

# A COMPARATIVE STUDY OF THE EMBRYONIC STAGES AT DIAPAUSE OF SOME SPECIES OF GRASSHOPPERS IN EAST TENNESSEE<sup>1</sup>

WILLIAM G. RUSSELL

Agricultural Biology, Agricultural Experiment Station  
The University of Tennessee<sup>2</sup>

## ABSTRACT

Eggs were collected daily from twelve species of Locustidae and incubated at a constant temperature of 16, 26, or 38 C. From each lot of eggs, beginning with the fourth week of incubation for that lot and every fourth week thereafter, one or two eggs were removed and examined to determine the stage of development. The grasshopper embryos were classified as to stage of development by comparing them with the diagrams of the morphological stages of embryos of a known temperature history. Eclosion occurred in some species, but in others the embryos entered diapause before blastokinesis or just before hatching.

## INTRODUCTION

In the eggs of some grasshoppers, growth is continuous from the time of oviposition until hatching, while in others, an extended embryonic diapause occurs during which morphological changes are at a standstill.

Diapause is an adaptive mechanism which insures the eggs of certain species of insects from hatching at a time when conditions for survival would be unfavorable.

Most previous work involving diapause has been from a physical and chemical point of view. This investigation was designed to relate the effects of different constant temperatures on the embryonic development of various species of grasshoppers of East Tennessee.

Carothers (1924) reported that *Melanoplus differentialis* (Thomas) and *Melanoplus femur-rubrum* (DeGeer) went into diapause before blastokinesis when incubated at 25 C., and that *Chortophaga viridifasciata* (DeGeer) hatched, after being incubated at 22-25 C, in approximately six weeks. Moore (1948) found that *Melanoplus mexicanus mexicanus* (Saussure) would go into diapause just before hatching if exposed to low temperatures.

## MATERIALS AND METHODS

To obtain eggs for these experiments, grasshoppers were collected in the field and reared in the laboratory.

Twelve species representing nine genera and three subfamilies of Locustidae were collected. They were as follows:

1. Acridinae
  - a. *Tryxalis brevicornis* (Linnaeus)
  - b. *Dichromorpha viridis* (Scudder)
  - c. *Syrbula admirabilis* (Uhler)
2. Oedipodinae
  - d. *Arphia sulphurea* (Fabricius)
  - e. *Arphia xanthoptera* (Burmeister)
  - f. *Chortophaga viridifasciata* (DeGeer)
  - g. *Hippiscus rugosus* (Scudder)
  - h. *Dissosteira carolina* (Linnaeus)
  - i. *Mestobregma thomasi* Caudell
3. Locustinae
  - j. *Melanoplus differentialis* (Thomas)
  - k. *Melanoplus femur-rubrum* (DeGeer)
  - l. *Melanoplus mexicanus mexicanus* (Saussure)

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The "hoppers" were kept in a sunny, warm, and well ventilated place. Of the three requirements, sunshine is the most important (Carothers, 1924). The food consisted of a mixture of grasses since time was not taken to find the particular food plant preferred by a particular species.

The eggs were collected daily and placed in Petri dishes containing moist sand. Different egg pods of each species were placed at each of the three different temperatures—16, 26, and 38 C. Temperatures were held within plus or minus 1 degree C.

From each group of eggs, beginning with the fourth week of incubation for that group and every fourth week thereafter, one or two eggs were removed and examined by cutting off the anterior end of the egg, squeezing the contents into water in a watch glass, and observing under a binocular microscope. It was later determined that the eggs could be cleared by leaving them in Chlorox (calcium hypochloride solution) for a minimum of five minutes. After clearing, the embryos could be observed within the eggs and classified as to stage of development with the aid of a dissecting microscope and transmitted light (Slifer 1945).

The embryos were classified as to stage of development by comparing them with the diagrams of the morphological stages of embryos of a known temperature history, as presented by Slifer (1932), of *Melanoplus differentialis* reared at 26 C, and Riegert (1961) of *M. bilituratus* reared at 30 C. Slifer's (1932) diagrams proved the most satisfactory for any species that entered diapause before blastokinesis and Riegert's (1961) for any that entered diapause after blastokinesis.

If the external structures of an embryo were found to be the same as those which prevailed four weeks previously, the embryo was considered to be in a state of arrest.

## RESULTS AND DISCUSSION

*Arphia sulphurea* (Fabricius), the sulphur-winged locust, inhabits dry upland pastures and meadows. It lays its eggs in the soil in late spring or early summer and these hatch in late summer and early fall (Blatchley 1920). The eggs are never subjected to low temperatures and do not ordinarily hatch if they are so treated (Bodine 1929). The nymphs, reaching the second or third instar, hibernate in the winter, attain full growth in the spring, and become mature in early summer.

Fifty-seven eggs were collected and incubated at 16 C. After four weeks of incubation, two eggs from each lot were opened and examined. Only a small clump of cells could be found. After eight weeks of incubation, the embryo's hind legs were N-shaped, the

labial palps well formed, the antennae segmented, the tergo-sternal suture prominent, and anatrepsis was completed. These characteristics are in harmony with Riegert's (1961, Fig. 7) portrayal of a nondiapause form of *Melanoplus bilituratus* when incubated at 30 C. However, after twelve weeks the entire group of eggs had perished. The destructive factor was probably the low temperature, since, under natural conditions, the eggs of this species are never subjected to continuous low temperatures.

Sixteen eggs were incubated at 26 C, from which five nymphs emerged on the thirty-second day and one nymph on the fiftieth day of incubation. It is possible that this late arrival was in diapause, and as a result of an electrical storm that caused a power failure, the slight rise in temperature broke the diapause. The emergence of this nymph occurred twelve days after the storm. Examination of the remainder of the eggs revealed that five eggs had perished and five contained dead embryos. The chorion had split on three of the eggs that contained the dead embryos.

From the thirty-one eggs incubated at 38 C, seventeen nymphs emerged on the twenty-first day. Six of the remaining eggs perished and eight contained dead embryos. The emergence of the seventeen nymphs is an indication that development at this high temperature is rather good and that a lack of sunshine has no apparent effect on eclosion for this species.

*Melanoplus differentialis* (Thomas) abounds in low, damp waste places, such as the margins of lakes and ponds, the borders of streams, fence rows and the margins of lowland cultivated fields (Blatchley 1920). The eggs are normally deposited in the late summer and hatch in the early spring. They are subjected during the winter to low temperatures but will hatch if kept at a constant high temperature from the time of oviposition (Bodine 1925). Slifer (1932) reported that some eggs of *M. differentialis* would not go into diapause at 26 C but would emerge after thirty-eight days of incubation at this temperature. In this experiment, nondiapausing embryos were not encountered at 26 C, but embryos in a state of diapause were.

Approximately 700 eggs were incubated at 16 and 26 C. Embryos were found at each temperature in a state of arrest at two levels of development. At 16 C, after twelve weeks of incubation, the head of the embryo in one group was still at the caudal end; its ventral surface extending anteriorly beneath the concave portion of the egg; and all dorsal parts were still missing. These characteristics were given by Slifer (1932, Fig. 14) as the normal state of arrest for this species when incubated at 25 C. In another group the embryos had their eyes almost fully pigmented, the body had "peppered" pigmentation, there was moderate chevron pigmentation on the femora, and there were pigmented denta on the mandibles. These characteristics are the same as those reported by Riegert (1961) for *M. bilituratus* after 14 days of incubation at 30 C. At first these embryos were thought to be of the nondiapause group; however, at the end of the fourth month, their

external characteristics had not changed from those of the previous month.

The eggs incubated at 26 C entered diapause within four weeks. They, like those at 16 C, showed diapause at two levels of development. One group had gone into diapause before blastokinesis and the other just before hatching. At the end of twelve weeks of incubation at 26 C, there was no change in the physical appearance of the embryos at either level of development (Fig. 1).

Eight hundred eggs were incubated at 38 C. The majority of these eggs, after four weeks of incubation, perished. However, thirty per cent of the eggs had embryos with characteristics similar to those of group one at 16 C, but at the end of eight weeks all of these embryos had died.

Thus, a constant temperature of 38 C is a destructive factor to developing eggs of *M. differentialis* in contrast to Bodine's (1925) results of an exposure to 36 C.

*Melanoplus femur-rubrum* (DeGeer) occurs almost everywhere in bluegrass pastures and meadows, along roadsides and borders of cultivated fields, on city lawns and in open woodlands (Blatchley, 1920). The eggs are deposited in the early summer and hatch in the early spring. Like those of *M. differentialis*, they are subjected to low temperatures during the winter, but according to Bodine (1925), they will hatch if incubated at a constant high temperature from the time they were laid.

One hundred and twenty eggs were incubated at 16 C. After twelve weeks of incubation, the eyes of the embryo were almost fully pigmented; there were pigmented denta on the mandibles; the dorsal closure was complete; and there was "peppered" pigmentation on the body surface. At the end of sixteen weeks there was no change in the physical appearance from that which they possessed at the end of twelve weeks. Thus, diapause occurred just at the completion of embryonic development.

One hundred eggs were incubated at 26 C. At four weeks the characteristics of the embryos at this temperature were the same as those at 16 C after twelve weeks of incubation. Thus, the developmental rate was more than double that at 16 C. At the end of twelve weeks of incubation the only notable change in embryo characteristics was the full pigmentation of the eyes (Fig. 2). The results at these temperatures are in contrast with those reported by Carothers (1924) who found that *M. femur-rubrum* went into diapause before the occurrence of blastokinesis.

Another one hundred eggs were incubated at 38 C. Although after four weeks, eighty per cent of the eggs had perished, twenty per cent contained embryos that still had the head at the caudal end, the ventral surface extending anteriorly beneath the concave portion of the egg, and all of the dorsal parts missing. At the end of eight weeks all the eggs had perished. Thus such a high constant temperature is detrimental to the development of embryos of this species.

*Melanoplus mexicanus mexicanus* (Saussure), from the latter part of May until late November, may be



Figure 1. *Melanoplus differentialis* (Thomas) embryo in diapause from egg incubated 12 weeks at 26° C. from the time of laying.

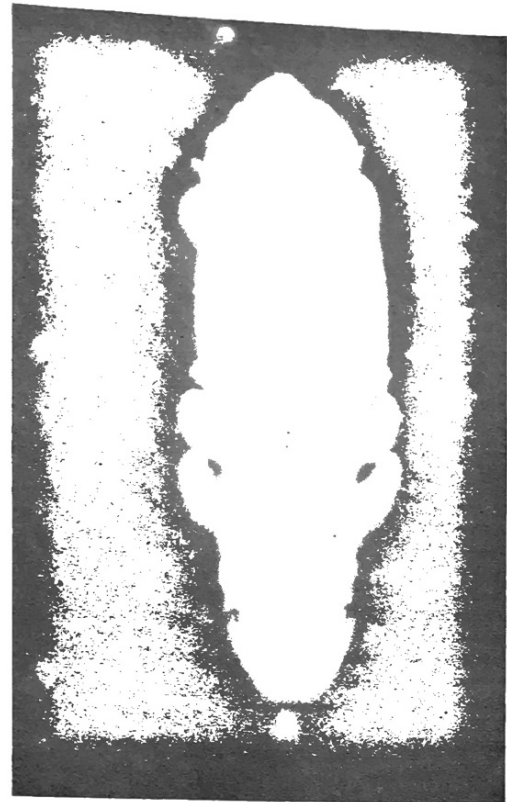


Figure 2. *Melanoplus femur-rubrum* (DeGeer) embryo in diapause from egg incubated 12 weeks at 26° C. from the time of laying.

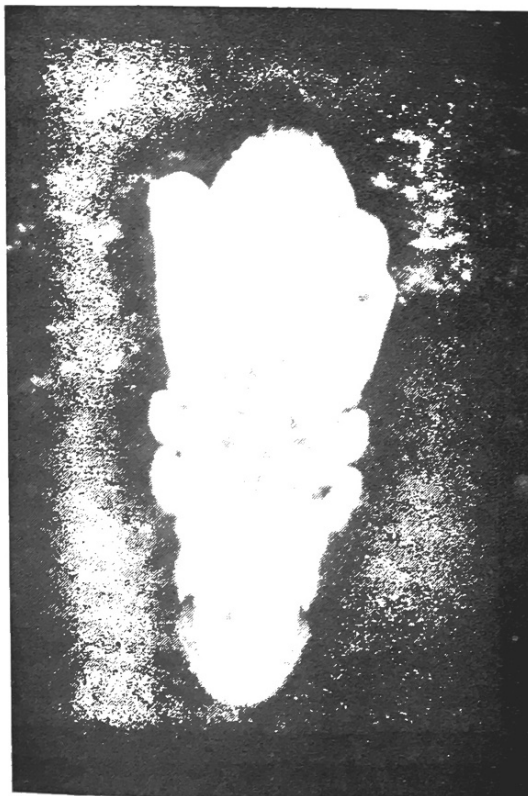


Figure 3. *Melanoplus mexicanus mexicanus* (Sauss.) embryo in diapause from egg incubated 12 weeks at 26° C. from the time of laying.

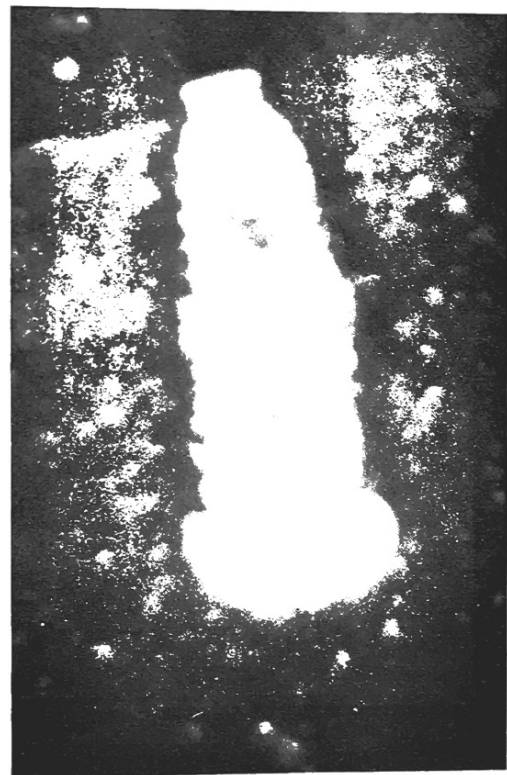


Figure 4. *Mestobregma thomasi* Caudell embryo in diapause from egg incubated 12 weeks at 26° C. from the time of laying.

noted almost anywhere in open bluegrass pastures and woods, borders of roadsides and cultivated fields, meadows and lawns (Blatchley 1920). The eggs are

deposited in late summer and early fall, and they hatch in the early spring. Moore (1948) reported that *M. mexicanus mexicanus* would enter diapause about six

days before hatching, but embryos had been found in the fall in stages of development varying from mere embryonic discs to practically fully developed embryos.

Thirty-eight eggs were incubated at 16 C for sixteen weeks. By the end of this time all embryos had developed to a point where the eyes were fully pigmented. There were pigmented denta on the mandibles; the dorsal closure was complete; and there was light pigmentation on the body surface. There were chevrons on the femur, but the tibio-tarsal spurs were not black. This condition is in agreement with Moore's (1948) finding.

Forty-one eggs incubated at 26 C for four weeks developed embryos to the same stage as those incubated for sixteen weeks at 16 C. When the embryos were examined at the end of eight and twelve weeks of incubation, the physical appearance had not changed. Therefore, the results obtained at 16 C and 26 C were the same as Moore's (1948), diapause occurring just before hatching (Fig. 3).

It was observed, from thirty-nine eggs incubated at 38 C, that the embryos would possess the following characteristics within twelve days: antennae elongate, labrum partly fused, legs bulbous, and abdominal segmentation incomplete. At the end of four weeks of incubation, however, all embryos were dead, and it was impossible to determine the stage at which they had died by the methods used in observing the normally developing eggs.

*Dissosteira carolina* (Linnaeus), commonly referred to as the blackwinged locust, frequents the highways and by-ways of man rather than the pastures and meadows where other grasshoppers are wont to congregate (Blatchley 1920). The eggs are laid in late summer or early fall and hatch in the spring. Blatchley (1920) believes there are two broods each year, or else the eggs hatch at irregular intervals, inasmuch as freshly molted individuals have been observed in September and as late as October 14.

At 16 C, the embryos developed for twelve weeks before entering diapause. During this time there was a great difference in the external characteristics from one month to the next. When the embryos were examined the fourth time, that is, after sixteen weeks of incubation, there was no apparent change from that which was noted after twelve weeks of incubation. The embryos, in diapause, possessed the characteristics of Slifer's (1932, p. 15, Fig. 16) illustration of *M. differentialis* at 25 C. The eye pigment was in the form of a small crescent covering not more than one-third of the eye; the dorsal closure was incomplete; the hind legs were N-shaped; revolution had not occurred; and from the dorsal aspect the short finger-like projections forming the stomodeum and proctodeum were very evident. These observations were made from 720 eggs incubated at 16 C.

The following evidence is in support of Blatchley's (1920) belief that two broods of *D. carolina* may occur

each year. From the 806 eggs incubated at 26 C, ninety-two nymphs emerged within thirty-two days from eggs collected before July 15. The embryos of all eggs collected after July 15 showed, within four weeks, the characteristics mentioned for those in diapause at 16 C. Although the diapausing embryos were observed for sixteen weeks, there was no notable change in the external characteristics. There is evidence, therefore, that *D. carolina* embryos will go into diapause just before blastokinesis, when incubated at or below 26 C, only if the eggs are laid in the latter part of the summer.

After twenty days at 38 C, twenty-one nymphs emerged from the 121 eggs collected before July 15. Although the remaining eggs perished, they contained embryos in various stages of development.

The embryos of the 652 eggs collected after July 15 developed within four weeks to a stage comparable to Slifer's (1932, p. 12, Fig. 14) illustration of *M. differentialis*. The abdomen of approximately 50 per cent of these embryos was greatly deformed. At the end of eight weeks, all eggs had perished.

*Tryxalis brevicornis* (Linnaeus) is a short-horned locust that frequents only the tall grasses and sedges along the margins of lakes, ponds, streams and swales, and in such localities is usually locally abundant (Blatchley 1920). The eggs are deposited in late summer or early fall and hatch the following spring. The nymphs reach maturity about July 20.

One hundred and two eggs were incubated at 16 C. The embryos developed slowly but steadily, and within four weeks a mass of cells with a short tail had formed. After eight weeks, the legs, optic lobes, and rudimentary head appendages were distinct, but the abdomen was not segmented. At the end of twelve weeks, the legs were turning in and showing constrictions at the sites of future joints. By the end of sixteen weeks, the eye pigmentation was a faint pink that formed a thin crescent and covered not more than one-third of the eye; the hind legs were N-shaped; the dorsal closure was incomplete; and the finger-like proctodeum and stomodeum were very noticeable. The head was still at the posterior end of the egg with the embryo lying along the ventral surface. The embryos had reached the point of development where the next stage is revolution, but instead they entered diapause.

A greater rate of development was shown by the 114 eggs at 26 C. Within four weeks, they had developed to the same stage that required sixteen weeks for those at 16 C. Also, after sixteen weeks at 26 C, they possessed the same external characteristics as they did when four weeks old. Therefore, one may conclude from the data just presented that the eggs of *T. brevicornis* will go into diapause just before blastokinesis when incubated at or below 26 C.

One hundred and eight eggs were incubated at 38 C. After four weeks, the notch on the posterior border of the labrum was distinct, the legs were bulbous but not N-shaped, and there were constrictions at the site of

future joints. However, by the end of eight weeks all eggs had perished.

*Mestobregma thomasi* Caudell frequents old abandoned fields, roadsides and bare rocky slopes, especially those on high wooded hills near streams (Blatchley 1920). This species was taken in July for the first time, but no doubt the adults had been mature for some time. If they had overwintered as nymphs, development was slow, but there are indications that the egg is the overwintering stage.

In the other eleven species studies in this experiment a clump of cells was usually found in the eggs after four weeks of incubation at 16 C, but if it were not present, an embryo was found by the end of eight weeks. This was not true of *M. thomasi*. At the end of twelve weeks, a cluster of cells could not be found even though the eggs appeared to be very fresh. This leads to a possibility that the eggs of this species may go into diapause very early in development when subjected to a temperature of 16 C. Due to the small number (twenty-one) of eggs that were incubated, it was not possible to verify this possibility. There is, of course, the chance that the eggs had not been fertilized.

The eggs of *M. thomasi*, like those of other species used for this experiment, developed within four weeks at 26 C to a condition where the eye pigment formed a thin crescent and the hind legs were not N-shaped. At the end of eight weeks, the eyes were not more than one-third pigmented; the hind legs were N-shaped; the dorsal closure was incomplete; and from the dorsal aspect, the finger-like proctodeum and stomodeum were prominent. At the end of twelve weeks, there was no change in the external characteristics. Thus, diapause occurs before blastokinesis (Fig. 4).

The eggs of *M. thomasi* and *A. xanthoptera* were the only ones, other than those where nymphs emerged, not to perish at 38 C. Twelve eggs were incubated, and within four weeks their characteristics were the same as those just presented for eight-week old embryos at 16 C. At the end of twelve weeks they still possessed these characteristics. Therefore, diapause occurs before revolution for *M. thomasi* eggs, when placed at constant temperatures of 26 and 38 C.

*Syrbula admirabilis* (Uhler), in East Tennessee, is commonly called the "two-lined locust." These grasshoppers frequent high, open uplands, where there is scant vegetation, though they are sometimes found in timothy meadows and along roadsides where the grass has been cropped short (Blatchley 1920). The males differ so much in size and color from the females, that the writer considered them a distinct species until a pair was found copulating.

It was very difficult to keep specimens alive in the laboratory. Several varieties of fresh grass were given each day. Even with this special care, the males would die after two days and the females would live for only approximately one week.

Two egg pods were collected on August 29; one was placed at 16 C and the other at 26 C. The rate of development of *S. admirabilis* at 16 C is similar to that of

other species that went into diapause before blastokinesis. A small clump of cells had formed at the end of four weeks, and at the end of eight weeks the rudiments of the antennae and labrum were visible. The tail was segmented at the upper end, and the mouth parts and thoracic segments were distinct. At the end of twelve weeks all eggs had perished.

When the "pod," incubated at 26 C, was examined, there were no eggs present; the mass that was thought to be an egg pod was only froth. Thus, there were no results for this species at 26 C.

The pod, incubated at 38 C, contained sixteen eggs. At the end of four weeks, the entire lot had perished.

*Arphia xanthoptera* (Burmeister), commonly called the autumn yellow-winged locust, frequents the stubble of wheat, clover and timothy fields, the banks along railways and the borders of high, dry, open woodlands and roadsides (Blatchley 1920). Unlike *A. sulphurea*, the egg over-winters and hatches about mid-July.

Nineteen eggs were incubated at 16 C and 39 at 26 C. Within four weeks, all the eggs at each temperature had perished. Either the embryos had never developed or they had decomposed. However, within four weeks the thirty-four eggs at 38 C possessed the following characteristics: hind legs N-shaped, labial palps well-formed, antennae segmented, eye pigment in the form of a thin crescent covering not more than one-third of the surface and the finger-like proctodeum and stomodeum visible as small projections when viewed from the dorsal surface. These characteristics had not changed after ten weeks at this temperature. Therefore, it may be concluded that *A. xanthoptera* eggs will go into diapause just before blastokinesis if subjected to a constant temperature of 38 C from the time of laying.

*Dichromorpha viridis* (Scudder) inhabits the borders of open woods, fence rows, roadsides and the coarse grasses which grow along the margins of lakes, ponds and other wet places (Blatchley 1920). The species is dimorphic and the females are much larger than the males. The eggs, which are deposited in late summer or early fall, hatch the following spring. The nymphs mature in early July.

Observations were made of 216 eggs at 16 C. The embryos were slow in developing, but within four weeks a small cluster of cells with a short tail had formed. The embryos developed within eight weeks to a stage where the legs, optic lobes, and rudimentary head appendages were distinct, but the abdomen was not segmented. By the end of sixteen weeks, the eye pigment was a medium red in the form of a crescent and covered not more than one-third of the eye; the hind legs were N-shaped; the dorsal closure was incomplete; the embryo's head was still at the posterior end of the egg; and the finger-like proctodeum and stomodeum were visible as small projections when viewed from the dorsal surface. Since no further changes were noted, it was concluded that the eggs of *D. viridis* entered diapause just before blastokinesis.

The characteristics for the 255 embryos of *D. viridis*, after four weeks at 26 C, were the same as those just

described for the embryos that went into diapause in sixteen weeks at 16 C. When the embryos were examined at the end of eighteen weeks, no other changes in the physical appearance were observed. From these results, it can be concluded that the eggs of *D. viridis* will go into diapause just before blastokinesis when incubated at or below 26 C.

Two hundred and seventy eggs were incubated at 38 C. Within four weeks, the notch on the posterior border of the labrum was distinct, the legs were bulbous but not N-shaped and there were constrictions at the site of future joints. However, this temperature must be beyond the point of tolerance for developing embryos of *D. viridis*, since all eggs had perished by the end of eight weeks.

*Hippiscus rugosus* (Scudder) inhabits dry upland localities, being especially abundant in open woodland pastures, timothy and clover meadows, along roadsides, and rail fences (Blatchley 1920). The eggs are laid in late summer or early fall and hatch the following spring. The grasshoppers mature about mid-July.

Approximately ninety eggs were incubated at 16 C. The rate of development was uniform, and within four weeks the embryos existed as a clump of cells with a short tail. After sixteen weeks, the eye pigment, covering not more than one-third of the eye, was a medium red and formed a thin crescent around the posterior edge of the eye; the hind legs were N-shaped; the dorsal closure was incomplete; the labial palps were well formed; the antennae were segmented; and the finger-like proctodeum and stomodeum were visible as small projections when viewed from the dorsal surface. Since these same characteristics prevailed at the end of twenty weeks, it was concluded that the state of arrest occurs before blastokinesis.

Within four weeks, the fifty-eight eggs at 26 C had developed to the stage where their characteristics were the same as those just described for the embryos in diapause at 16 C. When the embryos were examined at the end of ten weeks, there were no changes in physical appearance. The time required for the embryos to reach the stage of revolution, at 26 C, is approximately four weeks, but at 16 C it is sixteen weeks. The data collected are sufficient to conclude that *H. rugosus* embryos will go into diapause just before blastokinesis when incubated at or below 26 C.

The sixty-two eggs incubated at 38 C perished within four weeks.

*Chortophaga viridifasciata* (DeGeer), in East Tennessee, is the first locust to reach maturity in the spring from hibernating nymphs. It inhabits bluegrass pastures and grassy tracts along rail fences between upland woods and cultivated fields and meadows. From mid-April until early November mature individuals may occur anywhere in dry grassy places (Blatchley 1920).

Observations were made on 104 eggs incubated at 16 C. The embryos developed very slowly and had only attained, after six weeks, a stage where the rudiments of the antennae and labrum were visible and the "tail"

was segmented only at the upper end. At the end of ten weeks, the legs were turning inward, were more bulbous than tubular, and showed constrictions at the site of future joints; and segmentation of the abdomen was complete. After fifteen weeks, the head appendages were jointed; the legs were large, bulbous and jointed; there was no eye pigment; and the dorsal closure was incomplete. After nineteen weeks, the hind legs were N-shaped, the eye pigment formed a very thin crescent and revolution of the embryo had occurred. After twenty-three weeks, the dorsal closure was incomplete in some but complete in others, the eyes were approximately three-fourths pigmented, and there were lightly pigmented chevrons on the femur and a few small pigmented areas on the body surface. After twenty-five weeks the embryos died, thus hindering further evaluation of this species at this incubation temperature.

Two hundred and seven eggs were incubated at 26 C, from which 165 nymphs emerged on the thirty-second day of incubation. At the end of six weeks an examination of the eggs revealed that twenty-three had spoiled and nineteen had dead embryos.

From the 165 eggs incubated at 38 C, 114 nymphs emerged on the sixteenth day, eight of the remaining eggs were spoiled and forty-three contained embryos that had died before emerging.

The hatching of these nymphs at 26 and 38 C is an indication that development at these high temperatures is rather good. Embryos did not go into diapause when subjected to temperatures at or above 26 C, there being only a difference in the rate of development.

#### SUMMARY

The writer has listed twelve species of Locustidae, from which eggs were collected daily and incubated at constant temperatures of 16, 26, or 38 C (see Tables I, II, and III).

Nymphs did not emerge from any eggs incubated at 16 C. The eggs from *Melanoplus mexicanus mexicanus*, *M. femur-rubrum*, and *M. differentialis* went into diapause before blastokinesis. Other species that entered diapause before blastokinesis were *Hippiscus rugosus*, *Dissosteira carolina*, *Tryxalis brevicornis*, and *Dichromorpha viridis*. The eggs of *Arphia sulphurea* and *A. xanthoptera* perished, and the date were inconclusive for *Mestobregma thomasi* and *Syrbula admirabilis*.

Eggs of *D. carolina* (collected before July 15), *C. viridifasciata* and *A. sulphurea* incubated at 26 C yielded nymphs in approximately thirty-two days. The results obtained for eggs of *D. carolina* (collected after July 15), *H. rugosus*, *T. brevicornis*, *D. viridis* and three *Melanoplus* species were the same as those obtained at 16 C.

At 38 C eggs of *M. thomasi* and *A. xanthoptera* went into diapause within four weeks. Eggs of *A. sulphurea* and *C. viridifasciata* hatched into nymphs within twenty-one and seventeen days respectively. Those of *D. carolina*, collected before July 15, developed into nymphs

within twenty days. The eggs of all other species, including those of *D. carolina* collected after July 15, perished. Spoilage of eggs of some species may be attributed to a constant high temperature (Bodine, 1925).

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TABLE I  
 THE STATE OF OVERWINTERING AND THE EMBRYONIC EFFECT UPON THE EGGS OF GRASSHOPPERS WHEN INCUBATED AT 16° C.

|                                       | Over-wintering state |     | No. eggs incubated | No. hatched | Enters diapause before |          |
|---------------------------------------|----------------------|-----|--------------------|-------------|------------------------|----------|
|                                       | Nymph                | Egg |                    |             | Blastokinesis          | Hatching |
| <i>Chortophaga viridifasciata</i>     | +                    | -   | 104                | 0           | -                      | -        |
| <i>Arphia sulphurea</i>               | +                    | -   | 57                 | 0           | *                      | *        |
| <i>Arphia xanthoptera</i>             | -                    | +   | 19                 | 0           | *                      | *        |
| <i>Hippiscus rugosus</i>              | -                    | +   | 90                 | 0           | +                      | -        |
| <i>Dissosteira carolina</i>           | -                    | +   | 720                | 0           | +                      | -        |
| <i>Mestobregma thomasi</i>            | -                    | +   | 21                 | 0           |                        |          |
| <i>Tryxalis brevicornis</i>           | -                    | +   | 102                | 0           | +                      | -        |
| <i>Dichromorpha viridis</i>           | -                    | +   | 216                | 0           | +                      | -        |
| <i>Syrbula admirabilis</i>            | -                    | +   | 13                 | 0           |                        |          |
| <i>Melanoplus differentialis</i>      | -                    | +   | 700                | 0           | +                      | +        |
| <i>Melanoplus femur-rubrum</i>        | -                    | +   | 120                | 0           | -                      | +        |
| <i>Melanoplus mexicanus mexicanus</i> | -                    | +   | 38                 | 0           | -                      | +        |

\* Eggs perished

TABLE II  
 THE EMBRYONIC EFFECT UPON THE EGGS OF GRASSHOPPERS WHEN INCUBATED AT 26° C.

|                                       | No. eggs incubated | No. hatched | Enters diapause before |          |
|---------------------------------------|--------------------|-------------|------------------------|----------|
|                                       |                    |             | Blastokinesis          | Hatching |
| <i>Chortophaga viridifasciata</i>     | 207                | 165         | -                      | -        |
| <i>Arphia sulphurea</i>               | 16                 | 6           | -                      | -        |
| <i>Arphia xanthoptera</i>             | 39                 | 0           | *                      | *        |
| <i>Hippiscus rugosus</i>              | 58                 | 0           | +                      | -        |
| <i>Dissosteira carolina</i>           | 806                | 92          | +                      | -        |
| <i>Mestobregma thomasi</i>            | 14                 | 0           | +                      | -        |
| <i>Tryxalis brevicornis</i>           | 114                | 0           | +                      | -        |
| <i>Dichromorpha viridis</i>           | 255                | 0           | +                      | -        |
| <i>Syrbula admirabilis</i>            |                    | No data     |                        |          |
| <i>Melanoplus differentialis</i>      | 700                | 0           | +                      | +        |
| <i>Melanoplus femur-rubrum</i>        | 100                | 0           | -                      | +        |
| <i>Melanoplus mexicanus mexicanus</i> | 41                 | 0           | -                      | +        |

\* Eggs perished

TABLE III  
THE EMBRYONIC EFFECT UPON THE EGGS OF GRASSHOPPERS WHEN INCUBATED AT 38° C.

|                                       | No. eggs<br>incubated | No.<br>hatched | Enters diapause before |          |
|---------------------------------------|-----------------------|----------------|------------------------|----------|
|                                       |                       |                | Blastokinesis          | Hatching |
| <i>Chortophaga viridifasciata</i>     | 165                   | 114            | —                      | —        |
| <i>Arphia sulphurea</i>               | 31                    | 17             | —                      | —        |
| <i>Arphia xanthoptera</i>             | 34                    | 0              | +                      | —        |
| <i>Hippiscus rugosus</i>              | 62                    | 0              | *                      | *        |
| <i>Dissosteira carolina</i>           | 773                   | 21             | *                      | *        |
| <i>Mestobregma thomasi</i>            | 12                    | 0              | +                      | —        |
| <i>Tryxalis brevicornis</i>           | 108                   | 0              | *                      | *        |
| <i>Dichromorpha viridis</i>           | 270                   | 0              | *                      | *        |
| <i>Syrbula admirabilis</i>            | 16                    | 0              | *                      | *        |
| <i>Melanoplus differentialis</i>      | 800                   | 0              | *                      | *        |
| <i>Melanoplus femur-rubrum</i>        | 100                   | 0              | *                      | *        |
| <i>Melanoplus mexicanus mexicanus</i> | 39                    | 0              | *                      | *        |

\* Eggs perished