# THE INFLUENCE OF TEMPERATURE AND MOISTURE ON THE UPTAKE AND LOSS OF WATER IN THE EGGS OF *GRYLLULUS COMMODUS* WALKER (ORTHOPTERA—GRYLLIDAE)

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## I. INTRODUCTION

In almost all studies on the gain and loss of water by the eggs of insects, changes in the wet weight of the whole egg have been used as a measure of changes in water content. This procedure is admissible in the case of water uptake because the error introduced by the weight of the dry matter used in oxidative processes during development is always small compared with the changes in water content that occur. Furthermore, most insect eggs, being cleidoic, require no substances other than water from the environment, so that no error is introduced in this way (Needham, 1942). In the case of desiccation, the error introduced by the oxidation of dry matter is positive but may be neglected provided the changes in weight are fairly large in relation to the original weight, or if the period during which such changes occur is short.

Most authors, when studying changes in the water content of eggs, have estimated the average changes in the water content by weighing a number of eggs together. The method is convenient because a fairly large weight can be obtained and changes in weight are more easily measured. It suffers from the great drawback, however, that the variability in the behaviour of individual eggs during changes in water content cannot be measured. Since such individual variations are often important, it is preferable to weigh individual eggs, as has been done by Salt (1949 *b*).

## II. MATERIALS AND METHODS

The eggs used in this work were laid by some hundreds of crickets kept together in a large cage. The eggs were laid in trays of moist sand which were removed daily and new ones replaced. On removal of the trays the sand was sieved under water and the eggs washed, sorted and either used immediately or stored at 13° C. on wet blotting paper in an airtight jar.

During the experiments the eggs were kept in small numbered 'pockets' made in plaster disks which were either saturated with water when the experiment required a saturated atmosphere for the eggs, or were in equilibrium with the air in the desiccators when desiccating conditions were used. Thirty eggs were placed on one disk which was kept in an airtight jar in which the humidity was controlled with distilled water or sulphuric acid solutions. Temperatures were maintained constant within $\pm 0{\cdot}1^\circ$ C.

Individual eggs were weighed at daily or more frequent intervals on a torsion balance of 0–50 mg. capacity, calibrated in units of 0·02 mg. Care was taken during weighing to exert as little pressure on the eggs as possible, particularly when water was being lost and the eggs had become flaccid.

## III. RESULTS

### (*a*) *Uptake of water*

When the eggs of *Gryllulus commodus* are incubated soon after laying some develop and hatch promptly but others enter a diapause stage. Diapause is most rapidly and efficiently completed during an exposure to low temperature (Browning, 1952*a*). The proportion of eggs which manifest diapause and the intensity of the diapause is influenced by the environment of the eggs. For example, when eggs are incubated at a high temperature (about 30° C.) few enter diapause; at lower temperatures, (about 25° C.) most eggs enter diapause; whilst when temperature is quite low (about 20° C.), diapause occurs in virtually all the eggs. In all cases, however, an adequate exposure to an appropriate low temperature (13° C.) soon after laying results in the eggs becoming competent to complete their development and hatch promptly when incubated at higher temperatures. Nevertheless, an egg that is to be incubated at a low temperature (say 20° C.) requires to be exposed to 13° C. for a longer period than one that is to be incubated at a higher temperature (say 30° C.) if it is to have an equal chance of completing its development without diapause (Browning, 1952*b*).

#### (i) *Eggs incubated within 24 hr. of the time of laying*

In Fig. 1 is shown the course of increase in mean weight in eggs incubated on moist plaster at each of three constant temperatures, using thirty eggs at each temperature. Sigmoid curves of weight increase similar to these have been found for a number of insects (Banks, 1950; Johnson, 1937; Roonwal, 1936; Salt, 1949*b*).

The rate of water uptake, as measured by the time required by the eggs to reach the point where their weight ceased to increase significantly, was more rapid at higher temperatures than at lower. A similar situation was shown by Banks (1950) in the eggs of *Corixa*, and by Salt (1952) in the eggs of *Melanoplus*.

At 29·9° C. development was erratic. Out of a total of thirty eggs, thirteen hatched, whilst four entered diapause and eight did not increase in weight after the initial period of slow increase in weight. The remaining five eggs were injured during handling and died after having taken up their water. These eggs are very easily damaged at the time when water uptake is nearing completion, frequently bursting whilst being dried prior to weighing. This was not observed except at this temperature. At 26·5° C. only two eggs did not increase in weight beyond the initial stage, and one of these was injured on the second day. Only two eggs in this group hatched, the remainder entered diapause. At 20·9° C. six eggs did not increase in weight and all the remainder entered diapause.

(ii) *Eggs in which diapause development had been completed*

Eggs which had been stored at 13° C. for a period after laying were placed on moist plaster at each of the three constant temperatures, 29·9, 26·5 and 20·9° C., thirty eggs being used at each temperature.

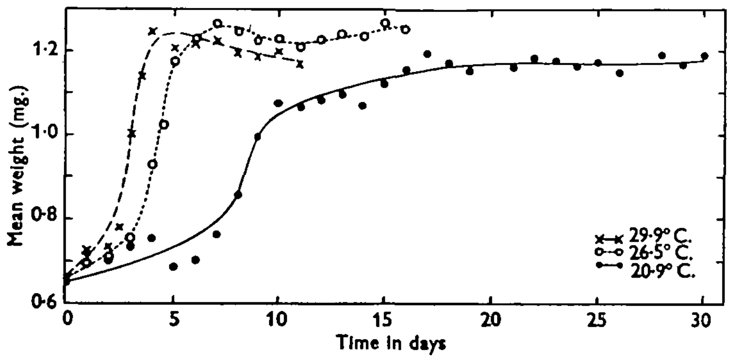


Fig. 1. Showing the changes in the mean weight of eggs during incubation at three different constant temperatures and incubated without preliminary exposure to low temperature.

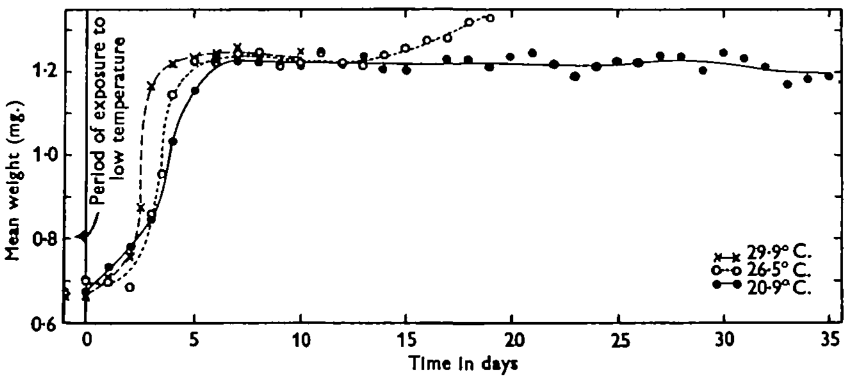


Fig. 2. Showing the changes in the mean weight of eggs during incubation at three different constant temperatures following a period of exposure to low temperature.

Those eggs which were to be incubated at 29·9° C. were held at 13° C. for 15 days, the corresponding period for eggs to be incubated at 26·5 and 20·9° C. being 17 and 39 days respectively. This was necessary to ensure that all eggs were comparable with respect to diapause at the beginning of the observations of water absorption (Browning, 1952*b*). Since over 90 % of the eggs hatched and there were no significant differences in the numbers emerging at the different temperatures, it was considered that this object had been achieved. The eggs increased in weight slowly whilst remaining at 13° C., so that those that were to be incubated at 20·9° C. were significantly heavier at the beginning of the incubation period than those in the other two treatments.

Fig. 2 shows the course of water uptake at the three temperatures which are very similar to those shown in Fig. 1 for newly laid eggs. The fact that the curve for eggs at 20·9° C. is not more markedly different from the other two may be explained by the fact that almost the whole of the initial period of slow water uptake had been completed by these eggs at 13° C., whilst this was not so for the other two groups, which had spent a shorter time at low temperature.

The chief difference between the eggs that had been exposed to low temperature before the incubation and those that had not was found in their variability. The former were much less variable than the latter, both with respect to the initial period characterized by a slow increase in water content and with respect to the rate at which the water content increased during the period of rapid increase. This was particularly true of the eggs incubated at 20·9° C. The decreased variability in eggs which had been exposed to low temperature before they were incubated is illustrated by the smoothness of the curves in Fig. 2 compared with those of Fig. 1.

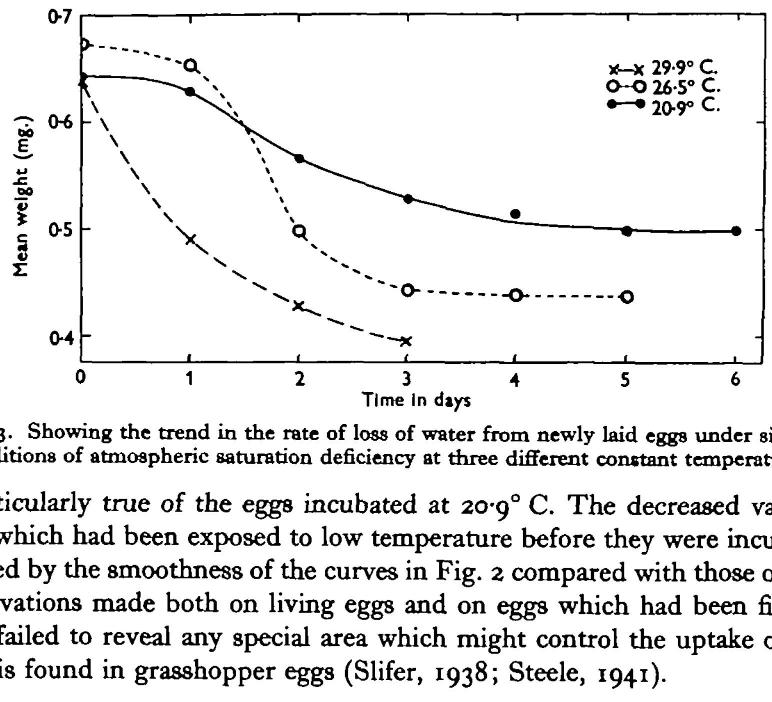


Fig. 3. Showing the trend in the rate of loss of water from newly laid eggs under similar conditions of atmospheric saturation deficiency at three different constant temperatures.

Observations made both on living eggs and on eggs which had been fixed and stained failed to reveal any special area which might control the uptake of water, such as is found in grasshopper eggs (Slifer, 1938; Steele, 1941).

## (*b*) *Loss of water*

### (i) *Newly laid eggs*

Three groups of twenty eggs each were placed on plaster disks in airtight jars containing sulphuric acid solutions whose concentrations were adjusted so that the saturation deficiency of the air in the jars would be 3·226 mm. of mercury at each of the three temperatures, 20·9, 26·5 and 29·9° C. The eggs were weighed each day, and the curves of Fig. 3 show the mean weights of each group during the period of observation. The variation in weight between individual eggs at any temperature at any one time was small, and so this method of presentation of the data has been used here (see § III(*b*) (ii) below).

During weighings it was noticeable that after the eggs had reached their lowest mean weight they began to increase in weight significantly during the following few days. This was attributed to the growth of fungi within the eggs rather than to an uptake of water by the eggs themselves.* Only the descending parts of the curves are shown in the figure.

The eggs lost weight most rapidly at the highest temperature, although the saturation deficiency of the air was similar at all temperatures. This contrasts with the view put forward by Mellanby (1935). The differences seem too great to be accounted for by the increased rate of diffusion of water vapour at the higher temperatures.

The eggs in these experiments were exposed to relatively moist air (between 82·5 and 90 % relative humidity, depending upon the temperature). In view of the rate at which the newly laid eggs lost water (Fig. 3) they must be considered extremely susceptible to desiccation. In nature the eggs are laid in heavy black clay soil which usually remains sufficiently moist throughout the winter and spring to enable many eggs to survive.

The eggs in these experiments, when replaced on moist plaster after 5 or 6 days, showed no sign of absorbing water and were evidently dead. An experiment was then done to determine how much water the eggs could lose without dying.

One hundred newly laid eggs were weighed and placed in an atmosphere of 90 % relative humidity at 29·9° C.; they were removed after 18–26 hr., weighed and placed on moist plaster at 29·9° C. Observations were made of the eggs that subsequently hatched or entered diapause and of those that died.

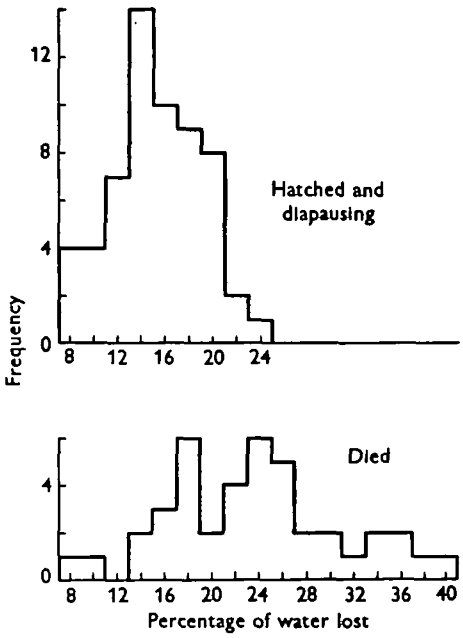


Fig. 4. Histograms showing the percentage of weight lost during a period of desiccation by fifty-nine eggs that survived the treatment and forty-one eggs that subsequently died.

In Fig. 4 are shown frequency histograms representing the percentage of the original weight lost during desiccation, of the eggs that either hatched or entered diapause and of those that died. It can be seen that the loss of more than about 20 % of the original weight was likely to result in an egg's death, whilst most eggs were able to withstand a loss of up to about 20 % of their original weight with no apparent harmful consequences.

* Fungi do not seem to influence directly the viability of eggs. High percentages of eggs were observed to hatch even when they were completely covered with fungal hyphae after long periods of storage at low temperatures. Dead eggs, however, seem to be readily invaded by hyphae.

(ii) *Eggs which had completed water uptake*

Three groups of thirty eggs which had been stored for 70 days at 13° C. and had reached a weight greater than 1·0 mg. were placed at 90 % relative humidity at 29·9, 26·5 and 20·9° C., and each egg was weighed daily until its weight fell below 0·6 mg. At this point they were considered to be dead and were discarded. This limit was chosen because, in a large series of similar experiments, no egg was found to recover after its weight had fallen to 0·6 mg. When the records of weight loss

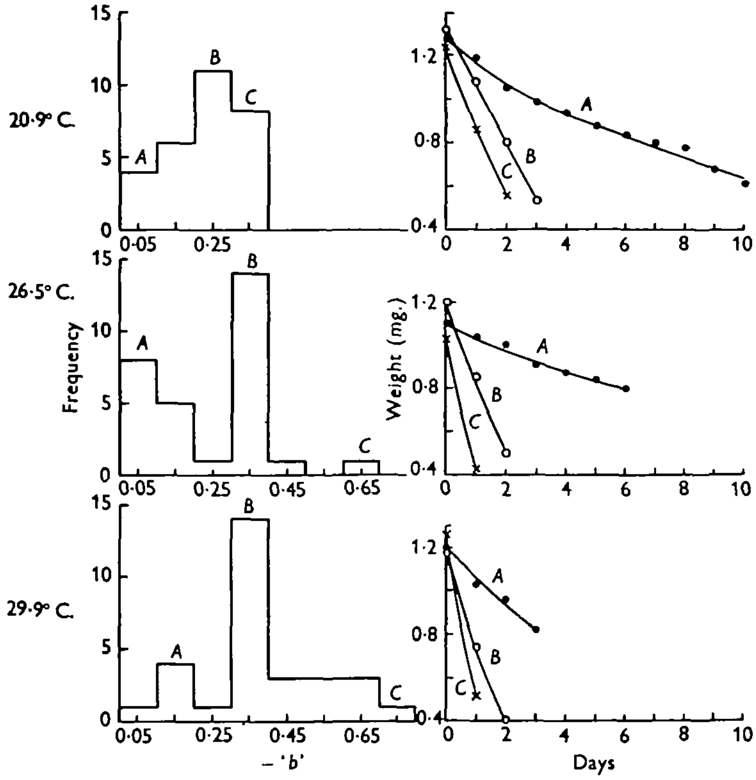


Fig. 5. Histograms showing the distribution of rates of loss of water, *b*, within samples of thirty eggs at each three constant temperatures and curves showing the actual course of water loss from representative individuals from groups labelled similarly in the corresponding histograms.

were examined it was found that at each temperature, individual eggs had lost weight at such different rates that the mean daily weight of the group was of little use in describing the trend of weight loss. This became emphasized after the first 3 days when many eggs had been discarded, leaving only a few quite heavy eggs to contribute to the means.

For this reason the regression of weight lost against time was calculated for each egg. Fig. 5 shows the distribution of *b*, the linear regression coefficient. Although the weight of an egg is not a linear function of the duration of exposure, yet it was considered that the linear regression, by accounting for a substantial part of the total variance, would provide an adequate statistic to enable comparisons to be made between individual eggs at the same temperature and between groups of eggs at different temperatures.

In Fig. 5 the right-hand set of diagrams shows the course of weight loss of representative individuals from groups labelled similarly in the corresponding histograms. The curves were drawn free-hand through the observed points.

At each temperature two distinct kinds of eggs may be distinguished, one which lost water very slowly and one which lost water quite rapidly. These do not seem to be merely the extremes of a very variable population, but rather to be representative of distinct populations which differ in their resistance to desiccation. When comparing eggs at one temperature with those at another, some of the differences—the loss of distinction between the two kinds of eggs at 20·9° C. for example—may have been due to the increased power of desiccation of the air at higher temperatures when the relative humidity was maintained constant.

In order to estimate the stage during desiccation at which eggs lost the power to reabsorb water and continue development to the point of hatching, 100 weighed eggs, which had completed water uptake and diapause development during storage for 70 days at 13° C. were desiccated at 90 % relative humidity and 29·9° C. for periods varying from 24 to 48 hr. At the end of this time the eggs were again weighed and transferred to moist blotting-paper at the same temperature. At the end of 12 days most of the eggs had either hatched or died, but a few remained turgid and appeared healthy but showed no visible signs of development. These eggs appeared to be in a state resembling diapause, even though they had received an exposure to low temperature estimated to be sufficient to enable them to complete diapause development.

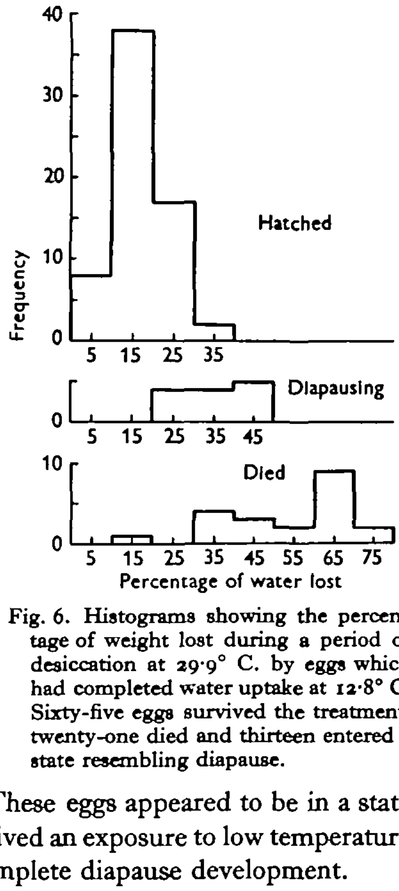


Fig. 6. Histograms showing the percentage of weight lost during a period of desiccation at 29·9° C. by eggs which had completed water uptake at 12·8° C. Sixty-five eggs survived the treatment, twenty-one died and thirteen entered a state resembling diapause.

Fig. 6 shows the frequency distribution of the percentage of weight lost by eggs that either hatched, entered diapause or died.

In order to study the unusual diapause which had occurred in these eggs the following experiment was done. Some hundreds of eggs, which had completed water uptake during an exposure of 4 months to 13° C., were desiccated at 90 % relative humidity at 29·9 C. for 56 hr. They were then returned to moist plaster and left until most of the hatchings were complete. 100 eggs were then carefully selected which were fairly turgid and which appeared, under a binocular microscope, to be in diapause. Twenty-five of these were placed at 29·9° C. as controls, and the remainder, in three lots of twenty-five eggs each, were placed at 13° C. At the end of 15, 25 and 35 days, twenty-five eggs were removed from the cold and placed at 29·9° C., and observations of hatchings were made each day.

This experiment gave no significant results in so far as the numbers of nymphs hatching in the four treatments were concerned. However, although about the same numbers of nymphs hatched in each treatment (between one and three) the numbers of eggs which developed to the point of having clearly visible eye-spots and cerci were considerably greater in the group of eggs stored at low temperature for 35 days compared with the controls. A number of embryos, particularly in the cold-treated groups, were found to have developed to the point where revolution was about to occur but to have been unable to undergo the movement round the posterior pole of the egg. In these cases morphogenesis had continued so that 'monsters' were produced, in which the development of the external form of the embryo was complete, or almost so, but the embryo was facing the posterior instead of the anterior pole of the egg. Similar monsters occurred in which the embryos underwent about half their revolution, and fully developed embryos bent backwards on themselves were found. In still other cases development continued to the point where hatching should normally occur, the embryo became darkened and hardened, but was unable to escape from the egg-shell. These cases are similar to those described by Andrewartha (1943) in eggs of *Austroicetes* which had been exposed to low temperatures for inadequately long periods to permit the completion of diapause development. It can be concluded then that these eggs of *Gryllulus* had been induced to enter a state closely resembling diapause. But it would seem best to regard this as a pathological condition because diapause is not naturally induced in *Gryllulus* in this way and because the treatment necessary to promote the further development of these eggs was not the same as that necessary to promote the completion of the normal diapause development which occurs in *Gryllulus* eggs.

(iii) *Eggs at different stages of development*

A large number of eggs which had been kept for 3 months at 13° C. and had completed water uptake during this period were placed at 29·9° C. on moist plaster. After the eggs had been at 29·9° C. for 0, 1, 3, 5, 7 and 9 days, samples of thirty eggs were taken and placed at 90 % relative humidity and 29·9° C. and each egg was weighed daily. The results are set out in Fig. 7, which was prepared in the same way as the histograms of Fig. 5.

The eggs, which initially lost weight rapidly, became more resistant to desiccation up to about the fifth day and then became less resistant. Similar changes have been shown for the eggs of *Austroicetes* by Birch & Andrewartha (1942) and for eggs of *Smynthurus* by Davidson (1932).

No eggs hatched among the thirty that were desiccated immediately after transfer to high temperature, nor in those that were incubated for 1 day. Two eggs hatched of those that had 3 days' incubation, ten of those that had 5 days, nine of those that had 7 days' incubation and none in the sample that was incubated for 9 days prior to desiccation. All the eggs that hatched lost water slowly. However, a number of eggs in the 0- and 1-day samples lost water quite as slowly as those that hatched in later samples, yet they had so much development to undergo that they died before completing it. The only cases in this experiment in which eggs hatched whose rates of water loss were greater than 0·099 mg. per day (those that did not fall within the first group interval of the histograms of Fig. 7) occurred among the sample that was incubated for 7 days initially and thus had only about 3 days development to complete. Even in these cases, however, the greatest rate of water loss among the eggs that hatched was 0·147 mg. per day, and most rates were much less than this. In all cases hatching occurred at least 1 day later than would be expected for eggs developing at the same temperature (cf. Birch, 1944).

A striking difference can be seen between the histograms for 0 day initial incubation in Fig. 7 and that for the loss of water from eggs at 29·9° C. which had completed water absorption at 13° C., shown in Fig. 5. The only difference in the treatment of these two groups of eggs was in the time spent at 13° C. The eggs in Fig. 7 were in a slightly more advanced stage of development than those of Fig. 5, having spent some 20 days longer at 13° C., and it can be seen that their modal rate of water loss was less rapid. Morphogenesis proceeds very slowly at 13° C. (eggs kept for a year at this temperature did not complete more than one-third of their morphological development), and this rather rapid development of the capacity to resist water loss in eggs exposed to this low temperature suggests that the two processes (morphogenesis and the capacity to resist water loss) may respond in quite different ways to changes in temperature.

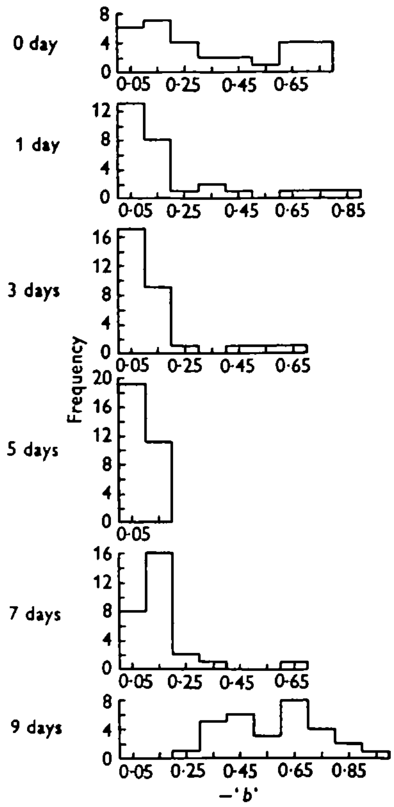


Fig. 7. Showing the distribution of the rates of loss of water, $b$, in samples of thirty eggs which had completed water uptake during storage at 12·8° C., and which were desiccated at intervals after removal to 29·9° C. for incubation.

## IV. DISCUSSION

In the development of eggs of *Gryllulus commodus* there was an initial period during which the weight of the eggs increased slowly, and this was followed by a period when the water content of the eggs increased rapidly. Similar changes have been observed for other species whose eggs absorb moisture during development. The changes in water content must be an expression of physiological changes taking place in the eggs during development. The nature of these changes is not understood, but it is known that the stage of development is important, since in *Gryllulus*, *Corixa* (Banks, 1950) and *Melanoplus* (Salt, 1952) the time after the egg is laid at which the change from slow to rapid increase in water content occurs is dependent upon the incubation temperature, being shorter at higher temperatures. There is evidence also that in *Locustana* water uptake occurs only when the egg has access to oxygen (Matthée, 1951). The osmotic pressure of the egg contents may also be important.

The course of increase in water content was similar, irrespective of whether the eggs entered diapause or developed without interruption. It would seem that the eggs either took up their water before entering diapause or water uptake continued during diapause. In most eggs that have been studied the period of rapid water uptake occurs either before the eggs enter diapause (Salt, 1949*a*) or is delayed until after diapause is complete (Birch & Andrewartha, 1942; Slifer, 1946). It would be unusual for most of the water uptake to occur during diapause, and this suggests that in *Gryllulus commodus* diapause supervenes after water uptake is complete.

In a number of species the resistance to water loss from the egg is associated with the presence of one or more layers of lipoid material in the egg shell (Beament, 1946, 1951; Matthée, 1951; Salt, 1952; Slifer, 1948). Salt (1952), however, has shown in *Melanoplus* that, after this mechanical waterproofing has been completed, the egg, as it develops, continues to change with respect to the rate at which water passes into it and out of it. Similarly, in *Gryllulus* the way in which the eggs become first increasingly and then decreasingly resistant to the loss of water when exposed in dry air suggests some mechanism, at present unknown, other than simple mechanical waterproofing.

The great variability in the resistance to water loss found within samples of eggs which had been treated similarly was comparable with the situation found by Salt (1952) in *Melanoplus* eggs. More must be known about the processes governing water exchange in insect eggs before an explanation of this situation will be possible.

The inception of a state resembling diapause, following the loss of a considerable proportion of their water, by eggs in which diapause development had been completed at low temperature was similar to that described by Salt (1947) for the larvae of *Cephus cinctus* that were exposed to high temperature. Such a phenomenon implies that the physiological state which is brought about gradually at low temperature and renders the organism competent to continue its morphological development, can be destroyed by unfavourable environmental conditions.

## SUMMARY

1. Eggs placed on moist plaster took up water slowly at first and then more rapidly until they had reached their maximum water content. The duration of the initial period of slow water uptake was dependent upon temperature, becoming shorter as the temperature was raised. Similarly the duration of the period of rapid water uptake decreased with temperature.

2. No special water-absorbing structure was identified in the eggs of *Gryllulus*.

3. The rate of water loss from newly laid eggs was more rapid at higher than at lower temperatures under conditions of constant atmospheric saturation deficiency.

4. Newly laid eggs died if they lost more than about 20 % of their original weight.

5. At 90 % relative humidity, eggs which had completed water uptake lost water at a higher rate at high than at lower temperatures. The rate of water loss was also dependent on the stage of development of the embryo.

6. Eggs that had completed diapause development at 13° C. and had completed water uptake were able to lose about 30 % of their weight through desiccation without being killed, but many of those that lost more than about 20 % of their weight failed to develop when replaced on moist plaster; it seemed as if they had entered a state resembling diapause.

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