157.1	29.3	18.7	18.0	100
	25	140.6	30.2	21.5	27.0	100
	30	133.4	32.7	24.5	29.0	100
<i>Pteronemobius fascipes</i>	20	165.0	35.4	21.5	28.3	138
	25	91.6	15.0	16.4	25.4	138
	30	89.1	29.6	32.2	34.8	138
<i>Pteronemobius taprobanensis</i>	20	104.5	15.4	14.7	13.1	168
	25	107.1	26.3	24.6	13.1	168
	30	94.3	30.8	32.7	22.0	168
<i>Pteronemobius ohmachii</i>	20	224*	—	—	19.6**	102
	25	>300	—	—	22.5**	102
	30	>300	—	—	52.0**	102

*Number of days required for 50 per cent hatching.

**Mortality at the end of ten months' observation.

Mortality

Highest mortality in the egg stage of the crickets was usually observed at the highest temperature. It ranged from about 20 to 35 per cent at 30°C and from 10 to 30 per cent at the lower temperatures. The difference in mortality of the same species at different temperatures are, however, statistically insignificant except in *P. ohmachii*. In this cricket only a half of the eggs had survived at 30°C throughout ten months' period of observation. As temperature was lowered to 20 or 25°C the proportion of spoiled eggs was reduced to about 20 per cent, and a number of eggs had been still persisting in diapause at the end of the experiment.

It should be noted that spoiled eggs often contained embryos that had fully developed, though failed to hatch. Most of them had turned black before they were judged as dead. These eggs were frequently found among those which had been kept at the higher temperatures, and this was the principal cause of the high mortality under these conditions in most species except *P. ohmachii*. In the latter more eggs died before absorbing water which marks the resumption of post-diapause development as will be mentioned later. Such black eggs were perhaps similar in nature to those reported by BROWNING (1952 a) in *Gryllulus commodus*. He further found deformed embryos in eggs of this cricket (see also ANDREWARTHA, 1943). Anomalous development of embryos was observed also in the present experiments, although it occurred only in a few cases and was of minor importance.

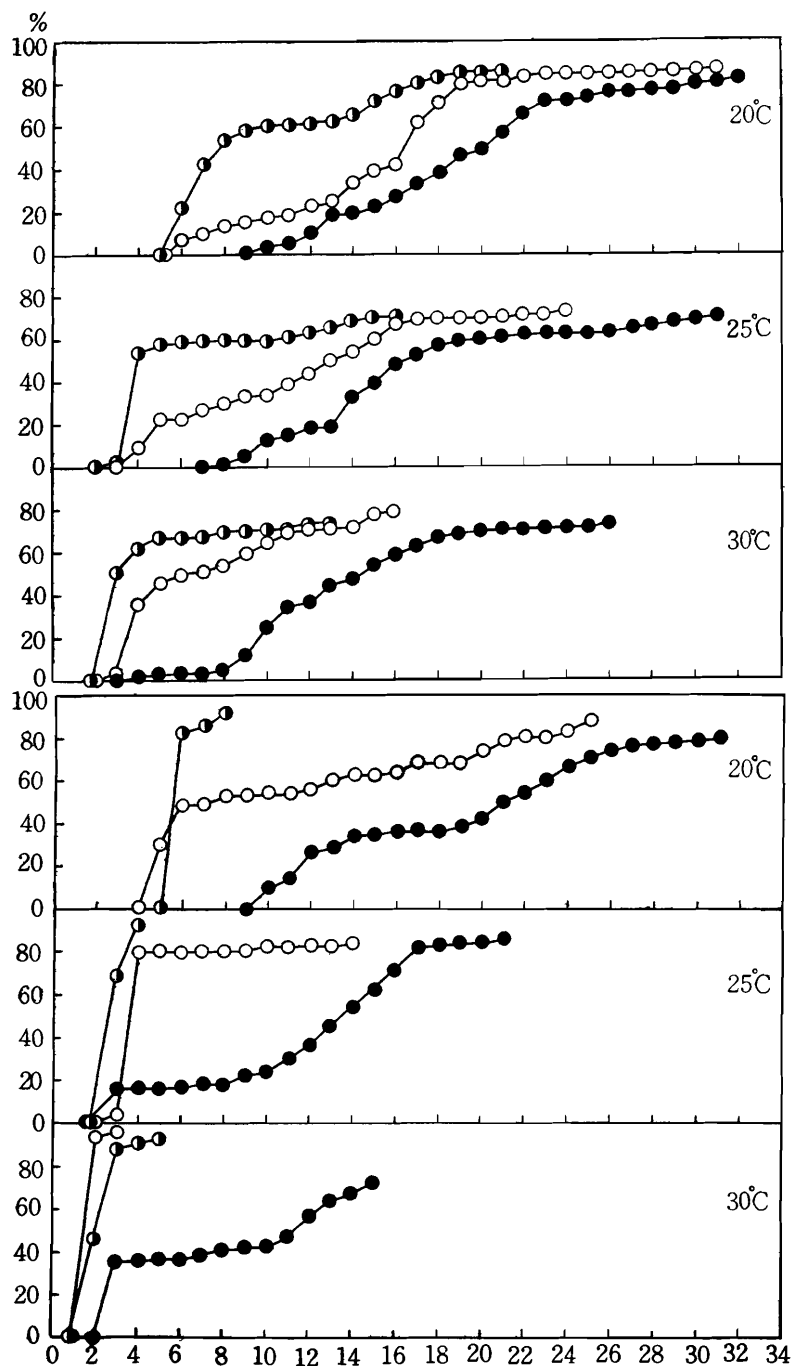


FIG. 2. Hatching curves of the Tambo-okame cricket, *Loxoblemus* sp. (upper) and *P. fascipes* (lower) at different incubation temperatures after different lengths of cold exposure (6-13°C). Length of cold exposure: closed circle, 2 weeks; open circle, 5 weeks; and half-closed, 8 weeks; Ordinate, cumulated percentage of eggs that had hatched; abscissa, time in weeks after cold exposure:

in mortality.

The effect of cold exposure

The effects of cold exposure and of subsequent incubation at different temperatures were more or less similar in the four species used in the experiments, as typically represented by the hatching curves of *Loxoblemus* and *P. fascipes* in figure 2. Although their diapause lasted for a very long time at warm temperatures, the eggs of *P. ohmachi* became competent to hatch in a relatively short time by this treatment. After five weeks in cold storage about a quarter of the eggs hatched promptly when incubated at 30°C. Almost all eggs hatched simultaneously when the treatment was lasted for eight weeks. The same influence was observed on hatching of the other species which could readily complete diapause at high temperatures as well. Thus many nymphs of *Loxoblemus*, *P. fascipes* and *P. taprobanensis* hatched within a short time at the high temperatures after eight weeks of cold exposure. It follows therefore that these species seem to be able to complete diapause in a wide range of temperature as compared with *P. ohmachi*. *S. aspersus* and *Gryllulus* were not included in these tests, but the results of another experiment, which will be published elsewhere, show that the latter is also able to complete diapause at a cold temperature at least higher than 5°C.

Throughout the tests of cold exposure the mortality of the eggs was generally lower than that at the constant warm temperatures.

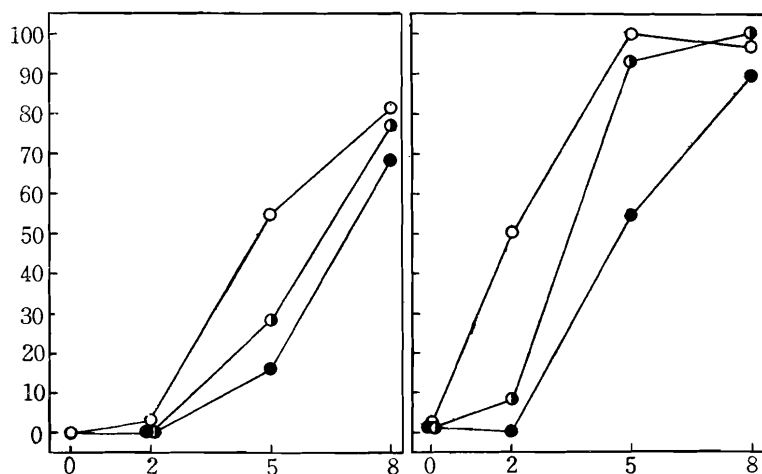


FIG. 3. Showing the relation between the length of preliminary cold exposure (abscissa, in weeks) and the percentage of eggs that hatched promptly (ordinate) in *Loxoblemus* sp. (left) and *P. fascipes* (right). The eggs were incubated at 20° (closed circle), 25° (half-closed circle) and 30°C (open circle), respectively, after cold exposure (6-13°C).

The effect of incubation temperature

In figure 2 it can be seen that the proportion of the eggs that hatched without delay after the same period of cold exposure varied with incubation temperature. This is more explicitly depicted by figure 3 in which the percentage of eggs that had hatched promptly at three different incubation temperatures is plotted against

the duration of cold treatment. From this figure it is clear that the higher the temperature at which the eggs were incubated, the greater was the number of emerging nymphs after cold exposure. After two weeks at the low temperature about a half of the eggs of *P. fascipes* resumed to develop at 30°C without delay; the proportion of such eggs was reduced to about one-fifth at 25°C and none at 20°C. When the treatment was prolonged to five weeks, almost all eggs hatched immediately at the two higher temperatures but a half still remained in diapause at 20°C. Under the same conditions about 70, 60 and 24 per cent of the eggs of *P. taprobanensis* hatched simultaneously at 30, 25 and 20°C, respectively. The eggs of *Loxoblemus* required a larger amount of cold treatment for a simultaneous hatching than do these *Pteronemobius*, and the influence of incubation temperature was most noticeable after five weeks of cold exposure; by this treatment 55, 28 and 16 per cent of the eggs began to develop immediately after incubation at 30, 25 and 20°C, respectively. The eggs that had previously been kept cold for eight weeks responded to incubation temperature in a similar way.

These results confirm the observation made by BROWNING (1952 b) with diapausing eggs of the Australian black field-cricket. It should be emphasized that the eggs which had been kept cold for an adequate period and would certainly hatch at a high temperature, say 30°C, were not necessarily free of diapause; for many of them would fail to hatch if incubated at a lower temperature, say 20°C. This can be interpreted as indicating that there are two phases of different thermal

TABLE 2. *Approximate length of post-diapause or non-diapause development in several species of crickets*

Species	Temperature (°C)	Length of development (days)			Number of eggs
		Mean	S. D.	Range	
Emma field cricket	20	46.2	4.09	39-54	22
	25	20.1	1.31	18-22	67
	30	16.8	1.77	16-19	16
Tambo-okame cricket	20	46.0	6.17	37-60	58
	25	24.4	2.99	20-36	58
	30	20.4	3.28	17-32	67
<i>Pteronemobius fascipes</i>	20	39.9	1.17	38-42	41
	25	20.5	1.36	19-23	46
	30	14.8	1.10	14-16	44
<i>Pteronemobius taprobanensis</i>	20	40.4	1.15	38-41	7
	25	22.8	1.64	20-27	35
	30	17.9	0.94	17-20	37
<i>Pteronemobius ohmachii</i>	30	14.2	0.64	14-16	42
<i>Nemobius yezoensis</i> *	20	32.9	1.05	30-37	126
	25	18.9	2.08	17-31	124
	30	13.0	0.63	12-16	139
Taiwan field cricket*	20	43.1	2.26	39-52	79
	25	25.3	1.04	24-28	88
	30	13.3	0.54	13-16	67

*The eggs of these crickets do not enter diapause.

requirements in diapause development and that the final phase proceeds very rapidly at higher temperatures (c. f. MASAKI, 1956 a, b). Such is probably the case also in *Melanoplus bivittatus*; CHURCH and SALT (1952) stated that in the eggs of this grasshopper there is certain necessary events which take place best at higher temperatures between the end of diapause and the beginning of morphogenesis. The sequence of events involved in the completion of diapause which leads to the resumption of morphogenesis and finally to hatching of the cricket nymphs under natural, daily and seasonally fluctuating, conditions of temperature might be much more complicated than it is under simplified experimental conditions. This should fully be analysed to make clear the role of diapause in the seasonal regulation of the life cycle through timing the hatching season.

Length of post-diapause development

From the results of the experiments stated above the duration of post-diapause development can roughly be estimated. This is represented by the mean length of incubation period of eggs that hatched simultaneously after an adequate period of cold exposure, as shown in table 2. The figures in the table are, however, not quite precise; since, as discussed above, the final short phase of diapause would be involved. Furthermore there might be no sharp distinction between the diapause and post-diapause stages.

The data given for *Gryllulus* in table 2 were based on the results of experiments with the Tsu population of this cricket (unpublished). It might slightly differ in physiological nature from the Hirosaki population. The length of the egg stage of *Nemobius yezoensis* and the Taiwan field-cricket (*Gryllulus* sp., see OHMACHI and MATSUURA, 1951), both of which have no embryonic diapause, are also presented for comparison. It will be noted that the durations of post-diapause development of the diapausing crickets are almost comparable to the egg stage of the non-diapausing species. The variability in length of the post-diapause development is much smaller than that for the entire egg stage, as indicated by the standard deviation. This might imply that the large variation in length of the diapausing egg stage at constant warm temperatures might mainly be derived from the great variability in the length of diapause.

Morphological stage of diapause

In *Gryllulus* diapause occurs at a stage during anatrepsis before the embryo undergoes the differentiation of body segments, the so-called dumb-bell shaped stage (UMEYA, 1946). This stage is reached by the 4th, 5th, 10th and 22nd days after oviposition at temperatures of 30, 25, 20 and 15°C, respectively, according to observations made with the Tsu stock (unpublished). The eggs of the Hirosaki population enter diapause at the same stage, but the precise rate of pre-diapause development was not determined. Although precise comparison could not have been made, the diapausing embryo of the Emma field-cricket seems to be morphologically a little younger than that of the black field-cricket, as judged from the descriptions by Australian authors (BROOKES, 1952; BROWNING 1952 a; HOGAN, 1959).

Morphological observations of the embryo indicated that *S. aspersus* seems to enter diapause also at around this stage. On the other hand, in most diapausing eggs of *Pteronemobius* no conspicuous body of the embryo could be detected after

they were fixed in hot BOUIN's solution. HOGAN's method (HOGAN, 1959) gave the same results. In a very few eggs of *P. fascipes* embryos at the stage just before the differentiation of segments were found. It was doubtful if these eggs were persisting in diapause at the time of fixation, for in this small crickets a few eggs occasionally completed diapause in a very short period (see fig. 1). Soon after the resumption of morphogenesis the embryo could easily be detected by either of the two methods mentioned above. Although the precise stage of diapause in these crickets should be determined in the future by microscopic sectioning technique, it might be suggested at present that the three species of *Pteronemobius* may enter diapause before the formation of the germ band. *Loxoblemus* seems to undergo diapause in the same state inasmuch as the embryo could not have been detected in the diapausing eggs.

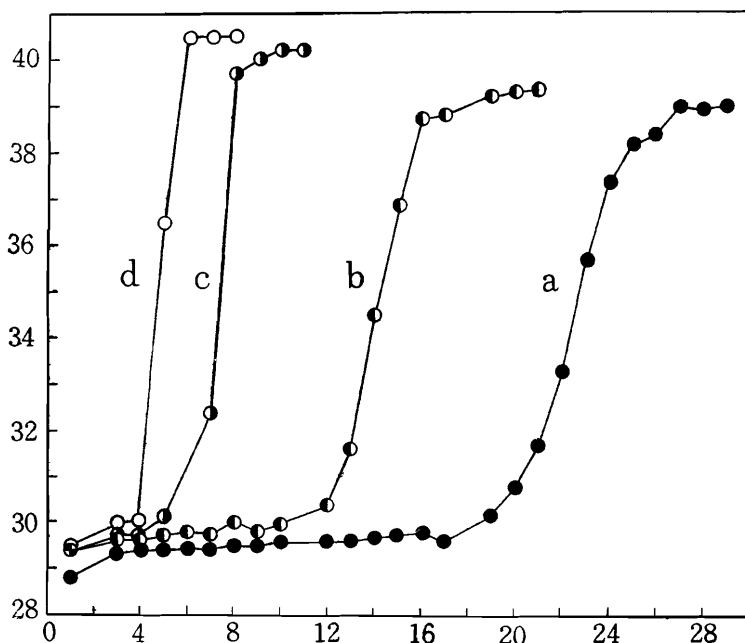


FIG. 4. Showing the increase in width during the pre-diapause development of the eggs of *Gryllulus* sp. (the Emma field-cricket), caused by water uptake. a. at 15°; b, 20°; c, 25°; and d, 30°C. Ordinate, mean width of 20 eggs in micrometer units; abscissa, time in days after deposition.

Water absorption

During the course of their embryonic development all the crickets used in the experiments absorb water at a definite stage as does their Australian relative (BROWNING, 1953). In all species the process is completed within a few days at a warm temperature after it has begun, and as a result the eggs enormously swell out. In *Gryllulus* and *S. aspersus* this takes place at about the time they enter diapause, while in *Loxoblemus* and the three species of *Pteronemobius* it occurs after the end of diapause. In the latter swelling of eggs caused by water uptake is the first and unmistakable visible sign of the resumption of development, which

is soon after followed by the appearance of eyespots.

Examples of water-uptake by the eggs of *Gryllulus* (Tsu stock) at various temperatures, as measured by the width of the egg, are given in figure 4. The curves are similar to those presented by BROWNING (1953). It can be seen from this figure that the eggs required about 28, 17, 8 and 6 days to complete the process at temperatures of 15, 20, 25 and 30° C, respectively. It seems important that they began to absorb water approximately at the time when the embryos

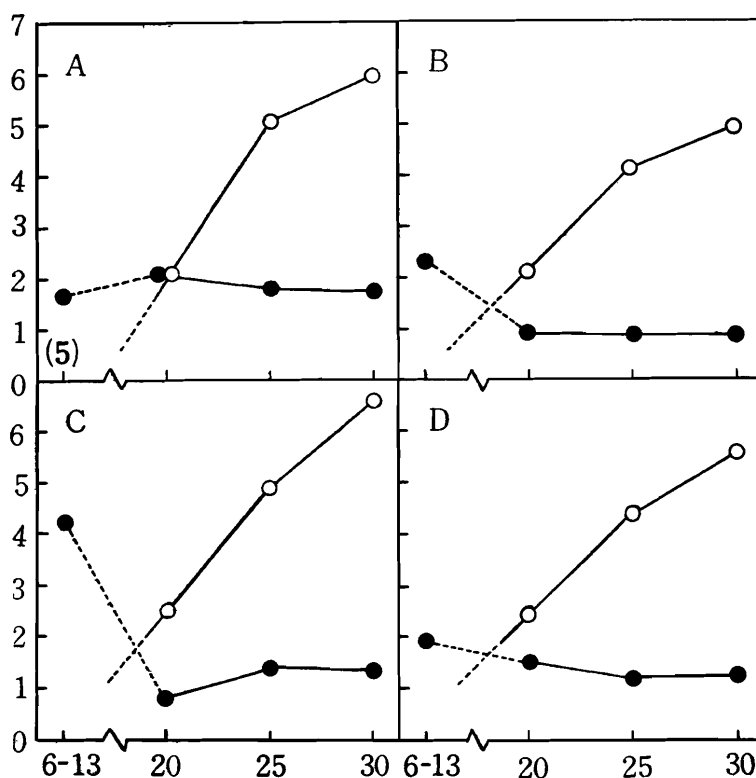


FIG. 5. Showing the rate of diapause development (closed circle) and of post-diapause development (open circle) in relation to temperature in (A) *Gryllulus* sp., (B) *Loxoblemus* sp., (C) *P. fascipes* and (D) *P. taprobanensis*. Ordinate, percentage of development per day; abscissa, temperature in centigrade degrees.

reached the dumb-bell shaped stage. It might be inferred that the water-absorbing mechanism, though it has not been unveiled in the crickets, would be completed or set in action around this stage of embryonic development. It will be reasonable to suppose that the same mechanism as in *Gryllulus* or *Scapsipedus* operates in the eggs of those crickets which undergo diapause at a younger stage. That is to say they postpone to take up water until after diapause, because the device is not existed or ineffective before they resume to develop and reach the significant morphological stage.

DISCUSSION

In estimating the efficiency of temperature for the completion of diapause, the first criterion to be considered is the speed of this physiological process. As suggested by the specific length of entire egg stage, the rate of diapause development as well as the pattern of reaction to varying temperature would vary in different species of crickets. In order to illustrate these relations more clearly, the duration of diapause was roughly estimated by subtracting the duration of post-diapause stage from the entire egg stage in *P. fascipes*, *P. taprobanensis* and *Loxoblemus*, for the length of their embryonic life before entering diapause is almost negligible. In *Gryllulus* the duration of pre-diapause stage was further deducted, though there is no evidence that diapause is not involved during this initial period of morphogenesis. The mean length of diapause at a temperature below the developmental zero was estimated by eye from the curves showing the relation between the percentage of prompt hatch at 25°C and the amount of preliminary exposure to cold. From the figures thus obtained the rate of diapause development was expressed as percentage of development per day and plotted against temperature in figure 5. The rate of post-diapause development is also given for comparison.

The range of temperature available for the present work was so limited that the complete relationship between the rate of diapause development and temperature could not be pictured, and it was impossible to estimate such important values as the limit and optimum of temperature for diapause development in each species. Certain interesting features can, however, be pointed out from the comparison of the data obtained in different crickets.

It seems rather noticeable that in most species studied the completion of diapause was not inhibited at all by a high temperature favouring rapid growth. In *Gryllulus* the rate of diapause development was kept even nearly constant over such a wide range of temperature as from 5 to 30°C, although in this case the possibility could not be excluded that there is a thermal optimum between 5 and 20°C at which the rate reaches its maximum, as in the case of *Gryllulus commodus* (BROWNING, 1952 b). In the other species cold exposure was more effective than a constant high temperature in bringing diapause to its end, if it was followed by growth-promoting warmth. Its efficiency considerably varied between species. Thus in *P. taprobanensis* the speed of diapause development was progressively, though slightly, increased as temperature fell from 30 to 6-13°C. In *Loxoblemus* it remained fairly constant between 20 and 30°C and was raised a little by chilling. The response of the eggs of *P. fascipes* was complicated; in this species the rate of the physiogenesis was reduced to a minimum at the moderate temperature of 20°C and increased on either side of this depression, and the diapause was terminated in a shortest time when the eggs were warmed after an adequate period of cold exposure. The action of low temperature upon the completion of diapause was most drastic in *P. ohmachii*. Among the crickets found at Hirosaki this was the only species whose requirement for low temperature seemed to be obligatory. The upper limit of temperature for its diapause development apparently lies not far above 20°C. Compared with the other crickets, *P. ohmachii* occupies very moist environments, marsh or the ridge of paddy field. Although the ecological

background of its relatively long and intense diapause is still obscure, it might be expected that the particular micro-climatic conditions of its habitat may have some bearing on this physiological characteristic.

It has been generally experienced by entomologists who have been studying diapause that diapausing insects often suffer high mortality when they are kept at such a high temperature which retards the progress of diapause development. In such a case mortality might be taken as a measure to indicate the deleterious effects of the warm temperature on that physiogenesis. In *P. ohmachii* this was clearly demonstrated by the high mortality of eggs that had been kept at a high temperature of 30°C at which diapause development was practically stopped, as indicated by the very low percentage of hatching over a long period. Hatching of nymphs at 25°C was suppressed to the same extent, but the number of eggs surviving at the end of ten months' period was two times as large as at the higher temperature. In the other species the influence of temperature upon the death of eggs was less conspicuous, but the highest mortality was usually observed at the highest temperature. We can not, however, properly estimate the action of temperature on diapause development by this criterion unless the cause of the death of eggs is fully understood. It is as yet not certain whether eggs died as a result of the abnormal or disturbed physiogenesis caused by adverse effect of high temperature; or whether they were killed by microbes whose activity was enhanced by rising temperature. It is possible that both these factors were involved.

In this context we should consider the *carry-over* effect of diapause which would cause death or physiological weakness of insects after they had passed through the morphological stage of diapause. This was first noticed by ANDREWARTHA and BIRCH (1954, p. 64) who observed that the nymphs of *Austroicetes cruciata* that had experienced diapause under certain artificial conditions were unable to feed while those hatched under natural conditions readily accepted food. In the crickets studied this was not the case, or at least not of common occurrence. Nymphs of *P. fascipes*, *P. taprobanensis* and *P. ohmachii* that had hatched from eggs kept constantly at a warm temperature were reared in order to obtain their eggs during winter; they grew at a normal rate, matured and laid fertile eggs, although no record on mortality during the nymphal stage was kept. The same is true in *Gryllulus*. This large univoltine cricket was held throughout its life-cycle, including the embryonic diapause stage, at a constant temperature of 30°C; even after three successive generations under these conditions adults were able to reproduce, though they laid a number of inviable eggs. On the other hand, the occurrence of black eggs and deformed embryos observed in the present work might be related to unhealthy completion of diapause, because they have been found only in a very small number of non-diapause eggs of *Nemobius yezoensis* and the Taiwan field-cricket (*Gryllulus* sp.). But this is not substantiated unless the physiological mechanism leading to these anomalies is analysed.

The frequency distribution of hatching is another important criterion to be considered, and the degree of diapause completion has often been expressed by this measure (BROWNING, 1952 a; MASAKI, 1956 b). It is particularly useful in estimating the effect of temperature below the threshold for growth. The completion of diapause at a cold temperature usually results in a remarkable reduction both in the hatching time and its variation at a warm incubation temperature. These results

are apparently brought about by the simultaneous initiation of growth of individuals that had completed diapause — or more precisely the first substantial phase of it — but that had been retarded to grow by the low temperature. This does not mean that the variability in the length of diapause itself is reduced at the low temperature. In *Gryllulus* the variability in the length of diapause at a subthreshold temperature was not smaller than at a warm temperature (unpublished observation). On the other hand, in *P. taprobanensis* and *P. fascipes* this seemed to be affected by temperature to a greater extent. Thus nymphs of these crickets hatched over a longer period at 30°C than at a lower temperature of 25°C in spite of that the mean length of egg stage was shorter at the higher temperature. The curves in figure 3 suggest further that in *P. fascipes* the variability for the duration of diapause seems to be still smaller at 6-13°C. The similar effect of cold was also observed in *Loxoblemus*. But in *P. taprobanensis* it was not so conspicuous, for nymphs began to hatch promptly after the cold exposure of two weeks but one-third or more of the eggs still persisted in diapause at 30 or 25°C even after the longest exposure lasting eight weeks. In *P. ohmachi* the spread of variation in the length of cold exposure necessary to develop promptly on incubation was very small compared with the tardy hatching of nymphs over a very long period at the constant high temperature. More precise interpretation would be made by using the method of probit analysis which could not have been carried out by the insufficient number of measured points on the curves. Even by this method it is difficult to measure the length of diapause and its variability at cold temperature, because the eggs must be warmed in order to make them hatch and to measure the length of their egg stage, so that the effect of incubation temperature upon the final phase of diapause can not be excluded.

It is certain, at any rate, that the spread of variation for the length of diapause is generally great compared with that for the other kind of development, morphogenesis. This might be connected to certain physiological characteristics of the process, but certain ecological conditions would be necessary to preserve it. The seasonal cycle of the environmental factors might have thus allowed the existence of such a wide range of variation for diapause. It is at least indispensable for the successful overwintering of the cricket eggs to remain in diapause from the time soon after being laid until the onset of cold weather. Once this is attained, the eggs will be kept in the resistant stage by winter cold inhibiting the resumption of postdiapause growth. On the other hand, the possible longest diapause may be extended until the return of the warm weather in the following spring. The eggs undergoing various lengths of diapause between these limits will be able to hatch successfully and uniformly after hibernation. Thus the range of allowance for the variation may be great, covering several months from late autumn to early spring. This may be one of the possible explanation of the highly variable length of diapause. Conversely speaking, it might be assumed that the range of variation is limited by the seasonal cycle of temperature.

If the variable length of diapause in a population of crickets would be determined by polygenes, like certain other quantitative characters such as body weight, body length, etc., this seems important in considering the adaptive nature of diapause. By their high variability in the duration of diapause, populations of

crickets might have potentialities to cope with climatic changes of a considerable degree as long as the length of hibernating season is concerned. In certain insects, indeed, patterns of diapause that had been concealed in natural populations could have been revealed by artificial selection (LE BERRE, 1953; HARVEY, 1957). In *Gryllulus* artificial selection of the short and long diapause lines are now in progress, but no change in the length of diapause has yet occurred by the third generation in either directions. The genetic nature and adaptive value of the variation in the length of diapause may perhaps be more clearly illustrated by studying local populations inhabiting localities of different climatic conditions.

SUMMARY

Diapause in the eggs of the Emma field cricket (*Gryllulus* sp.) and *Scapsipedus aspersus* occurs at around the so-called dumb-bell shaped stage while in those of the Tambo-okame cricket (*Loxoblemus* sp.), *Pteronemobius fasciipes*, *P. taprobanensis*, and *P. ohmachi* it supervenes at an earlier stage, probably before the formation of germ band. The eggs of the first two species absorb water before the onset of diapause while the others do so after the completion of diapause.

The eggs of these crickets, except *P. ohmachi*, could complete diapause at a warm temperature between 20 and 30°C. The length of diapause varied among species and so was the pattern of reaction to varying temperature. In all species, including *P. ohmachi*, temperatures below the threshold for morphogenesis were also effective in bringing diapause to its end; and it was observed that the efficiency of low temperature was considerably affected by the temperature of subsequent incubation. Thus the higher the temperature at which the eggs were incubated the larger was the proportion of eggs that hatched promptly after the same period of cold exposure.

In all the species studied, great variations in the length of diapause were observed under various conditions of temperature, and the factors responsible for these variations are discussed from the ecological point of view.

The writer is indebted to Prof. F. OHMACHI of the Mie University and Mr. I. MATSUURA of Tokyo for their kind communication about the biology of crickets which has been invaluable for the present work.

REFERENCES

- ALEXANDER, R. D. The taxonomy of the field crickets of the eastern United States (Orthoptera: Gryllidae: Acheta). *Ann. Ent. Soc. Amer.* **50**: 584-602, 1957.
- ANDREWARTHA, H. G. 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, 1943.
- ANDREWARTHA, H. G. Diapause in relation to the ecology of insects. *Biol. Rev.* **27**: 50-107, 1952.
- ANDREWARTHA, H. G. and BIRCH, L. C. *The Distribution and Abundance of Animals*. Chicago, 1954.
- BROOKES, H. The morphological development of the embryo of *Gryllulus commodus* WALKER (Orthoptera: Gryllidae). *Trans. Roy. Soc. S. Austr.* **75**: 150-159, 1952.

- BROWNING, T. O. The influence of temperature on the completion of diapause in the eggs of *Gryllulus commodus* WALKER. *Austr. J. Sci. Res. B* **5**: 112-127, 1952 a.
- BROWNING, T. O. On the rate of completion of diapause development at constant temperatures in the eggs of *Gryllulus commodus* WALKER. *Austr. J. Sci. Res. B* **5**: 344-353, 1952 b.
- BROWNING, T. O. The influence of temperature and moisture on the uptake and loss of water in the eggs of *Gryllulus commodus* WALKER. *J. Exp. Biol.* **30**: 104-115, 1953.
- CHURCH, N. S. and SALT, R. W. Some effects of temperature on development and diapause in eggs of *Melanoplus bivittatus* (SAY). *Canad. J. Zool.* **30**: 173-184, 1952.
- GHOURI, A. S. K. and MCFARLANE, J. E. Observations on the development of crickets. *Canad. Ent.* **90**: 158-165, 1958.
- HARVEY, G. T. The occurrence and nature of diapause-free development in the spruce budworm, *Choristoneura fumiferana* (CLEM.). *Canad. J. Zool.* **35**: 549-572, 1957.
- HOGAN, T. W. A rapid method for examining diapause embryos of *Acheta commodus* WALK. *Nature* **183**: 269, 1959.
- LE BERRE, J. R. Contribution a l'étude biologique du criquet migrateur des landes (*Locusta migratoria gallica* REMAUDIERE). *Bull. Biol. Fr. Bel.* **87**: 227-273, 1953.
- LEES, A. D. *The Physiology of Diapause in Arthropods*. Cambridge, 1955.
- MASAKI, S. The effect of temperature on the termination of pupal diapause in *Barathra brassicae* LINNÉ (Lepidoptera: Noctuidae). *Japan. J. Appl. Zool.* **21**: 97-107, 1956 a.
- MASAKI, S. The effect of temperature on the termination of diapause in the egg of *Lymantria dispar* LINNÉ (Lepidoptera: Lymantriidae). *Japan. J. Appl. Zool.* **21**: 148-157, 1956 b.
- OHMACHI, F. and MATSUURA, I. Okame cricket and its closely related species. *Kontyu* **18**: 4-5, 1950.
- OHMACHI, F. and MATSUURA, I. On the Japanese large field cricket and its allied species. *Bull. Mie Univ. Fac. Agric.* **2**: 63-72, 1951.
- UMEYA, Y. Embryonic hibernation and diapause in insects from the view point of the hibernating eggs of the silkworm. *Bull. Seric. Exp. Sta. Tokyo* **12**: 393-480, 1946.