# A COMPARATIVE STUDY OF TWO SYMPATRIC SPECIES OF FIELD CRICKET, GRYLLUS PENNSYLVANICUS BURMEISTER AND G. VELETIS (ALEXANDER AND BIGELOW) (ORTHOPTERA: GRYLLIDAE)

By

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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March 1961

TABLE OF CONTENTS

# I. GENERAL INTRODUCTION

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The first description of an American field cricket of the genus Gryllus was published by Fabricius in 1775 when he described Acheta assimilis from Jamaica. By ~~the~~ beginning of the 20th century no less than forty-seven New World species of Gryllus had been described (Blatchley, 1903), thirty-three of these were recognized as valid by Kirby (1906). Practically all the early workers on this group considered macro-morphology only: such characters as size, coloration, wing venation, body proportions, number and relative length of tibial spines, and the length of tegmina, wings, ovipositor and the hind femora. The wide range of variation existing in most of these characters, however, led Lutz (1908) and Rehn and Hebard (1915) to conclude after studying large series of specimens, that all American (or at least North American) forms belonged to only one highly variable species, Gryllus assimilis, and, until recently, this view has been generally accepted. For many years almost all authors used the specific name assimilis to the exclusion of any other, although there have been changes in the generic name within more recent times.

The sequence of changes in the generic name (Gryllus Linnaeus, 1758, to Gryllulus Uvarov, 1935, to Acheta Fabricius, 1775) has been discussed in detail by Gurney (1950, 1951). Chopard (1955), in a footnote but without full explanation, has more recently suggested that the genus Acheta [synonym Gryllulus] should not be separated from Gryllus and thus he would again

place all American species in the latter genus, although he does not refer to ~~these~~ them specifically. Gurney, however, (in a personal communication to Alexander, 1957) states, "I have decided to continue using Acheta until going into the matter further, but I expect the change will eventually be accepted here because the genera [Acheta and Gryllus] have been rather artificial." Randell (in press) on the basis of cytological studies, and the structure of the male genitalia, however, has now conclusively shown that Acheta is validly distinct from Gryllus but that American field crickets belong to the latter ~~genus~~ and not to the former genus, so that current usage is incorrect. In this thesis, therefore, the author reverts to the name Gryllus.

McNeill (1889) was the first investigator to note that the northeastern North American field cricket, in Illinois, is composed of two populations, one overwintering as a late instar nymph and maturing in spring, while the other overwinters in the egg stage and matures in middle or late summer. He also noted that the adult males of the nymph-overwintering population more often occupy burrows and are characteristically more solitary and more aggressive than those of the egg-overwintering population, and, further, that the ovipositors of the females in the nymph-overwintering population are usually shorter in relation to the length of the body than those of the females in the egg-overwintering population.

Blatchley (1903; 1920) supplied additional biological information regarding these crickets and remarked that, in Indiana and other northern states, most are represented in winter by the egg alone, but that a few pass the cold season as nymphs. He recognized the two types as belonging to distinct subspecies - Gryllus assimilis pennsylvanicus and G. a. vernalis. [The latter name, however, is applicable to a distinct species - see Alexander (1957).]

In Ontario, Walker (1904) considered that there are two species, the adults of one beginning to appear about the third week of May and continuing in the field until the end of July. The adults of the other begin to appear about the second week of August and are found until October. The adults of the former species are very numerous about ~~the~~ mid-summer but are very difficult to obtain, for they are not gregarious like the adults of the latter species.

Criddle (1925), in Manitoba, recognized two races, the spring cricket and the autumn cricket. He also pointed out that the two races do not interbreed. His conclusion was based on observations that one adult population is present in the field from about the first of May to the first of August and is then replaced by the other which appears about the first of August and remains until the onset of winter. He also pointed out that the spring cricket overwinters in the nymphal stage while the autumn cricket overwinters in the egg stage. Thus his observations are more or less similar to those of Walker (l.c.) regarding the appearance of adults of the species (of Walker) in the field.

Allard (1929a) noted that in Georgia and New England there are two broods which, in all features of behaviour, habitat, etc. appear to be as identical as they are in all external morphological characters which the taxonomist would care to consider. Since there are no macro-morphological differences between the two he did not regard these populations even as belonging to two distinct races. In South Dakota, however, Severin (1926; 1935) suggested that there might be two 'biologic races', one hibernating in the egg, the other in the nymphal stage. However, he did not eliminate the possibility of there being two generations.

Folsom and Woke (1939), in Louisiana, observed that field cricket eggs laid in late April and early May produce adults in late July and early August, and that the eggs from the second generation produce nymphs that overwinter. Thus they actually observed one generation succeeding the other. In all probablity they were dealing with what is now regarded as a distinct species which has two generations a year: Gryllus rubens Scudder. Cantrall (1943) suspected that, in Michigan, the spring and fall populations might actually represent two different variants, each having a single annual cycle although mating at separate times, but he also considered the possibility that the two populations were merely successive generations of a single variant which was two-brooded.

More recently Fulton (1952) published a study of the field crickets of North Carolina in which he described four populations

that differed in ecology, life history, song and distribution, but which he believed had no distinguishing morphological characters. These four populations failed to interbreed. However, he hesitated to name them as four distinct species and this stand was supported by Hubbell (1954; 1956). Alexander (1957), however, has now shown that the four populations mentioned by Fulton (l.c.) in fact represent four distinct species which show several differences, particularly in their song characters and he further described an additional new species. Thus he recognized five species from northeastern North America, namely, Acheta [Gryllus] pennsylvanicus (Burmeister), A. [G.] firmus (Scudder), A. [G.] rubens (Scudder), A. [G.] vernalis (Blatchley) and A. [G.] fultoni Alexander. Alexander's conclusions are based on ecological, biological and morphological characters as well as on differences in stridulation. He did not recognize the northern spring cricket as being specially different from the fall cricket A. [G.] pennsylvanicus. While describing the seasonal life-histories of the different species he mentions that no way of distinguishing the spring and fall "broods" of pennsylvanicus in Ohio could be found, other than the differences in their biology and a slight difference in the relative length of the body and the ovipositor in most females. Thus his observations, in this regard, are more or less similar to those of McNeill (1889). Alexander further noticed that the two "broods" occupy the same habitat and that there is a slight overlap in the occurrence of their adults in

mid-July. Bigelow (1958) also corroborated and refined McNeill's observations without materially altering his conclusions.

Prior to Fulton's work, various names had been applied to the two "pennsylvanicus" forms, but Fulton, Alexander, and Bigelow (ll.cc.) did not separate the two populations with formal nomenclature. Fulton (1952) remained quite uncertain about their status. Alexander (1957), unable to find any ecological, morphological and song differences between the two populations, states that "These two broods may interbreed in mid-summer, or probably in fall in the southern part of their range, or it may be that thay have been isolated for such a short time that no noticeable differences have yet appeared between them." The two populations are, in fact, so closely identical in most of their characters that Alexander was unable to recognize them as two species. Bigelow (1958) on the basis of differences between the developmental rates of the two populations states, "The distinctive differences between these two populations are more likely to become further consolidated than they are to break down through any future gene exchange. Therefore these two populations should be regarded as distinct species, however similar they might be morphologically." He was thus the first since Walker (1904) to recognize the two populations as two distinct species, but he still refrained from using formal nomenclature.

Very recently, however, Alexander and Bigelow (1960) have collaborated on the problem and have discussed the relationships

of these two populations in greater detail. They have now formally recognized them as two distinct species. They believe that these species have become reproductively isolated through a seasonal separation of adults initially imposed by elimination of all but two widely separated overwintering stages in the ancestral population and have called this 'allochronic speciation'. After a thorough review of the whole field, they have described the nymph-overwintering species as Acheta [Gryllus] veletis and have restricted the name pennsylvanicus to the egg-overwintering species. The morphological difference between the two species which they have been able to recognize so far lies in the size of the ovipositor (as noted by McNeill, 1889), and even this character is only applicable when the specimens of the two species are from the same locality. Some overlap occurs when specimens from different localities are compared.

From the review of literature, given above, it would appear that there are virtually no macro-morphological characters of significance which may be used to distinguish the two species, G. pennsylvanicus and G. veletis, from each other. The problem of identification is particularly difficult for a taxonomist since the two species are sympatric over much of their range, and the aim of the present studies ~~have~~ has therefore been to search for the micro-morphological, biological and physiological differences which may help to distinguish these species from each other. This thesis provides detailed information on the similarities and

differences between these two sibling species as well as giving some information on related species.

As already noted, Alexander (1957) has pointed out that song differences have proved better than any other single set of related characters for separating the North American species of the genus Gryllus distinguished so far, but G. pennsylvanicus and G. veletis cannot, apparently, be distinguished by this means. A comparative study of the sound-producing organs was nevertheless undertaken in the hope that some differences in their structure and character could be found. Certain differences in fact were discovered, but they were of minor nature.

Since these two species are so similar in morphology and song, and since, as Bigelow (1960b) has indicated, adults of both species are in contact throughout most of northeastern North America during late July and (or) early August and yet do not interbreed, it was thought that there might be some fundamental differences in their reproductive organs. A study of the structure and development was, therefore, undertaken, but this did not reveal any difference of significance between the two species. Randell (unpublished), however, has discovered certain differences in the shape of the male epiphallus.

Studies on the morphological development of the embryos were also undertaken, but, before attempting this comparison, another species, the West Indian Gryllus assimilis (Fabricius) sens. strict. was first studied because of its known regular development, both

in the egg and nymphal stages. Later the morphological development of the embryos of the two species was compared with that of G. assimilis and G. rubens Scudder, another more southerly North American species. These yielded some interesting results, and it was discovered that the embryos of G. pennsylvanicus enter their well-known diapause at a particular stage in their development. The embryos of G. veletis, G. assimilis and G. rubens develop without diapause.

The possibility of experimentally breaking the diapause of G. pennsylvanicus in the laboratory in order to compare the biological characters of this species with those of G. veletis then suggested itself. Experiments with this end in view were performed and different aspects of the diapause studied. Finally a preliminary study of the respiratory metabolism during embryogenesis of the two species was made, since it was considered that this might be used as a test for the presence or absence of diapause.

## Acknowledgments

I am greatly indebted to Professor D. K. McE. Kevan, Chairman of the Department of Entomology and Plant Pathology, for directing the research, for constructive criticism and for valuable advice. My thanks are also due to Dr. R. S. Bigelow for helpful suggestions and advice. The financial assistance received from the National Research Council of Canada and from an Agricultural Auxiliary Grant from the Province of Quebec is gratefully acknowledged.

# II. SOUND-PRODUCING ORGANS AND MECHANISM OF SONG-PRODUCTION IN FIELD CRICKETS OF THE GENUS GRYLLUS LINNAEUS

(Published in Canadian Journal of Zoology 38: 499-507, 1960, using the genus name Acheta Fabricius)

# II. SOUND-PRODUCING ORGANS AND MECHANISM OF SONG-PRODUCTION IN FIELD CRICKETS OF THE GENUS GRYLLUS LINNAEUS.

## 1. Introduction

Studies by Criddle (1925), Severin (1926), Fulton (1952), Alexander (1957), and Bigelow (1958) have shown that biological characters are of primary importance in the taxonomy of North American Gryllus species. All species of Gryllus are good singers and, according to Alexander (1957), song differences have proved better than any other single set of related characters for separating the North American species distinguished so far.

Much attention has been paid to the study of songs of insects by using expensive instruments, but the following points, which are of fundamental importance in explaining the audiospectrographs of the songs, still remain to be answered: (i) parts played by different structures of the song-producing organs, (ii) extent to which the file is scraped by the scraper at the time of stridulation, (iii) whether it is the outward or the inward movement of the tegmina that produces the song, or both these movements. It is hoped that this study of the song-producing organs will help to elucidate these points.

## 2. Materials and methods

The studies are based on both living and preserved specimens from cultures of the following species reared at Macdonald College: G. assimilis (Fabricius, 1775), G. pennsylvanicus Burmeister, 1838, G. rubens Scudder, 1902, and G. veletis (Alexander and

Bigelow, 1960). The measurements were taken in the following way: The length of the tegmen was measured from the base of its dorsal part to its tip and its width along a line drawn parallel to the file, while the resonator was measured from the anterior to the posterior corner and from the left to the right corner. The length of the part of the $A_1$ vein having teeth was considered as the length of the file. In counting the number of teeth, even the smallest tooth, which could be seen under a magnification of about 500 times, was taken into account. All the measurements except those of the file were made directly by means of an oculometer, while the file was measured with the help of a camera lucida.

### 3. Structure of the song-producing organs

A general description of the stridulatory organs of crickets is found in almost all text books of Entomology and a detailed account of these organs has been given by Tsuchiyama (1932). The song-producing organs of G. pennsylvanicus, a common species in Quebec, are illustrated (Fig. 1) and described briefly as a basis for comparison with those of other species of the genus. It may be noted that, in Gryllus, song-producing organs are found exclusively in males; they are present, and similar, on both tegmina. The song-producing structures may be divided into two groups: the structures which produce the characteristic song, viz., the stridulating organs (file and scraper); and the auxiliary structures (tegmen, resonator and ~~the~~ harp). The tegmina of

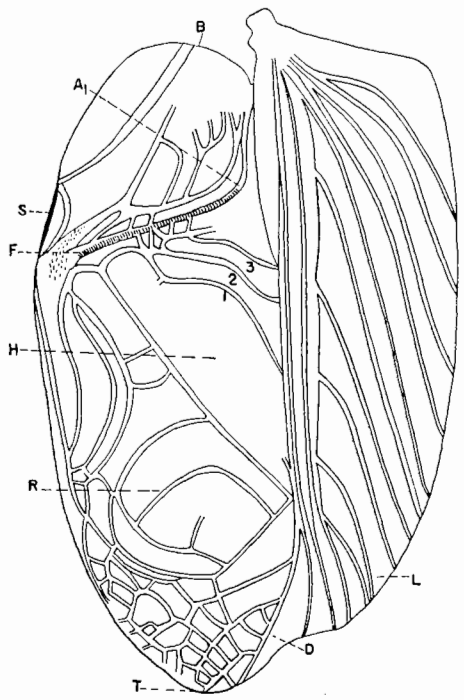


Fig. 1. Left tegmen of G. pennsylvanicus, Ventral view. $A_1$, first anal vein; B, base of dorsal part of tegmen; D, dorsal part of tegmen; F, file; L, lateral part of tegmen; R, resonator; S, scraper; T, tip of dorsal part of tegmen; 1, 2, 3, veins of harp.

crickets are tough and sclerotized and each is divisible into two parts, the dorsal and the lateral, the dorsal part bearing the song-producing organs. The file lies near the base of the tegmen and is formed from the under surface of the part of $A_1$ vein which is more or less transverse in the region of the file. The teeth of the file are not evenly spaced but are somewhat closer together in the apical region. They project from the tegmen at approximately a right angle and are separable into three groups: largest and elongated in the middle region of the file, small and more or less elliptical in its basal region, while towards its apex they become progressively smaller although remaining elongated. The scraper is situated near the apex of the file and is a small thickened zone on the anal margin in its basal part. The resonator lies near the tip of the tegmen, is more or less rectangular in shape, and may be traversed by one or two veins. The harp is a triangular area occupying the space between the file and the resonator and is traversed by three veins which are also connected to the stridulating vein. The first of these ~~three~~ veins demarcates the limit up to which the file is scraped. Usually the right tegmen overlaps the left one and therefore the file of the right is scraped by the scraper of the left to produce the characteristic song.

The right and left tegmina are usually similar in size but the length of the file and the size of the resonator sometimes differ, while the number of teeth is generally different on each

tegmen. The size of the resonator and the length of the file in many cases are more or less directly proportional to the size of the tegmen. When the tegmina of ~~the~~ two individuals are of the same size and the number of teeth varies, the length of the file is directly proportional to the tooth number.

4. Comparison of the song-producing organs of different species of Gryllus

The size of tegmen, harp[1], resonator, and file show great individual variation within each species and there is much overlap in these characters between species. The size of harp, resonator, and file appear to be directly proportional to the size of tegmen. In all species of Gryllus examined the file teeth are separable into three groups, as in G. pennsylvanicus (vide supra). Their exact shape is difficult to describe adequately in words, but figure 2 will give some idea of the differences in size and shape. Although the number of the file teeth in different individuals of a species is variable, the mean differences are considerable. The mean numbers of the file teeth and the standard deviations for each species (where N = 50) are listed below:

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1 However, Randell (unpublished) has recently discovered that there are some small differences in the venation, particularly of the harp, between G. pennsylvanicus and G. veletis, but it is not yet known if such differences are constant.

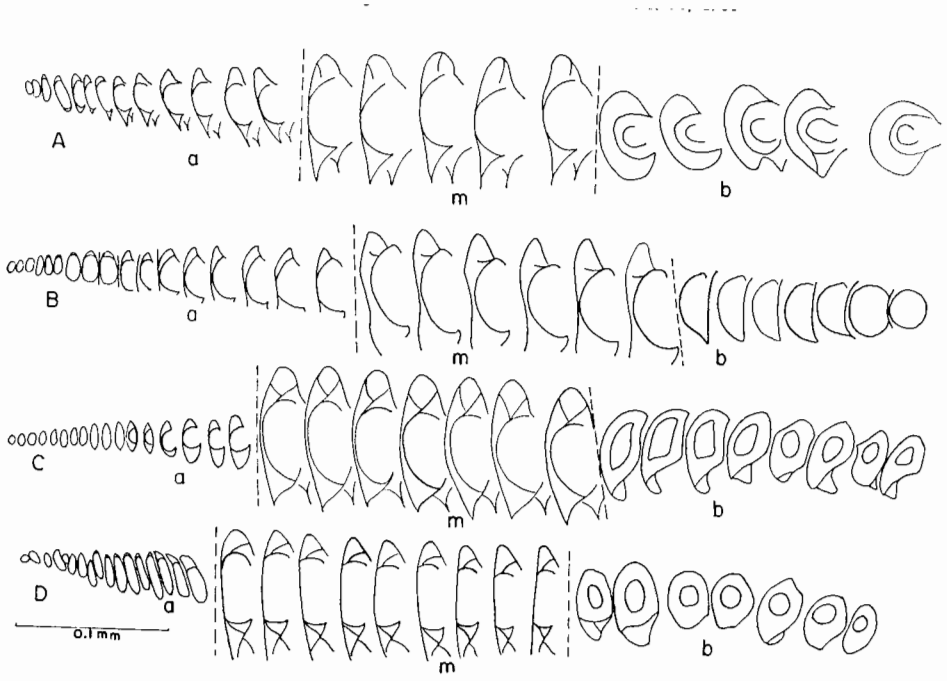


Fig. 2. Teeth in the file of G. rubens (A); G. assimilis (B); G. veletis; and G. pennsylvanicus (D); a, apical region; b, basal region; m, middle region.

| | |
|---|---|
| G. rubens | 98±5 |
| (mid-point 109) | |
| G. assimilis | 120±5 |
| (mid-point 132) | |
| G. veletis | 143±11 |
| (mid-point 153) | |
| G. pennsylvanicus | 162±9 |

The mid-points between the means cited above are 109, 132 and 153 respectively. Assuming representative samples and normal distributions, 98 per cent of all G. rubens specimens will have fewer than 109 file teeth; 98 per cent of all G. assimilis specimens will have more than 109 and fewer than 132 file teeth; 86 per cent of all G. veletis specimens will have more than 132, and 81 per cent fewer than 153 file teeth; 84 per cent of all G. pennsylvanicus specimens will have more than 153 file teeth. The above-noted analysis shows that the number of teeth in the file is an excellent taxonomic character for some species.

## 5. Experiments

Of approximately 50 individuals of each species examined, 90 to 96 per cent were found to be right-winged, i.e., the right tegmen overlaps the left tegmen. When they were made left-winged by reversing the position of the tegmina in the laboratory, 92 per cent of the individuals of G. rubens and 68 per cent of those of G. veletis became right-winged again within 24 hours. Individuals

which did not revert to the right-winged condition during this period remained left-winged for the remainder of their lives. But,even so, artificially left-winged individuals sometimes began to stridulate almost immediately. At first the song was soft, almost exactly like the first song of the adults after their final ecdysis. Within one to three days, however, the left-winged individuals began to sing normally. In G. assimilis all individuals became right-winged again within one hour.

When the lateral part of the right tegmen of G. veletis was cut away such mutilated individuals stridulated as efficiently as normal males, but the song so produced was not so loud. Thus the removal of the lateral part of the tegmen did not affect the modulation quality but only the volume of the song.

The removal of the lateral part of the left tegmen and the apical part (including the resonator) of both tegmina of G. veletis individuals again did not affect the modulation quality of the song although its loudness was further diminished, i.e., the song became even softer than it was when only the lateral part of the tegmen was removed. Even when both the lateral and apical parts of both tegmina were removed the insects were able to stridulate; the sound so produced was very soft but the characteristic quality of the song was maintained.

When the scraper of the left tegmen of G. veletis was removed the mutilated individuals tried to stridulate but could not, at first, produce any sound, and it is interesting to note that 80 per cent subsequently became left-winged and produced a song similar to that of the normal individuals.

The adults of G. assimilis and G. veletis begin their first singing on the fourth and sixth day of their adult life respectively. By this time the spermatophore has not yet developed, so that the presence of a spermatophore in the spermatophore chamber is not essential for stridulation to begin. When the spermatophore was removed artificially from the genital cavity, singing was not inhibited; individuals sometimes began to sing almost immediately after the removal.

It was observed that at the time of stridulation the tegmina are raised from the back at an angle of about 30 to 45° and there appears to be some difference in the songs produced at various angles. It has been noted that when an insect sings 'spontaneously' it keeps its tegmina at an angle of about 30° from its back; this is probably a 'calling song', but when another male also begins to sing, the first raises its tegmina to an angle of about 45° to produce the 'fighting song'. Thus the kind of song seems to depend on the angle of elevation of the tegmina.

It was observed that at the time of stridulation the insect uses only about one-third of the file, i.e., about one-third of the file is engaged by the scraper and the remaining two-thirds do not come in contact with it. The scraper runs laterally nearly to the first vein of the harp. This was determined for G. assimilis and G. veletis in the following manner: the insect was held in the left hand by the thorax which was pressed a little, causing the tegmina to move upwards at an angle of about 45°. The left

tegmen was then moved outwards with a forceps and freed so that it moved inwards, either by itself, or assisted a little by the thumb. By this means a characteristic chirp may be produced, and it can be observed that the left tegmen can move underneath the right tegmen only up to about the middle vein of the harp in G. assimilis and up to the first vein in G. veletis. When the tegmina are held at an angle of about 30 to 40° the left tegmen cannot move below the right tegmen beyond the positions mentioned above. The left tegmen cannot move beyond these limits by itself. However, when the tegmina are parallel to the back the left tegmen can be moved well beyond these limits by means of forceps and the sound thus artificially produced is not characteristic of the species. It is, however, very similar to the first song of the adult.

To discover whether it is the outward, inward, or both movements of the tegmina that produce the song, an adult male G. assimilis was held in the manner described above, keeping the tegmina at an angle of about 45° from the back. First the left tegmen was pulled out and then allowed to move inwards by itself. The characteristic sound was produced as the tegmen returned to the resting position. When a similar experiment was done with the right tegmen, however, no sound was heard. Only the inward movement of the tegmina produced the song. The insect first pulls its tegmina apart and then allows them to come together. It is only when they move towards each other that the characteristic song is produced.

A sound can be produced by moving the tegmen both inwards and outwards by means of forceps, but the sound so produced is not characteristic.

## 6. Discussion

As noted above, individual crickets show great variation in tooth-number in the file. It is, in fact, difficult to find two individuals having the same number of teeth. However, the limits of variation in each species appear to be fixed around a characteristic mean number, and the mean differences in some cases are considerable. There is little or no overlapping in tooth number (fig. 3), for example, between G. rubens and any of the other three species. The same is true of G. assimilis. Although overlapping is considerable in the case of G. veletis and G. pennsylvanicus there is a distinct difference in the means and the range of variation in tooth number between the two species, G. pennsylvanicus having more teeth on the average. Overlapping is so great, however, that tooth-number is useless as a taxonomic character in the case of these two species. If the number of file teeth affects the song character of a species, one would expect little or no difference between the song of G. veletis and G. pennsylvanicus. This is in fact the case, and is one of the main reasons why Alexander (1957) did not separate these two species. There appear to be, however, distinct differences in the size and shape of the file teeth (Fig. 2 ).

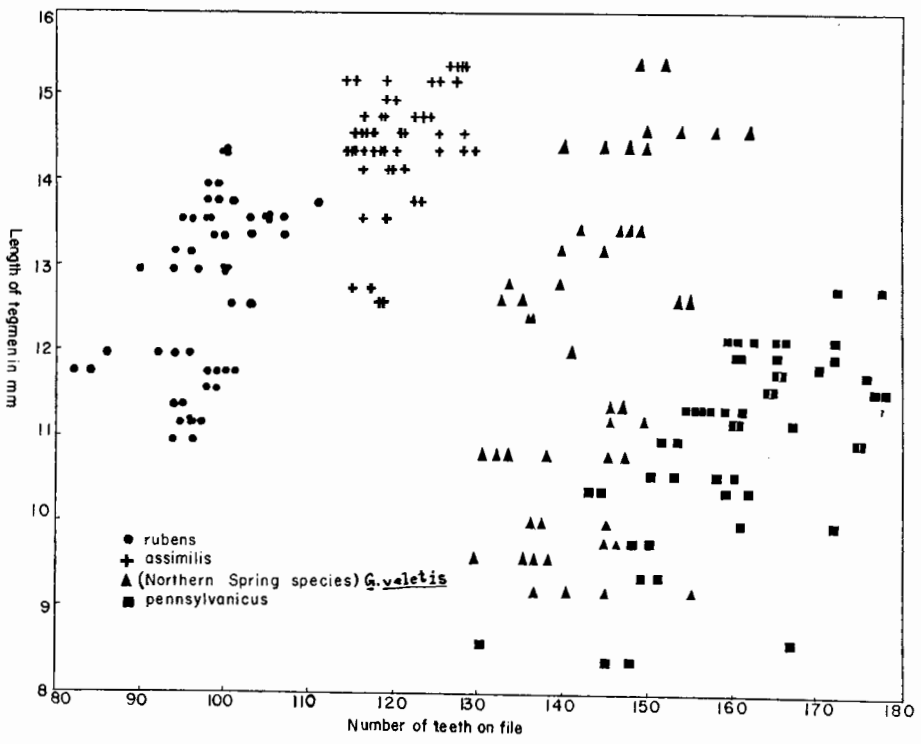


Fig. 3. Distribution of teeth in the file of 50 tegmina of each of G. rubens, G. assimilis, G. veletis and G. pennsylvanicus.

The experimental studies reveal that the stridulatory organs of both tegmina are equally efficient, either set being able to function as well as the other after a little practice. The removal of the lateral or apical parts of either or both tegmina (including the resonator) lowers the intensity of the song. This corresponds with the observations of Loher (1957) for Acrididae. The change in the intensity of the song appears to be of no great influence in the life of crickets (under laboratory conditions) since males in which intensity of the song was lowered experimentally could attract females. In the laboratory, it may be noted that, particularly in G. assimilis, more than 25 per cent of males break the apical part of their tegmina accidently.

It has been observed by many workers that there is sometimes variation in the quality of song of a species from different localities. Loher (1957) and Frings and Frings (1957) have found clear-cut differences in the songs of different individuals of Neoconocephalus ensiger (Harris) (Tettigoniidae). It is believed by Pumphrey and Rawdon-Smith (1939, 1940) and by Frings and Frings (1958) that the tympanic organs lack frequency discrimination of pure tones but are sensitive to modulated sounds, and that this modulation quality remains the same for a given species. The present experiments support this view, and it is probably not possible to change the modulation quality of the songs of crickets experimentally. It may be suggested that the number of teeth in the file is probably (to a great extent responsible) for

determining the modulation quality of the song of a particular species. The overlap in tooth number in G. veletis and G. pennsylvanicus keeps them very close in song character although there are other differences, particularly in the shape and size of teeth. There may be differences in the song due to the size and shape of teeth which the human ear cannot detect.

There is no previous record of the extent to which the file is used for stridulation in Gryllus, but Bonner (1954) has argued that about 40 per cent of the file is used in stridulation in Neoconocephalus ensiger. His conclusion is supported by the present observations, but the basis of his arguments does not appear to be very sound, for according to him each note contains about 40 pulses and probably each pulse represents a tooth being struck. Probably he means by 'note' a pulse and by 'pulse' a spike. By examining a number of audiospectrographs of several species, published by a number of workers, and including some of those mentioned above, it was found that each pulse consists of a number of vertical 'spikes' and I am of ~~the~~ opinion that each spike is produced by a tooth being struck. If a pulse shows a large number of spikes this means that a large number of teeth is scraped to produce the pulse. Comparing Alexander's (1957) figures 11 and 14 of audiospectrographs of Acheta [Gryllus] pennsylvanicus and A. [G.] rubens, the audiospectrograph of the former shows a large number of spikes in a pulse while that of the latter shows a smaller number. This is due to the fact that G. pennsylvanicus

has a greater number of teeth than G. rubens on the one-third of the file scraped. Further, these spikes are responsible for the modulated quality of the pulse and, since the number of teeth in the part of the file scraped by the scraper is more or less fixed, the modulated quality of the song for a species becomes fixed.

Allard (1929b) and others believe that the notes are made only by the inward movement of the file. Recently Borrer (1954) has discussed this point at length and believes that the song is probably produced by one movement of the scraper. Frings and Frings (1957) also feel that the sound is produced by unidirectional movement and that the silent period is a period of return of the tegmina to a starting position. However, this was not demonstrated by these authors. The present observations have conclusively shown that the song is indeed produced by unidirectional movement, namely the inward movement of the tegmina, in Gryllus species. This observation also appears to be of great importance in interpreting the audiospectrographs of various insects. A study of these audiospectrographs reveals that the pulses are spaced; this spacing coincides with the outward movement of the tegmina when no sound is produced. Further, this period is different for different species, depending on the rapidity with which the tegmina are moved apart. In some insects the pulse period and non-pulse period are equally spaced, which would mean that both inward and outward movements of the tegmina take place with equal speed. In other insects in which the pulse and non-pulse periods

are unequal, the speed of movement of the tegmina inwards and outwards is different. Since the spacing is fixed for a species, the quality of the song becomes distinctive for a species. This may be regarded the second quality of the song.

The third quality of ~~the~~ song is the number of pulses comprising a phrase, i.e., the number of successive pulses before a break in the song. For example, in G. assimilis a few pulses are emitted, then a break occurs before the singing is repeated.

It may be concluded that the quality of the song of a particular species depends firstly on the number of teeth of the file scraped by the scraper, secondly on the speed by which the tegmina are moved outwards and inwards, and thirdly on the number of pulses per unit time; and the kind of the song on the elevation of the tegmina.

One question still remains to be answered, namely, the function of the remaining part of the file which is not scraped by the scraper. It is possible that this 'unused' part may further help in producing the specific modulated frequency in a way analogous to what occurs in our stringed instruments, in which only a part of the string is engaged by the bow. When even a small peg is put on the string, the modulated quality of the string changes. The tooth-number probably performs a similar function in the stridulatory mechanism of crickets.

# III. STRUCTURE AND DEVELOPMENT OF THE REPRODUCTIVE ORGANS OF *GRYLLUS VELETIS* AND *G. PENNSYLVANICUS*

# III. STRUCTURE AND DEVELOPMENT OF THE REPRODUCTIVE ORGANS OF GRYLLUS VELETIS AND G. PENNSYLVANICUS

## 1. Introduction

The structure of the reproductive organs of the Gryllidae has been studied by a number of workers. Spann (1934), who describes the structure of these organs for Gryllus "assimilis"[1], has given a complete review of the literature up to date. Since then Snodgrass (1933, 1937), Qadri (1940) and Gupta (1948) have described the structure and development of these organs in Gryllus "assimilis"[1], Gryllulus [now Acheta] domesticus (L.) and Gymnogryllus erythrocephalus (Serville), and Gryllulus [Gryllodes] sigillatus (Walker) respectively. The aim of the present study, as in other parts of the thesis, was to discover differences, if any, in the development of the reproductive organs of the two closely related North American species Gryllus pennsylvanicus and G. veletis, particularly ~~in~~ with respect ~~of~~ to late instar nymphs.

In G. veletis and G. pennsylvanicus the duration of the penultimate and the last nymphal instars (nymphs with short wing-pads and long wing-pads, usually in their eighth and ninth instars respectively) is very variable and differs in the two

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1 Not Gryllus assimilis (F.) but one of the common North American species, possibly G. pennsylvanicus. Spann's material was from Kansas.

species. Under the laboratory conditions used by the author[1] the duration of these instars in G. veletis was found to vary from 5 to 29 and 6 to 31 days respectively, while in G. pennsylvanicus the corresponding periods of development were 7 to 10 and 8 to 11 days; the durations of other instars of the two species were more or less similar (unpublished observations - but see also Jobin (1961) for a fuller study of this aspect). As noted elsewhere, G. pennsylvanicus overwinters in the egg stage but G. veletis enters hibernation as a nymph, probably in the eighth or ninth instar. In spite of the differences between the species during these critical instars, the present studies unfortunately reveal virtually no difference in the structure and development of their reproductive organs, so that little additional information of value to the taxonomist has been obtained[2]. However, in view of the confusion in the literature regarding the species of the assimilis group studied by previous authors, a re-examination of the problem is fully justified.

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1. The nymphs were reared on crushed "baby rabbit pellets" (sold by Ogilvie Flour Mills Co. Ltd. Montreal) in one-gallon candy jars. A vial of 40 ml. capacity, containing water, tightly plugged with cotton wool, served as a source of water in the jars.

2. However, Randell (unpublished) has noted certain differences in the shape of the male epiphallus of the two species in Quebec populations, but it is not yet known if such differences are constant for the whole distributional range. Similarly Alexander and Bigelow (1960) have pointed out that the length of the ovipositor differs between the two species, provided thay come from the same area.

The study also clarifies some controversial points in the morphology of the reproductive organs, which have also been described briefly for the adult insects as a basis for discussing their development and morphology in the nymphs. Since the two species under review are similar in structure and development, the following account is equally applicable to both of them.

## 2. Materials and methods

Adult G. veletis and G. pennsylvanicus were collected locally in the vicinity of Ste Anne de Bellevue, Quebec, at the beginning of May and August respctively, and their progeny (first generation) was used for the study. The eggs of G. pennsylvanicus were given cold treatment at 6-7°C. for three months to ensure their regular development in order to obtain normal nymphs (see Section VI). The reproductive organs were studied after making dissections of both the adults and nymphs in 70 per cent alcohol. Diagrams were made using a camera lucida.

## 3. Female reproductive organs

The ovaries are more or less spindle-shaped extending from the second to the fifth abdominal segment. Each ovary consists of a large number of closely packed ovarioles which open into the oviduct. The two oviducts run first posteriorly and then turn towards the middle line to meet the common oviduct. The common oviduct is short, wide at its base and pointed at its tip, and extending posteriorly to open into the genital cavity. The gonopore is wide and dorsal being situated in the dorsal wall of the common

oviduct. The spermatheca is pear-shaped, thin-walled sac, from the middle region of the left side of which leads a much convoluted spermathecal duct. This duct opens on the ventral side of the spermathecal spout. The spout is conical, dorsally convex and strongly sclerotized, and ventrally concave and membranous. The concavity of the spout remains filled with fat body. The opening of the spermathecal duct is situated, not at the tip of the spout, but a little anterior to it and is considerably finer than the gonopore. The spout lies in the dorsal part of the genital cavity a little posterior to gonopore. The genital cavity lies between the subgenital plate on the ventral side and the ninth sternum on the dorsal side.

The subgenital plate is pocket-like and closed posteriorly. Its ventral wall is heavily sclerotized, the anterior margin of which is fused with the posterior margin of the seventh sternum. The dorsal wall of the pocket is sclerotized only postero-laterally, leaving the anterior and median part membranous. This latter remains folded, and in this fold opens the gonopore. The anterior margin of the dorsal wall of the subgenital plate is fused with the membranous eighth sternum, which also forms a fold, like that of the subgenital plate. In the posterior part of the fold of the eighth sternum lies the spermathecal spout. Thus the genital cavity actually lies between these two folds which, being fused anteriorly, form a membranous chamber where the fertilization of the egg takes place. Thus the dorsal wall of the subgenital plate

and the membranous eighth sternum separate the common oviduct and the spermathecal duct from each other, except near their openings.

The ovipositor consists of three pairs of valves, the anterior and lateral pairs are long and perform the function of oviposition, whilst the inner pair is rudimentary and remains concealed between the anterior and lateral pairs. The eighth sternum is membranous except for the lateral parts which form the first pair of valvifers; their postero-mesial corners remain articulated with the anterior ovipositor valves. The spermathecal spout, in the middle part of the sternum, probably forms the first intervalvula. The ninth sternum becomes divided into a number of sclerites: namely, the anterior and posterior intervalvulae; a pair of valvifers which remain fused with the bases of the lateral and inner pairs of the ovipositor valves; and a pair of antero-lateral pieces which become fused with the anterior valvifers. There are no basivalvulae since no distinct sclerite becomes differentiated at the base of any ovipositor valve.

Development of the ovipositor valves: The sexes cannot be distinguished externally in the first instar. In the second instar the sexes can be separated rather easily, as the ninth sternum of the female appears divided into three parts, but there are no traces of the ovipositor valves. In the third instar (Fig. 4A), two pairs of the ovipositor buds appear, one at the posterior margin of the eighth and the other at the posterior margin of the

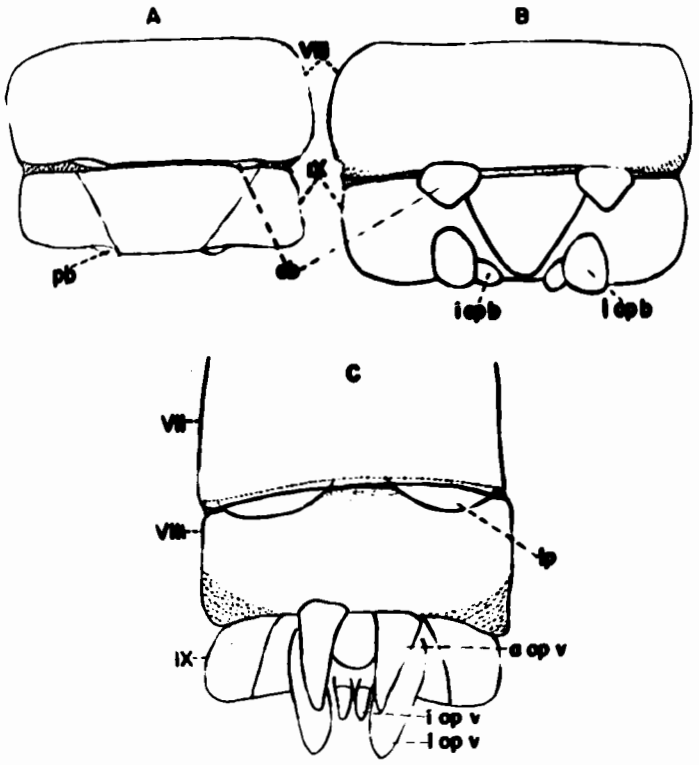


Fig. 4 Developmental stages of ovipositor and subgenital plate of G. veletis. A, surface view of eighth and ninth sterna of third instar; B and C, same of fourth and fifth instar. ab, anterior bud; aopv, anterior ovipositor valves; iopb, inner ovipositor bud; iopv, inner ovipositor valve; lopb, lateral ovipositor bud; lopv, lateral ovipositor valve ; lp, lateral pouch; pb, posterior ~~ovipositor~~ bud; VII, VIII, IX, number of sterna.

ninth sternum. The buds of the eighth sternum lie far apart, while those of the ninth are a little smaller than those of the eighth and are closer to each other. In the next instar (Fig. 4B) the buds of the eighth sternum, which form the anterior pair of the ovipositor valves, grow and become more or less triangular. Each bud of the ninth sternum divides into a large outer and a small inner part, the outer part becomes the lateral ovipositor valve while the inner one forms the inner ovipositor valve. In the fifth instar (Fig. 4C) the three pairs of the rudiments assume the characteristic shape of the ovipositor valves, the anterior and lateral pairs become elongated, while the inner pair remains small. In the subsequent instars (Fig. 5 A and B) the anterior and lateral pairs of valves grow further, but the inner pair, instead of growing, becomes reduced and therefore remains small and concealed between the former pairs of valves in the adult.

Development of the subgenital plate, valvifers and intervalvulae: In the first instar the sterna of the genital segments are more or less similar to those of the pre-genital ones. In the second instar the ninth sternum, which is the smallest, appears to be divided into a median and two lateral parts due to the median part being a little raised up. In the next instar (Fig. 4A) the eighth sternum is smaller than the seventh, while the ninth remains the smallest and its division becomes more distinct.

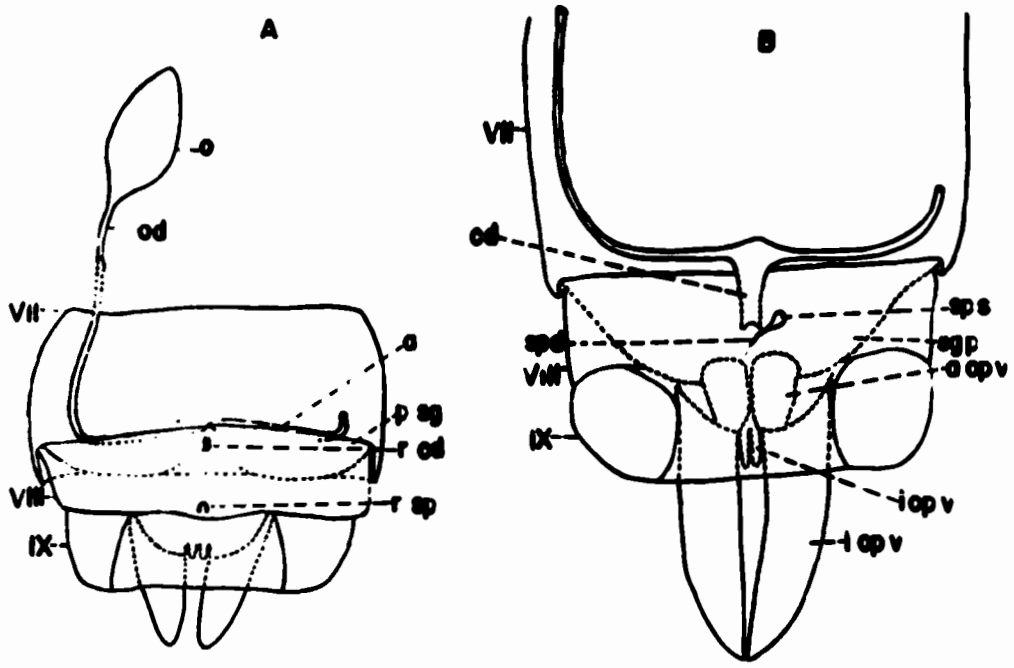


Fig. 5. Developmental stages of subgenital plate, common oviduct and spermatheca of G. veletis. A, inner view of last three sterna of sixth instar; B, same of eighth instar. a, ampulla of oviduct; aopv, anterior ovipositor valve; cd, common oviduct; iopv, inner ovipositor valve; lopv, lateral ovipositor valve; o, ovary; od, oviduct; psg, pouch of subgenital plate; rcd, rudiment of common oviduct; rsp, rudiment of spermatheca; sgp, subgenital plate; spd, spermathecal duct; sps, spermathecal sac; VII, VIII, IX, number of sterna.

In the fourth instar (Fig. 4B) the postero-lateral margins of the eighth sternum become membranous, while the ninth sternum becomes divided into three distinct parts: one median and two lateral, the latter bearing the ovipositor buds. In the next instar (Fig. 4C) the seventh sternum becomes the largest. From the intersegmental membrane between the seventh and eighth sterna a pair of lateral pouches is formed. The eighth sternum becomes further membranized in the antero-median and postero-lateral regions. The ninth sternum remains divided into three regions but the median region now becomes more or less membranous.

In the sixth instar (Fig. 5A) the seventh sternum remains the largest. The intersegmental pouches grow further and their mesial ends meet. The ventral walls of the pouches become sclerotized while their dorsal walls remain membranous. In this manner the intersegmental membrane which gives rise to the pouches becomes double-walled having its ventral wall sclerotized. The eighth sternum becomes further membranized leaving only the antero-lateral and a small postero-median region sclerotized. In the postero-median region is formed the invagination of the spermatheca. There is no change in the ninth sternum.

In the seventh instar the two pouches fuse forming a single large pocket, the entire ventral wall of the pocket becomes sclerotized like the seventh sternum, while the dorsal wall remains membranous and covered completely by the eighth sternum. The pocket forms the subgenital plate. The eighth sternum becomes

almost completely membranous except the small postero-mesial and antero-lateral parts. The ninth sternum also becomes membranous except the lateral regions. In the subsequent instars (Fig. 5B) the subgenital plate becomes adult-like. From the three sclerotized areas of the eighth sternum the median area forms the spermathecal spout, while the lateral areas form the first pair of valvifers. The lateral regions of the ninth sternum become divided into two parts, the anterior and posterior. The anterior part fuses with the first valvifer which thus becomes a composite structure, while the posterior part forms the second valvifer. At the same time the two intervalvulae are also formed in the ninth sternum. The ninth tergum remains connected with that piece of the ninth sternum which is fused with the first valvifer.

Development of the genital ducts: In the first instar the ovaries are very small and globular lying in the fourth segment. The oviducts extend up to the posterior margin of the seventh sternum where each terminates in a small hollow ampulla, the ampullae of the two sides lying very close to each other. In the next four instars these structures grow further. In the sixth instar (Fig. 5A) drastic changes take place. A mesial thickening is formed on the ventral surface of the dorsal wall of the subgenital plate, which is the rudiment of the common oviduct. An invagination develops at the base of the anterior ovipositor valves in the eighth sternum forming the rudiment of the spermatheca. In the eighth instar (Fig. 5B) the rudiment of the

common oviduct grows anteriorly and fuses with the ampullae of the oviducts and in the subsequent instars it develops the characteristic shape. The spermathecal rudiment, in the seventh instar, grows and forms a short duct with a small sac at the anterior end and in the next instar the duct becomes a little longer. In the ninth instar the sac assumes the adult form but the duct still remains short and less convoluted.

Discussion of the female reproductive organs: Qadri (1940) and Gupta (1948) have given detailed accounts of the development of the reproductive organs of certain species of cricket , but their accounts appear to be inaccurate in places. This inaccuracy has arisen, firstly, because probably neither author conducted adequate breeding experiments with the result that there was confusion regarding the precise nymphal instar in which a particular structure developed, and, secondly, because the authors mentioned studied only certain nymphal instars and not others, the instars studied by the two authors not necessarily being the same.

The rudiments of the ovipositor valves, one pair at the posterior margin of the eighth and the other pair at the posterior margin of the ninth sterna, arise at the same time in the third instar of G. pennsylvanicus and G. veletis. However, Qadri and Gupta (ll.cc.) describe their origin in the second and first instar respectively for the species studied by them. Gupta maintains that each rudiment of the anterior ovipositor valves divides into two and that the inner division becomes absorbed shortly after-

wards. In G. pennsylvanicus and G. veletis these rudiments do not divide. Each third and fourth nymph took three to four days to reach the fourth and fifth instar respectively, during which period nymphs were examined daily in order to observe any division of the rudiments. The rudiments of the anterior ovipositor valves did not divide at any stage, however, and it is inconceivable that any divided condition of the rudiments could be so short lived - less than 24 hours. It also seems improbable that Gupta could have secured such a stage without rearing experiments. Admittedly Gupta was using crickets of a genus other than Gryllus, but this kind of division has not been observed in any other insect and he was surely wrong in his opinion that other workers like Nel (1930), Metcalf (1932), D'Rozario (1942) and myself (Rakshpal, 1945) had missed this stage. Subsequent development of the ovipositor valves in G. pennsylvanicus and G. veletis, however, is similar to that described by Qadri and Gupta for Acheta domesticus, Gymnogryllus erythrocephalus and Gryllodes sigillatus.

The subgenital plate develops as a pair of pouches in the intersegmental membrane between the seventh and eighth sterna as described by Qadri. This author has described their origin in the second instar of the species studied by him (A. domesticus, G. erythrocephalus), but this requires confirmation, since, in G. pennsylvanicus and G. veletis they arise in the fifth instar. Becker (1932) regards the formation of the subgenital plate in insects as being due to the fusion of the appendages of the

seventh segment, but he probably confused the paired pouches of the intersegmental membrane with the appendages of the seventh segment. Gupta, on the other hand, believes that the subgenital plate in Gryllodes is formed from the posterior part of the seventh sternum, but it would seem probable that he missed the instars in which the paired origin of the subgenital plate from the intersegmental membrane can be distinguished.

The common oviduct is formed in the sixth instar in G. pennsylvanicus and G. veletis, although Qadri and Gupta regard its formation as occuring in the third and second instar respectively in the species studied by them. It develops as a thickening on the ventral surface of the dorsal wall of the subgenital plate as shown by Qadri, whereas Gupta (presumably in error) describes its formation on the dorsal wall of the intersegmental membrane. In G. pennsylvanicus and G. veletis the rudiment of the spermatheca appears in the sixth instar. Qadri and Gupta, however, mention its origin in the third instar in the species studied by them. This structure develops on the eighth sternum as described by Gupta, and not on the ninth as assumed by Qadri. Gupta mentions the formation of the rudiment of the accessory gland in the ninth sternum in the second instar and its absorption subsequently. However, my observations show that no such rudiment arises either in the second or in the subsequent instars in G. pennsylvanicus and G. veletis.

## 4. Male reproductive organs

The male reproductive organs consist of a pair of testes lying in the third to sixth abdominal segment and meeting in the mid-dorsal line. Each testis has a large number of follicles which overlie one another and are enveloped in a delicate peritoneal membrane. The follicles open in the intra-testicular part of the vas deferens through fine vasa efferentia. After emerging from the testis the vas deferens runs posteriorly to the ninth segment where it loops below the cercal nerve and then turns forward to enter the mass of the accessory gland tubules. Here it becomes thickened to form the compactly coiled epididymus and then runs a little posteriorly to open dorsally into the ejaculatory duct near its anterior end. The ejaculatory duct is a wide tube opening into the genital cavity. Into the extreme posterior end of the duct opens a pair of pear-shaped lateral vesicles. At the mouth of the ejaculatory duct are present two lateral lips which become extended when the spermatophore is present in the genital cavity. There is a median ventral fold which remains upturned, covering the lateral lips and gonopore and occupying the cavity of the spermatophore chamber. The accessory glands consist of a mass of tubules arising from the lobe formed by the fusion of the posterior ends of the vasa deferentia. The dorsal tubules of the gland are somewhat smaller than the lateral and ventral ones.

The genitalia (Fig. 6) consist of a broad, sclerotized, recurved, median dorsal prong - the pseudosternum of Walker (1922), pseudoepiphalle of Chopard (1920), or ancre of Snodgrass (1937) - and a pair of bifid prongs (ectoparameres of Walker). From the median dorsal prong extends a long arm (ramus of Walker) anteriorly on each side. On the ventral side of the median prong is present a slender, pointed spermatophore guide extending into a large thin-walled pouch, the spermatophore chamber; a U-shaped structure (endoparamere of Walker) lies on the dorsal surface of the guide. The subgenital plate is formed by the trough-like ninth sternum.

Development of the genitalia and subgenital plate: In the first and second instars (Fig. 7A) the posterior margin of the ninth sternum is slightly convex, but, in the next instar, the margin becomes more or less flat. In the fourth instar the narrow posterior part of the ninth sternum turns inwards and becomes membranous, while the posterior margin of the sclerotized part develops a median depression. In the next instar (Fig. 7B) the margin of the sternum becomes slightly more depressed and the inturned part grows further, thus forming a pocket, the ventral wall of which consists of the sclerotized sternum, while the dorsal wall is formed by the inturned part. Due to further growth of the inturned part, the intersegmental membrane behind the inturned part becomes folded. In the sixth instar (Fig. 7C) the membrane develops a pair of sclerotized penis lobes in its dorsal part, and in its ventral part a median

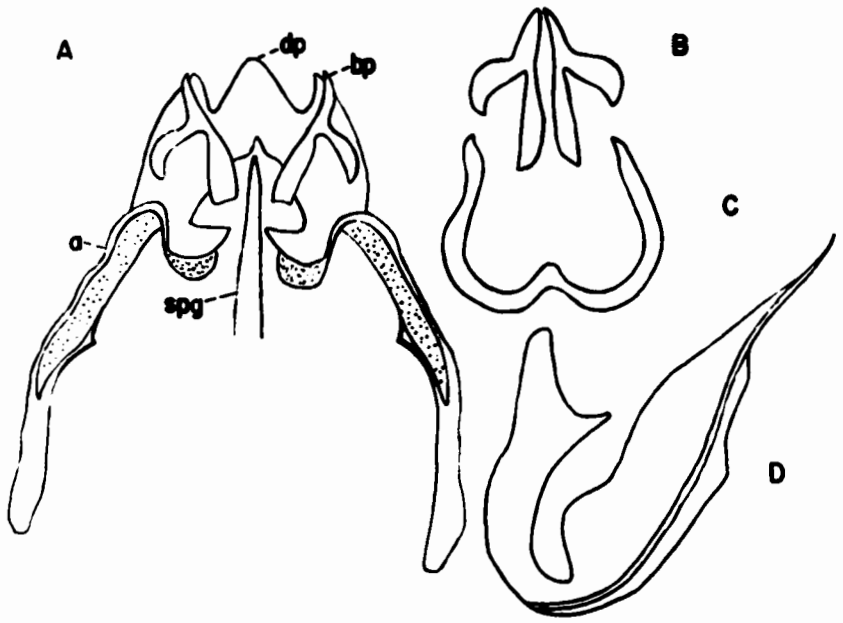


Fig. 6. Male genitalia of G. veletis. A, ventral view of dorsal and bifid prongs; B, ventral part of bifid prongs; C, U-shaped sclerite; D, spermatophore guide; a, arm of dorsal prong; bp, bifid prong; dp, dorsal prong; spg, dorsal part of spermatophore guide.

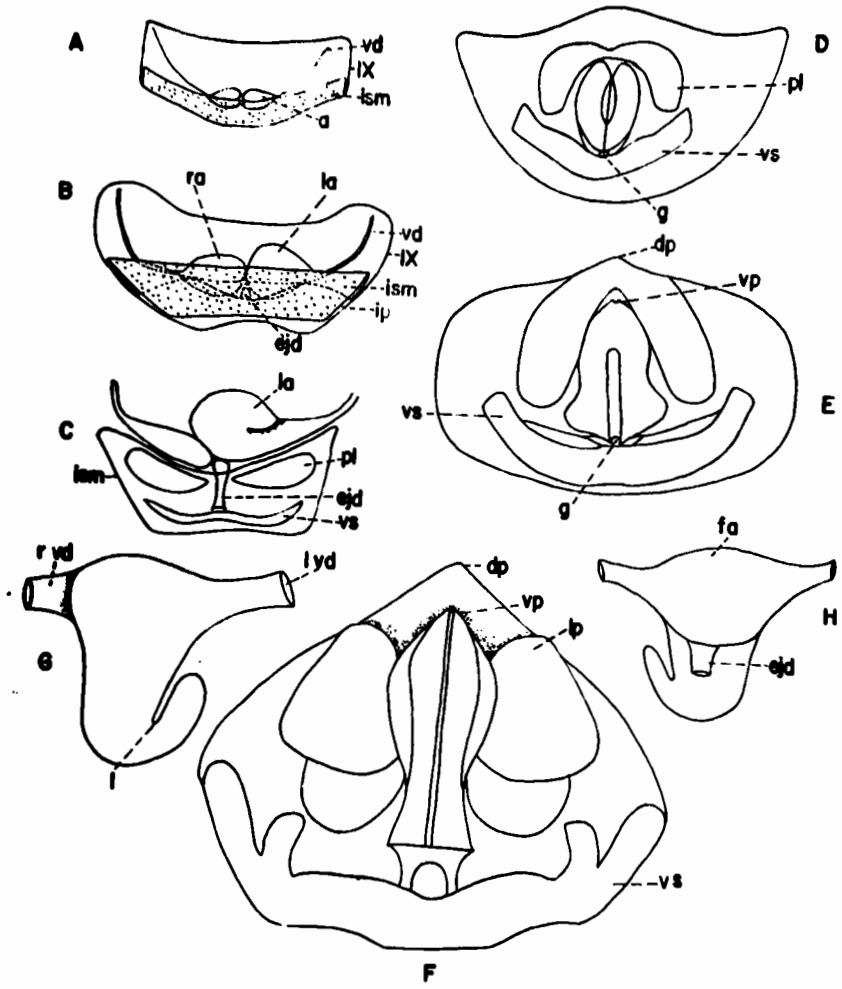


Fig. 7. Developmental stages of male genitalia and genital ducts of G. veletis. A, inner view of ninth sternum of second instar; B, same of fifth instar; C, postero-ventral view of genitalia and ventral view of ampullae of vasa deferentia of sixth instar; D, E, F, postero-ventral view of genitalia of seventh, eighth and ninth instar; G, dorsal view of ampullae of vasa deferentia; H, ventral view of same of ninth instar. a, ampulla of vas deferens; dp, dorsal prong; ejd, ejaculatory duct; fa, fused ampullae of vasa deferentia; g, gonopore; ip, inturned part of ninth sternum; ism, intersegmental membrane; l, loop of ampulla of left vas deferens; la, left ampulla; lp, lateral projection; lvd, left vas deferens; pl, penis lobe; ra, right ampulla; rvd, right vas deferens; vd, vas deferens; vp, ventral prong; vs, ventral sclerotization; IX, ninth sternum.

sclerotization extending on both sides. Between the dorsal and ventral sclerotizations in the membrane a median depression appears, in the ventral part of which lies the gonopore. The inturned part of the ninth sternum remains more or less membranous.

In the seventh instar (Fig. 7D) the inturned part, due to its growth covers the posterior half of the sclerotized ninth sternum. The anterior margin of the inturned part is depressed in the middle, while the corners, which are more or less rounded, remain projecting anteriorly. The space between the sternum and the inturned part becomes narrow, and the inturned part itself remains almost completely covered by the intersegmental membrane. The intersegmental membrane, due to its folding, forms a shallow pouch. The penis lobes fuse in the middle line, while the ventral sclerotization grows laterally.

In the eighth instar (Fig. 7E) the inturned part covers almost three quarters of the sternum from the posterior margin, and the posterior margin of the inturned part becomes sclerotized. The anterior median part of the two fused penis lobes evaginates dorsally forming the dorsal median prong. Similarly the area between the two penis lobes, along with their mesial margins, grows above the general surface forming a short ventral prong, the double nature of which remains distinct due to the slight bifid condition of the tip.

In the ninth instar (Fig. 7F) the dorsal prong grows and becomes a distinct pointed structure. On each side of the ventral

prong arises a projection whose tip is flat, and, as the prong grows, the projection remains at a lower level. Two or three days before the final moult the ventral prong gives rise to the spermatophore guide, while the lateral projections form the bifid lateral prongs, and the dorsal prong assumes the shape of the adult structure. All these structures are distinctly visible inside the nymphal structures. After the formation of the spermatophore guide, the membrane between the guide and the gonopore becomes depressed, forming the spermatophore chamber; and, due to the depression, the base of the guide comes to lie in the dorsal wall of the chamber. At the same time two lips are formed one on either side of the gonopore, and these normally remain contracted, becoming extended only when the spermatophore is present in the genital cavity. The ventral fold is formed by the ventral sclerotization which ~~becomes~~ turns dorsad, covering the gonopore and the lateral lips, and coming to occupy the cavity of the spermatophore chamber. The ventral fold when inside the spermatophore chamber assumes the form of a hollow, ribbed cylinder having six ridges alternating with six furrows, and probably forms a mould for the spermatophore plate. When the spermatophore is completely formed the ventral fold is extruded and forms a ventral support for the spermatophore, the lateral lips being released and thus extended at the same time, forming lateral supports.

Development of the genital ducts: In the first instar the small testes lie in the third abdominal segment, while the vasa

deferentia extend to the posterior margin of the ninth sternum where they terminate in two hollow ampullae of equal size. In the fourth instar the left ampulla becomes a slightly larger than the right one, while in the next instar (Fig. 7B) it becomes quite distinctly larger. In the sixth instar (Fig. 7C) the left ampulla becomes looped and thus appears to consist of two parts, while in the seventh instar the ampulla of the right side fuses with the overgrown part of the left ampulla, so that, after fusion, a large lobe is formed. The dorsal part of this lobe grows posteriorly more towards the left side than towards the right, and thus the whole structure becomes asymmetrical. In the eighth instar the extended part loops ventrally towards the left side, and in the ninth instar (Fig. 7G and 7H) a distinct loop is formed opening to the left. The anterior part of the entire structure remains globular, and in this part the ejaculatory duct opens ventrally. Thus the posterior overgrown part of the vasa deferentia conceals the ejaculatory duct. The accessory glands develop from the posterior fused part of the vasa deferentia and are therefore mesodermal in origin.

The common ejaculatory duct is formed in the third instar in the intersegmental membrane behind the ninth sternum; later on it extends anteriorly and fuses with the ampullae of the vasa deferentia.

Discussion of the male reproductive organs: Snodgrass (1937) and Qadri (1940) have described the development of the male

reproductive organs in Gryllus "assimilis"[1] and Acheta domesticus respectively. In G. pennsylvanicus and G. veletis the penis lobes arise in the fifth instar in the form of a pair of buds as indicated by Snodgrass. Qadri, however, mentions their presence in the first instar of A. domesticus and he further states that the ampullae of the vasa deferentia rest on the buds. In fact the ampullae of G. pennsylvanicus and G. veletis lie on a thick layer of fat body which has the appearance of two small buds and it seems probable that Qadri mistook them for the penis lobes. From his diagram of a cross-section passing through the ampullae of vasa deferentia, and showing the histology of the nymph, it is quite evident that the structure beneath the ampullae is the fat body. Qadri maintains that each penis lobe of A. domesticus divides into two. In G. pennsylvanicus and G. veletis it never divides, however, and it would seem that he has probably confused the ventral sclerotization with the ventral buds. He gives no diagram in support of his view. Snodgrass considers that the ventral sclerotization in Gryllus "assimilis"[1] arises as a pair of buds which fuse shortly afterward. However, in G. pennsylvanicus and G. veletis this structure is a single median structure from the very beginning. As in other insects the entire genitalia are formed by the penis lobes and the ventral sclerotization does not take any part in their formation. Therefore the latter cannot be part of the penis lobes. The spermatophore guide is comparable

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1. See footnote on page 29.

with the aedeagus of other insects, since it is formed by the fusion of the mesial parts of the penis lobes and also performes a similar function by directing the duct of the spermatophore to the opening of the spermatheca, thus transfering the spermatozoa to the female. The bifid lateral prongs are comparable with the parameres of other insects since they are formed by the outer parts of the penis lobes and probably function in ~~the~~ opening the genital cavity. The ejaculatory duct arises in the third instar of G. pennsylvanicus and G. veletis; Qadri, however, describes its presence in the first instar of A. domesticus.

## 5. General discussion

There are two views regarding the origin of the genitalia in insects (for literature see Matsuda, 1958). One view is that the embryonic abdominal appendages persist in the post-embryonic stages and give rise to the ovipositor of the female and the phallic organs of the male (Wheeler, 1893; Else, 1934; Roonwal, 1937 - on the basis of the embryological studies - and Verhoeff, 1896; 1902; Börner, 1921; and others, including Scudder, 1957 - on the basis of comparative morphology). The other view is that these structures are absolutely new arising in the post-embryonic stages (Heymons, 1896; 1897; 1899; Zander, 1900; 1901; 1903; and others). I have already shown that the rudiments of the ovipositor and the phallic organs of homopterous, hymenopterous, lepidopterous and coleopterous insects arise as new structures in the post-embryonic stages (Rakshpal, 1941; 1943; 1944; 1945; 1946a; 1946b).

Further, my studies of morphogenesis of four American species of Gryllus (See Sections IV and V) show that abdominal appendages arise on either side of each abdominal segment after the completion of anatrepsis, but that all these except those of the first and last segments (namely the pleuropodia and cerci) are resorbed before katatrepsis begins. These observations and the present studies show conclusively that the ovipositor and phallic organs have nothing in common with the embryonic abdominal appendages and that they arise as new structures in the post-embryonic stages as has been recently discussed on the basis of previous literature by Mastuda (1958).

Wheeler's (1893) view, that the appendages of the eighth, ninth and tenth abdominal segments take part in the formation of the ovipositor, and that the appendages of the tenth form the male genitalia of insects, has been supported by Else (1934), Roonwal (1937) and, more recently, by Dupuis (1950) and Gustafson (1950). However, my previous observations (Rakshpal, ll.cc.) and present studies, along with those of other workers, clearly show that the ovipositor is formed by the rudiments arising from the eighth and ninth sterna, and the phallic organs of the male by those arising from the ninth sternum or from the intersegmental membrane posterior to the ninth sternum.

It is interesting to observe that the view, that the ovipositor is formed by the persisting embryonic appendages of the eighth, ninth and tenth abdominal segments and that the male

genitalia are formed by the persisting appendages of the tenth abdominal segment, is based on the studies of the embryonic and post-embryonic development of Xiphidium [Conocephalus] (Tettigonioidea), Melanoplus and Locusta (Acrididae) and that no even evidence has been found to support the view in other groups of Orthoptera (namely Gryllidae). There appears, therefore, to be reason to doubt the accuracy of the observations on these insects and a re-investigation of their morphogenesis would seem to be called for.

# IV. MORPHOGENESIS AND EMBRYONIC MEMBRANES OF *GRYLLUS ASSIMILIS* (FABRICIUS)

# IV. MORPHOGENESIS AND EMBRYONIC MEMBRANES OF GRYLLUS ASSIMILIS (FABRICIUS)

## 1. Introduction

In order to compare the embryonic development in G. pennsylvanicus and G. veletis, it was considered that a proper prior knowledge of the morphogenesis of the genus should be obtained. For this reason a preliminary investigation of the development of the eggs of a non-diapause species from Jamaica, Gryllus assimilis (Fabricius), sensu stricto, was undertaken. This species was selected because it reproduces readily in the laboratory and is known to have an uninterrupted development. It was considered that the study might help to explain differences in the respiratory metabolism and nature of metabolites in the two kinds of eggs (diapause and non-diapause) found in G. pennsylvanicus and G. veletis. It was also thought that a study of embryonic membranes, particularly of the serosal cuticle, might possibly help to explain the resistance of eggs of different ages to cold and drought.

The eggs of G. assimilis and other field crickets (unpublished observations) are particularly well suited for studying the morphological development of the embryo without killing or injuring the eggs. The chorion is thin and transparent and therefore dechorionation is not necessary. Neither does the serosal cuticle obscure the embryo and for the greater part of its life the embryo lies on the surface of the yolk. The eggs can develop without any injury even when they are submerged in water. The

entire development of the embryo may be divided into ten main stages to facilitate the comparison of this with other species of Gryllus.

In the Orthoptera the development of the embryo has been studied mostly in locusts and grasshoppers, for example, by Slifer (1932a, 1932b, 1937) in Melanoplus differentialis (Thomas), Roonwal (1937) and Shulov and Pener (1959) in Locusta migratoria migratorioides (Reiche and Fairmaire), Steel (1941) in Austroicetes cruciata (Saussure), Bodenheimer and Shulov (1951) in Dociostaurus maroccanus (Thunberg), and by Matthée (1951) in Locustana pardalina (Walker). Amongst crickets only Gryllulus [Acheta] commodus (Walker)[1], a diapause species, has been studied in any detail (Brookes, 1952), although various earlier workers, notably Heymons (1895), have referred to the embryology of Gryllidae, including the genus Acheta [Gryllus domesticus auctt.].

## 2. Materials and methods

Ten females and four males were kept in a large candy jar with a dish containing 30 c.c. of sand moistened with 10 c.c. of water. The females began laying eggs almost immediately after the sand-dish was placed in the jar. Within half an hour quite a large number of eggs had been laid, when the dish was taken out and the eggs with sand were kept on a piece of paper towel in a small jam jar and incubated at room temperature (23-26°C.). Whenever

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[1] Randell (in preparation) indicates that commodus belongs to a genus different from both Acheta and Gryllus.

required the eggs were collected with a camel-hair brush dipped in water, and fixed within a few minutes. The eggs for fixing were collected every morning so that the exact age of the eggs could be known. The eggs were fixed in hot Bouin's picro-formol and left in the fixative for 18 to 24 hours. After washing with water, 30, 50 and 70 per cent alcohol the eggs were preserved in 70 per cent alcohol for future use. Whole eggs were stained in bulk in Borax carmine and destained in acid alcohol. It was necessary to puncture the chorion to allow penetration of the stain. The eggs were studied in 70 per cent alcohol under a stereoscopic binocular microscope. To study the early stages of the embryo it was found to be of great advantage for the light to fall on the eggs from the side. The diagrams were made using a camera lucida.

Each sample contained eggs in various stages of development, particularly in the late stages. From each sample the stage selected was shown by the majority of the eggs, and this stage was taken as representative of that age of the egg. Most of the eggs hatched at room temperature (23-26°C.) on the seventeenth day. In all, twenty-two samples were fixed in three series and it is interesting to note that each sample of eggs of a particular age showed the same 'representative' stage of development. Twenty-five eggs from each sample were examined.

## 3. Description of egg and embryonic stages

The freshly laid egg (Fig. 8A) is about 2.5 mm. long, tapering at both ends, with the anterior pole slightly pointed and the posterior pole blunt. The dorsal surface is convex and the ventral surface a little concave. The chorion is stiff and transparent and shows no sign of a 'cap' (see below). The yolk is finely granular during the first three days of incubation, after which its particles become grouped into larger polyhedral masses.

The entire morphological dvelopment of the embryo may be divided into ten main stages which are distinct even when the embryo is alive and inside the chorion. In the fixed embryos, after their removal from the chorion, many other features become distinct, and the main stages may be subdivided.

Stage I (Figs. 8 and 9 ):- About 36 hours after the egg is laid the embryo appears as a minute speck of cells at the posterior pole. Although the embryo is not visible when inside the chorion, a small space containing some fluid at the posterior pole suggests the presence of the embryo. Within the next twelve hours (Figs. 8 and 9 Ib, Ia) the embryo occupies the entire posterior end of the egg, becomes pear-shaped, shows no signs of differentiation and is about 0.3 mm. long.

Stage II (Figs. 8 and 9):- During the next twelve hours the embryo comes to lie on the dorsal surface of the yolk, extending up to about one-third of the length of the egg from the posterior pole. It becomes elongated and develops a constriction which divides it into a protocephalic and a protocormic regions.

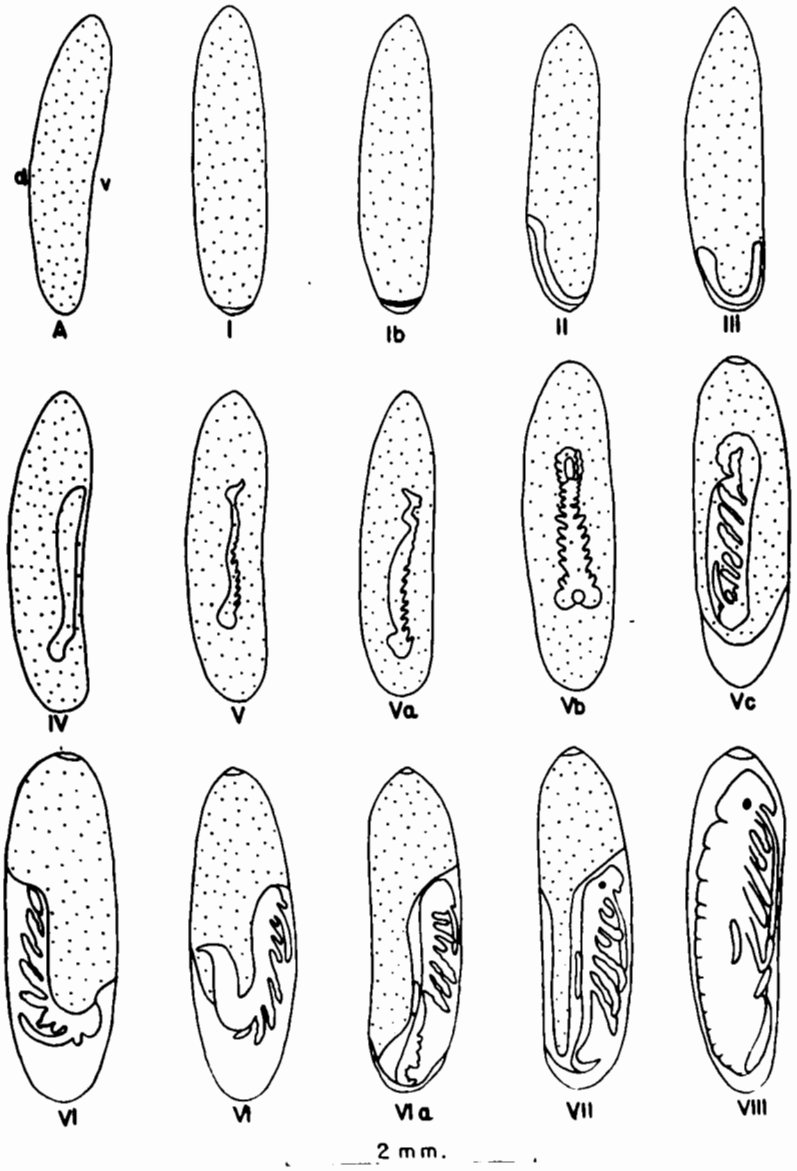


Fig. 8. Developmental stages of G. assimilis, showing the position of the embryo inside the egg after different periods of incubation. d, dorsal side of egg; v, ventral side of egg.

The protocephalic region, with its flat margin, is shorter but broader than the protocormic region, and the caudal end of the protocormic region is rounded.

Stage III (Figs. 8 and 9):- On the fourth day of incubation the embryo becomes bent in a U-shape and lies at the posterior pole. It elongates further, the protocephalic lobes becoming distinct through a lateral expansion of the anterior part of the protocephalic region and due to the formation of an anterior median depression.

Stage IV (Figs. 8 and 9 ):- Within the next twelve hours the embryo occupies the middle region of the concave ventral surface of the egg, and by this time has completed about one-fifth of its development; it becomes about half as long as the egg. The protocephalic lobes become thickened and between them, at their extreme end, the labrum develops as a small globular swelling leaving the stomodeaum exposed. Primary segmentation begins, proceeding gradually from the anterior to the posterior end and demarcating successively the antennal, mandibular, two maxillary, three thoracic and eleven abdominal segments. After completing primary segmentation the embryo sinks into the yolk.

Stage V (Fig. 8):- During the next period of five to six days of incubation the embryo remains immersed in the yolk and therefore not visible. An egg of this stage, however, is distinguishable from the eggs of early stages due to the presence of polyhedral masses of yolk, and a distinct cap. On the fifth day of incubation

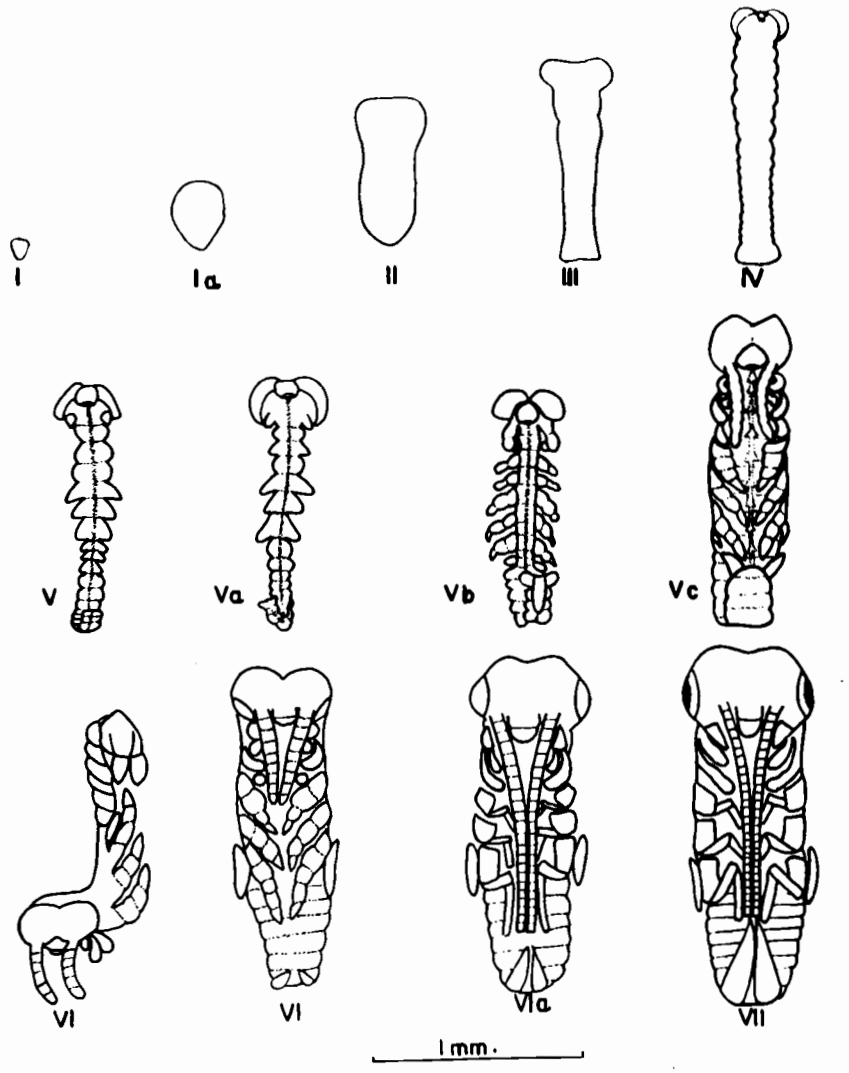


Fig. 9. Development of the body form of the embryo of G. assimilis during the first ten days of incubation.

the posterior abdominal segments of the embryo become convoluted and ventrally flexed (Fig. 9, Va). The embryo appears to become shorter due to flexion but it begins to widen as secondary segmentation begins. The rudiments of the antennae and three pairs of gnathal, three pairs of thoracic and three pairs of abdominal appendages appear. Within the next twelve hours indications of the other abdominal appendages also appear. On the sixth day of incubation the labrum moves downward and conceals the stomodæum (Fig. 9, Vb). Segmentation appears in the gnathal and thoracic appendages. The appendages of the first (pleuropodial) and the last (cercal) abdominal segments become distinctly marked off from other appendages, assuming the shape of small buds. Each pleuropodium arises as a lateral evagination from the pleural membrane. On the seventh day of incubation, and before the embryo prepares for revolution (see below), all abdominal appendages except the pleuropodia and cerci are resorbed (Fig. 9, Vc). The pleuropodia become reniform in outline with a narrow projection by means of which they remain connected to the pleural membrane. The cerci become conical and lie close to the ventral wall of the body. The antennae become elongated, extending up to the posterior margin of the prothorax. The gnathal and thoracic appendages become further segmented and the latter become longer than the former.

Stage VI (Figs. 8 and 9):- The eighth day of incubation sees the beginning of revolution which is described more fully below.

The embryo becomes bent into the form of a J or a U and its appendages project into the clear space at the posterior end of the egg. During revolution only a few developmental changes appear. On the ninth day of incubation the embryo becomes thickened and the free margins of the body wall begin to fuse in the mid-dorsal line, beginning at the posterior end (Figs.8 and 9, VIa). The appendages become further elongated but do not assume their characteristic shape. The labrum shows a transverse groove in the middle line. The pleuropodia become further elongated and the cerci, though small, become distinctly conical. The conclusion of this stage marks the end of blastokinesis.

Stage VII (Figs. 8 and 9):- On the tenth day of incubation the compound eyes, due to the development of pigmentation, appear as two orange-brown specks at the lateral margin of the head and are visible near the middle of the egg. The antennae become very long and extend as far as the sixth abdominal segment, when the appendages begin to assume their characteristic shape. The cerci become further elongated and lie along the ventral surface of the body. By growth the antennae and cerci become more elongated, and the tips of the former begin to touch those of the latter.

Stage VIII (Figs 8 and 9):- On the eleventh day of incubation the embryo becomes greatly elongated, occupying almost the whole length of the egg, and therefore the orange coloured compound eyes are visible near the anterior end of the egg. The head becomes more or less conical. The antennae and cerci elongate further.

The distal parts of the antennae lie under the metathoracic legs (when viewed from the ventral side) and their tips on the outer side of the cerci.

Stage IX (Fig. 10):- On the thirteenth day of incubation, a pair of black lines marking the thickening in the embryonic cuticle at the lateral margins of the labrum, is visible; the posterior half of these lines is serrated. A cuticle is secreted by the embryo over the entire body except the pleuropodia. The head becomes more conical, the eyes very distinct with their margins clearly defined, and the embryo attains its full length.

Stage X (Fig. 10):- On the sixteenth day of incubation the body and appendages become covered with small, regularly arranged bristles. Pigmentation increases over the entire body and the cerci and antennae become blackish in colour. On the seventeenth day, i.e., just before hatching, the head and face become brown, and the eyes, antennae and cerci black in colour.

The developmental stages of the embryo of G. assimilis may be summarized as follows:-

Stage I ($1\frac{1}{2}$-2 days): Embryo not visible; yolk finely granular.

Stage II ($2\frac{1}{2}$ days): Embryo small, straight, visible on dorsal side of egg at about one-third of distance from the posterior pole; yolk finely granular.

Stage III ($3\frac{1}{2}$ days): Embryo longer, U or J-shaped at the posterior pole; yolk grouped in polyhedral masses.

Stage IV (4 days): Embryo on the ventral side of egg, occupying the middle region.

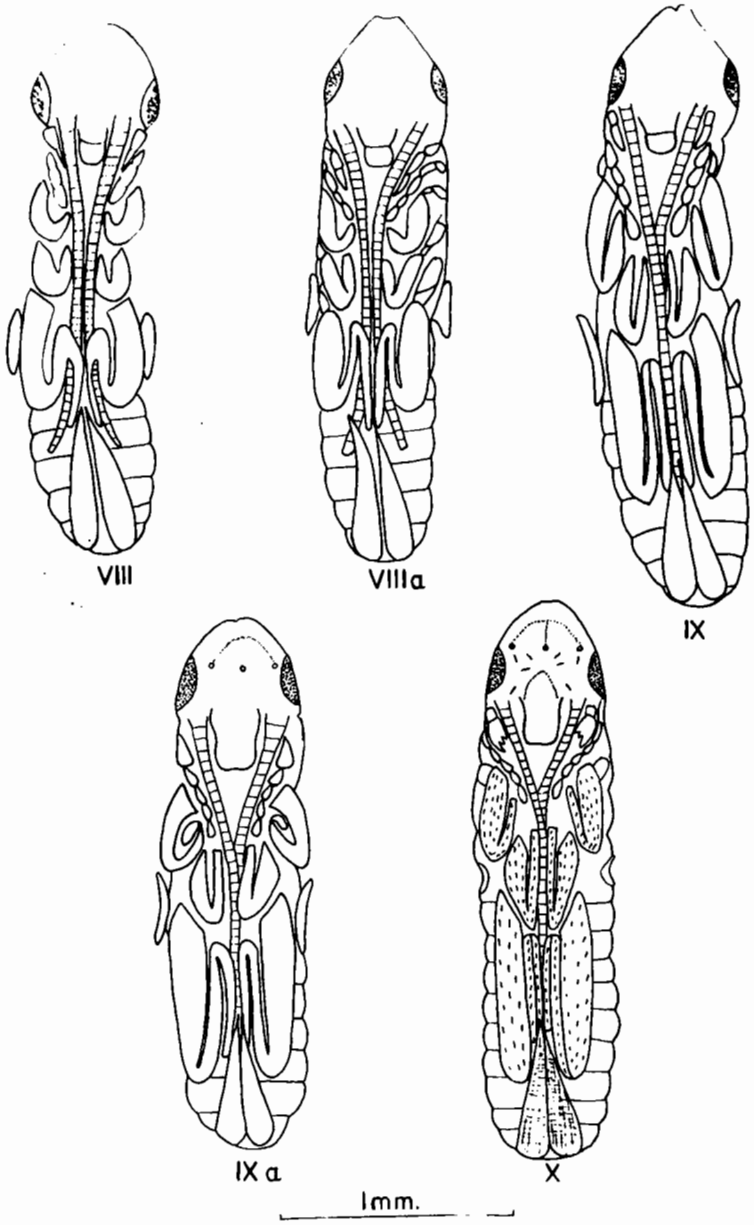


Fig. 10. Development of body form of the embryo of G. assimilis from the eleventh to sixteenth day of incubation.

Stage V (5-7 days): Embryo not visible, yolk grouped in polyhedral masses.

Stage VI (8-9 days): Embryo bent into the form of a J or U and its appendages projecting into the clear space at the posterior end of the egg.

Stage VII (10 days): Embryo with orange-brown coloured compound eyes visible near the middle of the egg.

Stage VIII (11 days): Embryo occupying the whole length of the egg, orange coloured compound eyes visible near the anterior end.

Stage IX (13 days): Embryo showing a pair of black lines in the embryonic cuticle at the lateral margins of the labrum.

Stage X (16 days): Body and appendages of the embryo covered with small bristles.

## 4. Blastokinesis

Blastokinesis was observed in several living eggs kept in Ringer's solution, however, the timings were noted in only five eggs and in these cases the process took more or less the same time. The embryo is of the immersed type, i.e., the yolk penetrates between the amnion and serosa (see below). The embryo, which is first visible after about 36 hours of incubation comes to occupy the whole of the posterior end of the egg by the end of the second day (Fig. 8, Ib). During the next twelve hours, due to its further growth, the embryo comes to lie on the dorsal surface of the yolk

extending to about one-third of the length of the egg from the posterior pole which is then occupied by the caudal end of the embryo (Fig. 8, II). At this stage anatrepsis begins. The embryo first moves its caudal end around the posterior pole of the egg. After about one and a half hours, the yolk at the posterior pole becomes slightly transparent and a small space containing fluid is formed between the pole and the embryo. As the embryo moves its caudal end towards the ventral surface of the yolk the quantity of fluid in this space increases. In approximately five hours, about one-quarter of the embryo comes to lie on the ventral surface of the yolk, and the embryo as a whole is bent in the shape of a J. After about two more hours the caudal arm of the bent embryo is about half the length of the cephalic arm. In the next four hours the embryo becomes U-shaped, the cephalic and caudal arms being equal (Fig. 8, III). In this position the protocephalic part lies on the dorsal surface of the yolk and the caudal part of the protocormic region on the ventral surface. Thus in about eleven hours the caudal half of the embryo comes to lie on the ventral surface of the yolk. During the next ten hours the remainder of the embryo also reaches the ventral surface. After about 21 hours anatrepsis is completed and the caudal end of the embryo points towards the anterior pole of the egg (Fig. 8, IV). The embryo remains visible on the surface of the yolk until the completion of anatrepsis.

Three or four hours after the completion of anatrepsis the embryo sinks into the yolk until only the most posterior part is still on the surface. During the next three hours this caudal part of the embryo also sinks into the yolk. The embryo becomes completely immersed and its movements can no longer be observed. However, by studying the fixed specimens (Fig. 8, V, Va, Vb) it is clear that the embryo moves further forward and in some cases the caudal end reaches a point about one-fifth of the length of the egg from the anterior pole. Further irregular convolutions along the length of the embryo, a spiral twisting, and the orientation of different embryos either towards the dorsal or the lateral surface of the yolk suggest that the embryo continues to move after its immersion in the yolk.

On the seventh day of incubation the embryo straightens out (except for the posterior abdominal segments) and its ventral surface comes to face the dorsal surface of the yolk (Fig. 8, Vc). When this occurs the embryo is usually preparing to undergo katatrepsis. Before katatrepsis begins the anterior part of the embryo comes to lie on the surface of the yolk and the head, labrum and antennae are distinctly visible. The yolk contracts to about one-eighth of the length of the egg from the posterior pole, leaving only a small gradually decreasing quantity of yolk on the top of the head between the cephalic amnion and serosa. A space, containing amniotic fluid, appears in front of the head, and, as the quantity of the fluid increases the amnion in front of the

head forms a bulge, ultimately meeting the serosa (Fig. 11). The two membranes then fuse together to form the amnio-serosa. The embryo remains in this condition for about ten hours, after which the amnio-serosa ruptures.

About half an hour later the protocephalic lobes become curved and face the ventral surface of the egg, the labrum is directed towards the posterior pole, projecting between the two antennae (Fig. 8, VI). The antennae, which were formerly touching the body, become arched into a semi-circle which conceals the labrum. The posterior part of the embryo still remains covered by yolk and is not visible. About one hour later the antennae subtend an angle of about 45 degrees with the head; the mandibles and the two pairs of maxillae begin to face the posterior pole. The labrum becomes perpendicular but continues to point towards the posterior pole. The yolk remains separated from the dorsum of the embryo due to the presence of the ental membrane (see below).

In about three hours after the rupture of the amnio-serosa the head becomes further and further bent towards the ventral surface. Each antenna is now at right-angle to the head and together with the mouth-appendages, points towards the posterior pole. The antennae now lie on the ventral surface of the yolk and a little later, together with the labrum, abut the ventral surface of the egg. About half an hour later the three pairs of legs begin to point towards the posterior pole and the embryo becomes U-shaped with both arms equal and after about another hour has

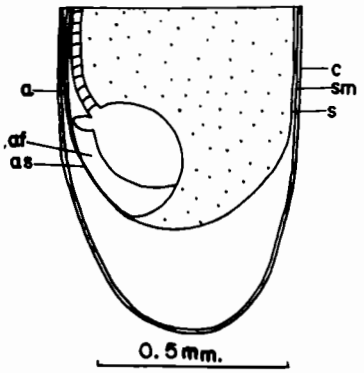


Fig. 11. Posterior part of egg of G. assimilis after seven days of incubation. a, amnion; af, amniotic fluid; as, amnio-serosa; c, chorion; s, serosa; sm, serosal cuticle.

elapsed the embryo becomes J-shaped with its third pair of legs and the pleuropodia still pointing towards the anterior pole. As the head moves further towards the anterior pole the cerci separate from the body, but the posterior part of the body remains bent on itself, the terminal abdominal segments remaining adpressed. After about one hour more the head becomes stationary at a point two-thirds of the length of egg from the posterior pole.

After the head becomes stationary the caudal portion of the embryo begins to extend towards the posterior pole beyond the bent part of the main body, and the cerci begin to point towards the ventral surface of the egg. The bend in the abdomen decreases as the caudal part is extended towards the posterior pole. As the caudal part is thus extended posteriorly its tip becomes slightly bent towards the ventral side of the egg, but the cerci continue to point towards the ventral surface. Now the caudal part instead of extending towards the posterior pole, begins to bend, causing the cerci to point towards the anterior pole, approaching nearer and nearer the metathoracic legs. In this condition the embryo rests for about an hour, after which the bent caudal end extends slightly further towards the posterior pole of the egg, leaving only a small space between the two. Only one bend remains in the abdomen, between the sixth and seventh abdominal segments. The yolk extends only upto this bend, which suggests that the dorsal closure has started at the posterior end of the embryo by the fusion of its two lateral walls in the mid-dorsal line.

The movements so far described during katatrepsis have been designated by Wheeler (1893) as the revolution of the embryo. Slifer (1932) has observed vigorous movements of the embryo in Melanoplus differentialis during revolution. The embryo of G. assimilis, however, does not show any movement either of the body or of the appendages at this time and these observations confirm those of Brookes (1952) on the embryo of "Acheta" commodus. Actual movements in the body wall are seen for the first time after the completion of revolution, i.e., on the eighth day, and before hatching heart beats are distinctly visible. As the embryo elongates due to growth it continues to move its head further forward until this reaches the anterior pole of the egg on the twelfth day of incubation.

5. Development of the embryonic membranes

Serosa:- By the end of the first day of incubation of the eggs the embryonic membranes, the amnion and serosa, are formed. Between the amnion and serosa there is some yolk at the sides, but in the middle the two envelopes are nearly in contact with one another. The serosa lines the chorion and encloses the yolk. It is distinctly visible in stained preparations of the entire egg, consisting of large cells with elongated nuclei. As the growing embryo assimilates the yolk the serosa gradually contracts from the posterior end of the egg. By the time the embryo is ready to revolve the serosa becomes contracted to about one-eighth of the length of the egg. Shortly before the revolution of the embryo

the serosa and amnion become fused in the head region to form the amnio-serosa (Fig. 11). At the time of revolution a rent appears in the amnio-serosa in front of the head region. The margins of the rent, however, remain fused so that the edges of the amnion and serosa adhere to each other both before and behind the cephalic region of the embryo. After the rent appears the serosa opens at the posterior end of the egg. During the course of revolution the serosa continues to contract and when revolution is completed it lies at the top of the head of the embryo (Fig. 12). It then becomes thickened, forms a constriction in the yolk at the back of the head region, and remains adhering to the embryo through the cephalic amnion, ventrally, and the everted amnion, dorsally. As the embryo grows the serosa contracts further, ultimately forming a small cap over the unenclosed yolk at the head end of the embryo. At this stage the cells of the serosa become tightly packed together. Ultimately the serosa is withdrawn into the head.

Amnion:- The amnion originates at the posterior pole of the egg and covers the embryo ventrally. As the embryo grows the amnion also extends with it, always covering the embryo ventrally and remaining continuous with its margins. When the caudal flexure of the embryo develops, the amnion spans the abdominal arch. Hence, when the caudal portion is already flexed under the abdomen, the posterior amniotic cavity within the flexure is bounded dorsally and ventrally by the embryo itself and only laterally by the

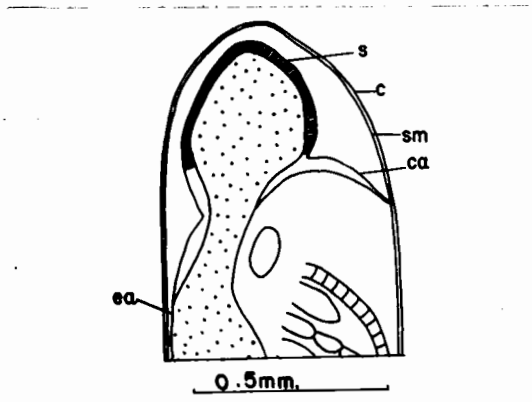


Fig. 12. Anterior part of egg of G. assimilis after ten days of incubation. c, chorion; ca, cephalic amnion; ea, everted amnion; s, serosa; sm, serosal cuticle..

amnion, i.e., the amnion is not folded within the flexure. With the revolution, the amnion turns inside-out and comes to lie on the dorsal surface of the embryo, forming its dorsal wall or 'second provisional dorsal closure' (Roonwal, 1937). After blastokinesis the amnion encloses the yolk dorsally and is gradually replaced by the body wall as the latter grows dorsally.

Serosal cuticle:- After the formation of the serosa the development of the serosal cuticle begins. It is secreted by the serosa, lies beneath the chorion and encloses the entire contents of the egg. The secretion of the cuticle begins during the second day of incubation. Before it is formed it is practically impossible to dechorionate a fixed egg without disrupting the contents. By the end of the second day it forms a complete lining to the chorion, to which it remains adhering. It is very thin, and it may be regarded the incipient serosal cuticle. It appears homogeneous at this stage, as it has not developed the typical pattern. Though still fragile, it is already tough enough to be separated from the chorion by keeping the egg in a dilute solution of sodium hypochlorite for two minutes. This dissolves the chorion but not the cuticle. On the third day of incubation the cuticle thickens and develops its typical pattern. The pattern is distinctly visible, even without removing the chorion, and consists of regularly arranged spots, each of which shows minute ridges and tubercles (Fig. 13). On the fourth day the cuticle becomes sufficiently thick to permit separation from the chorion by the

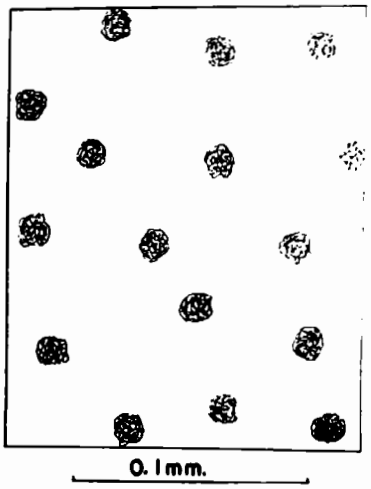


Fig. 13. A portion of serosal cuticle of egg of G. assimilis after four days of incubation.

help of fine needles. It continues to thicken for some time and becomes stiff; as it stiffens the chorion becomes weak and brittle.

Ental membrane (Miller, 1940):- On the fifth day of incubation a very thin membranous flap arises from each side of the trough-like dorsum of the embryo. The flaps can be seen distinctly in dissected embryos viewed laterally, but do not appear continuous throughout the lateral margins. The two flaps fuse in the mid-dorsal line on the sixth day. The membrane so formed is visible only in places and thus does not appear to be continuous. It covers the dorsum of the embryo separating the latter from ~~the~~ contact with the yolk. The membrane forms the 'first provisional dorsal closure' (Roonwal, 1937).

## 6. Discussion

In the order Orthoptera the development of the embryo has been studied mostly in Acrididae, and in the Gryllidae only in a diapause species, "Acheta" commodus, has any recent work been published. Apart from different rates of development, the appearance of different structures at different times, and differences in certain structural details between locusts and grasshoppers on the one hand and crickets on the other, development is more or less similar in the two groups. The development of the serosal cuticle was first observed by Slifer (1937) in Melanoplus differentialis and later by Matthée (1951) in Locustana pardalina, but its time of origin is different in the two genera. Recently Brookes (1952) has also observed the presence of the serosal cuticle in "A." commodus, but she has not given the exact time of

its origin. In G. assimilis it originates on the second day of incubation. Recently Slifer (1956) has used the name 'first embryonic cuticle' for the serosal cuticle, but I prefer the use of the latter term for the following reasons:- firstly, the cuticle is actually secreted by the serosa to cover the yolk and not by the embryo proper; and secondly, its structure is not exactly similar to that of the cuticle subsequently secreted by the embryo itself and which covers its body.

Blastokinesis has been studied in the living eggs of Melanoplus differentialis by Slifer (1932) and revolution has been briefly described in "Acheta" commodus by Brookes (1952). In G. assimilis the movement of the posterior part of the abdomen is the most interesting feature of katatrepsis, since it enables the embryo to occupy the entire length of the egg and thus to grow its maximum size. It is possible that this important movement was overlooked by Brookes (l.c.). She also states that in "A." commodus the embryo first appears on the dorsal surface of the yolk. In G. assimilis, however, the embryo is visible for the first time at the posterior pole of the egg and only later on does come to lie on the dorsal surface of the yolk, as in "A." commodus.

The pleuropodia appear to be of great interest. Slifer (1938) has made cytological studies of these structures in Melanoplus and is of opinion that they produce a hatching enzyme. The pleuropodia of G. assimilis progressively increase in size until the end of the twelfth day when the formation of the embryonic

cuticle is completed; then they begin to atrophy and become very much reduced before hatching. Brookes (1952) has not noted the progressive reduction of the pleuropodia in "Acheta" commodus after the formation of the embryonic cuticle. In G. assimilis the pleuropodia remain uncovered by the embryonic cuticle. This also has not been noted by Brookes in "A." commodus. After the formation of the embryonic cuticle the growth of the embryo virtually stops and at the same time the pleuropodia also begin to atrophy. This observation suggests that the pleuropodia have something to do with the growth of the embryo.

# V. MORPHOLOGICAL DEVELOPMENT OF THE EMBRYO IN DIAPAUSE AND POST-DIAPAUSE EGGS OF *GRYLLUS PENNSYLVANICUS* AND A COMPARISON WITH NON-DIAPAUSE SPECIES OF THE GENUS *GRYLLUS*

# V. MORPHOLOGICAL DEVELOPMENT OF THE EMBRYO IN DIAPAUSE AND POST-DIAPAUSE EGGS OF GRYLLUS PENNSYLVANICUS AND A COMPARISON WITH NON-DIAPAUSE SPECIES OF THE GENUS GRYLLUS.

## 1. Introduction

Gryllus pennsylvanicus is a species in which the development of the embryo is interrupted by a diapause whereas G. veletis is not, and the main objects of the present part of this thesis were to compare the rate of development of the embryos in diapause eggs of the former species with that of eggs of non-diapause species, especially G. veletis. At what stage of morphological development do the embryos enter diapause, what period is required to complete that stage, and what is the extent of development of the embryos during the (completion of diapause) cold treatment? The morphological development of the embryos of two related species, G. assimilis (Fabricius) and G. rubens Scudder, were also compared with those of G. veletis and G. pennsylvanicus in order to discover any morphological characters of the eggs and embryos which might help to distinguish the different species at different stages of their development.

## 2. Materials and methods

The eggs of laboratory stocks of G. rubens and G. veletis reared continuously at room temperature (23-26°C.) were obtained, fixed and studied in the manner described previously (page 55) for G. assimilis and, in addition, eggs of G. pennsylvanicus, also kept at 23-26°C. and not subjected to cold treatment (see Section VI), were fixed daily until the fortieth day of incubation.

In order to study the morphological development in post-diapause embryos, eggs of G. pennsylvanicus were incubated first at 23-26°C. for five days (when they entered diapause), then exposed to 6-7°C. for a minimum period of three months, during which they completed diapause, finally again incubated at 23-26°C. (see Section VI). After the cold treatment, during the final incubation, these eggs were fixed every day until hatching began; in this way fourteen samples of post-diapause eggs were fixed.

All the studies, except for those of the early embryos of G. pennsylvanicus, are based on fixed material. The stages of development of the embryos referred to (Stages I, II, etc.) are those described previously for the eggs of G. assimilis (Section IV). Measurements were made directly by means of an oculometer.

### 3. Development of the embryo in diapause eggs of G. pennsylvanicus

At 23-26°C. the embryos of G. pennsylvanicus completed stages I to III of development (pre-diapause stages) in about 84 hours and during this period their behaviour was exactly similar to that of the embryos of G. assimilis. After a few hours they sank into the yolk and were no longer visible. After four days of incubation the embryos were either in a state of development between stages III and IV or in stage IV. After five days of incubation, some had reached a stage intermediate between IV and V, while others still remained in stage IV or between stage III

and IV. Since the stages of development in G. pennsylvanicus after three days are not exactly similar to those of G. assimilis the two intermediate stages may, for convenience, be separately designated IIIa and IVa.

Stage IIIa (Fig. 14):- The protocephalic lobes are thick; primary segmentation, demarcating the antennal, mandibular, two maxillary and three thoracic segments, is distinct.

Stage IV (Fig. 14):- The embryo has become somewhat elongated, and the abdominal segmentation now develops, but the rudiments of the labrum are still not visible; the gnathal and thoracic segments are now a little thickened at their ends.

Stage IVa (Fig. 14):- The labrum is now visible as a globular swelling at the extreme end of the protocephalic lobes; the rudiments of the antennae are distinct; the gnathal, thoracic and first three abdominal segments are further thickened at their ends to form traces of the rudiments of their respective appendages. The posterior abdominal segments have become flexed and slightly thickened at their ends.

Ten embryonated eggs for each incubation period from four to forty days were studied and the number of the embryos at each stage of development for the different periods is given in Table I. The different stages of G. pennsylvanicus subsequent to stage IV also differ slightly from those of G. assimilis but are sufficiently similar to be assigned to comparable stages as indicated in the Tables I and II.

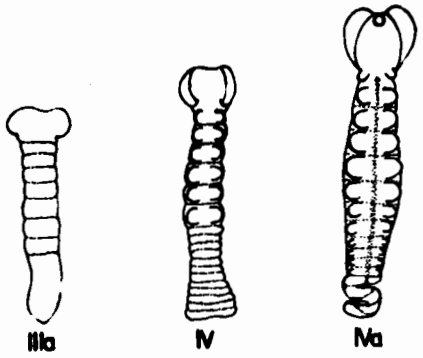


Fig. 14. Stages of development of the embryo of G. pennsylvanicus at 23-26°C. IIIa, 92 hours; IV, 96 hours; IVa, 100 hours.

From Table I it may be seen that from the fourth day of incubation onwards development of the embryos ceases for a time in any one of the three stages IIIa, IV, or IVa, but chiefly in the last. The number of embryos whose development remained arrested at stage IIIa was only about 3.2 per cent. About 15 per cent of the eggs were found to contain embryos in the stage IV, while 75 per cent contained embryos in stage IVa. It may therefore be concluded that most of the eggs stop developing when the embryos are in this last stage which may be regarded the stage at which the embryos enter diapause. Twenty-three or more days of incubation (and in one case as little as 17 days) brought about further development of a small proportion of the embryos, but this was very irregular and after 40 days of incubation not a single egg was found to be in fully developed condition. The most advanced stage reached by any embryo was stage IX. In this experiment the eggs started hatching on the 37th day of incubation and continued to hatch till 69th day, while in other experiments the hatching started as early as on the 28th day and continued as late as 140th day (see Section VI).

4. Development of the embryo in post-diapause eggs of G. pennsylvanicus

Eggs of G. pennsylvanicus which were cold treated at 6-7°C. for a minimum period of three months and thus had completed diapause (see Section VI) had a distinct 'cap' at their anterior end (see page 59), their chorion was generally cracked, and the

Table I. Development of Gryllus pennsylvanicus embryos in eggs not subjected to cold treatment.

| Incubation period in days | Number of embryos out of sample of 10 in each stage* | | | |
|---|---|---|---|---|
| | Stage IIIa | Stage IV | Stage IVa | Later stages |
| 4 | 3 | 7 | - | - |
| 5 | 1 | 3 | 6 | - |
| 6 | 1 | 2 | 7 | - |
| 7 | 1 | 2 | 7 | - |
| 8 | - | 4 | 6 | - |
| 9 | - | 4 | 6 | - |
| 10 | 1 | 3 | 6 | - |
| 11 | - | 3 | 7 | - |
| 12 | - | 2 | 8 | - |
| 13 | - | 2 | 8 | - |
| 14 | 2 | 2 | 6 | - |
| 15 | - | 2 | 8 | - |
| 16 | - | 2 | 8 | - |
| 17 | - | 2 | 7 | 1 Vc |
| 18 | - | 1 | 9 | - |
| 19 | 1 | 2 | 7 | - |
| 20 | - | 1 | 9 | - |
| 21 | - | - | 10 | - |
| 22 | - | 1 | 9 | - |
| 23 | 2 | - | 7 | 1 VI |
| 24 | - | 2 | 6 | 1 V, 1 Vb |
| 25 | - | 1 | 8 | 1 Vb |
| 26 | - | 2 | 7 | 1 Vc |
| 27 | - | - | 9 | 1 VIIIa |
| 28 | - | 2 | 6 | 1 V, 1 Vb |
| 29 | - | 2 | 7 | 1 VIIIa |
| 30 | - | - | 8 | 1 VIa, 1 IX |
| 31 | - | - | 9 | 1 VII |
| 32 | - | - | 9 | 1 Vb |
| 33 | - | - | 6 | 2 Va, 2 Vb |
| 34 | - | 1 | 7 | 1 Va, 1 VIa |
| 35 | - | 1 | 8 | 1 VIa |
| 36 | - | - | 9 | 1 VIa |
| 37 | - | 1 | 8 | 1 IX |
| 38 | - | 1 | 8 | 1 V |
| 39 | - | - | 9 | 1 VII |
| 40 | - | - | 8 | 1 VII, 1 IX |
| Total | 12 | 56 | 276 | 26 |

* For definition of stages, see text (pages 59-63, 82)

yolk was in polyhedral masses and contracted so that it fell short of the posterior pole by about one-twelfth of the length of the egg. Immediately after the cold treatment all the eggs contained embryos in stage IVa, and it is interesting to note that, out of the 50 examined, not a single egg had the embryo either in stage IIIa or in stage IV at this time. None of these eggs which failed to hatch showed either of these two stages; unhatched eggs were found to contain embryos which had completed katatrepsis, or no embryo was present. After about 24 hours of post-diapause incubation at 23-26°C. the yolk further contracted and reached only to about one-sixth to one-eighth of the length of the egg from the posterior pole. Subsequent development at this temperature was very regular and most of the eggs hatched on the fourteenth day (see Section VI). The stage of development reached after each day of post-diapause incubation is indicated in Table II. Ten eggs were examined after each incubation period; the stage of development indicated in the Table for the appropriate period of incubation was that reached by the majority of the embryos concerned.

5. Comparison of the embryonic development of G. assimilis G. rubens, G. veletis and G. pennsylvanicus

The morphological development of the embryos of the four species of Gryllus studied was found to be so similar as to be virtually identical. Such differences as occurred were in size and in the time of appearance of the pigmentation of the compound

Table II. Development of Gryllus pennsylvanicus embryo in post-diapause eggs.

| Post-diapause incubation period in days | Stage of embryo | Remarks |
|---|---|---|
| 0 | IVa | |
| 1 | V | |
| 2 | Va | |
| 3 | Vb | |
| 4 | Vc | |
| 5 | VI | Embryo straight |
| 6 | VIa | pigmentation in eyes develops |
| 7 | VII | |
| 8 | VIII | |
| 9 | VIIIa | Embryo reaches its full length and cerci become orange coloured |
| 10 | IX | |
| 11 | IXa | |
| 13 | X | |

eyes. There were, however, differences in the relationship between time and the stage of development reached. G. assimilis and G. rubens were almost exactly alike, G. veletis deviated slightly (see below), while G. pennsylvanicus was very different from the other three species due to the occurrence of the diapause.

Development continued more or less similarly in all four species until the embryo reached stage III, at which point the embryos of G. pennsylvanicus sank into the yolk and disappeared. The embryos of the other three species, however, completed stage IV before sinking entirely into the yolk and continued their development further without interruption - unlike those of G. pennsylvanicus which enter diapause shortly afterwards.

At 23-26°C. the eggs of G. veletis hatch on the eighteenth, whereas those of G. assimilis and G. rubens hatch on the seventeenth day. The pigmentation in the compound eyes of the embryos of G. rubens and G. veletis appears on the ninth and eleventh day respectively when they are in stage VIII, while in G. assimilis the pigmentation in the eyes appears on the tenth day when the embryo is still in stage VII. The embryos of G. rubens, G. assimilis and G. veletis reach their full length on the tenth, eleventh and twelfth day of incubation respectively. Another slight difference between the three species is also found; namely, the black line at each lateral margin of the labrum in the embryonic cuticle, appears in G. veletis on the fourteenth day (stage IXa); in G. assimilis on the thirteenth (stage IX); and in G. rubens on

the twelfth day of incubation (stage IX).

The eggs of G. pennsylvanicus develop very slowly and irregularly unless they are first exposed to a period of cold (see Section VI). As the pre-diapause development of the eggs require about four days at 23-26°C. and the post-diapause development about fourteen days of incubation at the same temperature, the actual developmental period is very similar to that of G. veletis. The pigmentation in the compound eyes in the embryo of G. pennsylvanicus appears on the tenth day of total (pre-diapause and post-diapause) development and the embryo reaches its full length on the thirteenth day (thus differing very slightly from G. veletis). The embryonic cuticle at the lateral margins of the labrum develops black lines on the fourteenth day of total development, as in G. veletis.

### 6. Distinguishing characters in the eggs and embryos of the four species

There appears little difference in the shape or in the sculpture of the chorion of the eggs of the different species of Gryllus studied. However, some difference in their size is noticeable although there is much overlapping (Table III) so that this character is not of much value in distinguishing the eggs of one species from those of another. The eggs of all the four species absorb water and thus become a little stouter and more elongated before the embryo sinks into the yolk.

Table III. Egg size of four species of Gryllus (in mm.)

| G. assimilis | | | | G. rubens | | | |
|---|---|---|---|---|---|---|---|
| One day old eggs | | Eggs after absorption of water | | One day old eggs | | Eggs after absorption of water | |
| Length | Breadth | Length | Breadth | Length | Breadth | Length | Breadth |
| 2.18 | 0.56 | 2.59 | 0.68 | 2.12 | 0.57 | 2.53 | 0.68 |
| 2.18 | 0.53 | 2.37 | 0.71 | 2.12 | 0.57 | 2.62 | 0.71 |
| 2.31 | 0.59 | 2.50 | 0.68 | 2.12 | 0.56 | 2.50 | 0.71 |
| 2.12 | 0.59 | 2.56 | 0.68 | 2.18 | 0.58 | 2.69 | 0.74 |
| 2.18 | 0.59 | 2.56 | 0.65 | 1.93 | 0.58 | 2.59 | 0.74 |
| 2.28 | 0.56 | 2.53 | 0.68 | 2.18 | 0.56 | 2.62 | 0.68 |
| 2.34 | 0.53 | 2.43 | 0.71 | 2.12 | 0.57 | 2.53 | 0.68 |
| 2.18 | 0.53 | 2.62 | 0.65 | 1.18 | 0.56 | 2.53 | 0.71 |
| 2.21 | 0.56 | 2.62 | 0.65 | 2.03 | 0.57 | 2.50 | 0.68 |
| 2.34 | 0.56 | 2.56 | 0.68 | 2.18 | 0.58 | 2.53 | 0.74 |
| 2.23 | 0.56 | 2.53 | 0.67 | 2.02 | 0.57 | 2.55 | 0.71 Average |
| G. pennsylvanicus | | | | G. veletis | | | |
| 2.25 | 0.62 | 2.87 | 0.81 | 2.21 | 0.53 | 2.75 | 0.73 |
| 2.37 | 0.65 | 2.93 | 0.81 | 2.33 | 0.56 | 2.90 | 0.76 |
| 2.18 | 0.59 | 2.81 | 0.84 | 2.30 | 0.53 | 2.75 | 0.76 |
| 2.31 | 0.59 | 2.81 | 0.84 | 2.36 | 0.50 | 2.90 | 0.76 |
| 2.43 | 0.59 | 2.84 | 0.84 | 2.36 | 0.56 | 2.87 | 0.73 |
| 2.40 | 0.65 | 2.87 | 0.83 | 2.06 | 0.56 | 2.87 | 0.73 |
| 2.28 | 0.65 | 2.84 | 0.84 | 2.24 | 0.53 | 2,90 | 0.73 |
| 2.18 | 0.62 | 2.93 | 0.83 | 2.30 | 0.50 | 2.75 | 0.70 |
| 2.43 | 0.65 | 2.84 | 0.84 | 2.21 | 0.53 | 2.84 | 0.70 |
| 2.31 | 0.62 | 2.93 | 0.83 | 2.33 | 0.53 | 2.81 | 0.70 |
| 2.31 | 0.62 | 2.86 | 0.83 | 2.27 | 0.53 | 2.83 | 0.73 Average |

In the early stages of embryonic development it is scarcely possible to differentiate between the embryos of the four species. Immediately before the formation of the embryonic cuticle, however, the embryos of G. pennsylvanicus can be distinguished from those of the other three species by the fact that, on the twelfth day of total development, the scape becomes brownish and a dark brown band appears at the base of the postpedicel of each antenna and that the cerci become orange coloured.

## 7. Discussion

The eggs of the four species of Gryllus studied absorb water and become enlarged after the completion of anatrepsis. The absorption of water by the eggs probably makes the yolk a little thinner and thus facilitates the sinking of the embryo into it. In three of the species this occurs after the completion of primary segmentation, i.e., after about four days of incubation at 23-26°C. In G. pennsylvanicus, however, the embryo sinks a little earlier, at a stage when the abdominal segments are not demarcated. In the eggs of G. assimilis, G. rubens and G. veletis the development of the embryo continues without interruption and hatching takes place in about seventeen to eighteen days. In G. pennsylvanicus, however, development is arrested early when the embryo is in any one of the three following stages: Stage IIIa, when the primary segmentation is present only in the gnathal and thoracic regions (at about 92 hours); Stage IV, when the primary segmentation is complete and the traces of the gnathal and thoracic appendages appear (at about 96 hours); and Stage IVa,

when the posterior abdominal segments become flexed (at about 100 hours).

In other words, in the eggs of G. pennsylvanicus there is apparently no absolute rigidity regarding the exact stage of embryonic development at which growth stops. This occurs at stage IIIa only in about 3.2 per cent of eggs and this may mean that only a very small number of embryos stop development at this stage. Alternately it may imply that, after remaining for a time in this stage, development proceeds further before being again arrested in stage IV or IVa. The second explanation appears to be more plausible since the number of the embryos in stage IIIa was greatest at the beginning of the incubation period. As this period increased the number of stage IIIa embryos declined until the 23rd day of incubation, after which no egg remained in this stage of development. It is probable that all such embryos proceed to the next stages after 23 days at most. Stage IV continues to occur among eggs incubated up to 38 days, but,after the first four days and up to 40 days of incubation the maximum number of embryos was found to be in stage IVa of development. It may therefore be presumed that most of the eggs of G. pennsylvanicus stop development when the embryos are in stage IVa, which may thus be regarded as the diapause stage. Further development of the embryo beyond the diapause stage, may sometimes occur after an incubation period of at least seventeen days, but this seems to be unusually rapid. The stage which is thus completed corresponds that attained on the seventh day of incubation in the non-diapause eggs of other species.

In G. pennsylvanicus, therefore, there is a period of at least (about ten days or more during which time no development occurs and it may be concluded that all eggs enter a diapause of several days of duration at least. As the incubation period increases beyond about three weeks, embryos of more advanced stages occur, but these are not all in the same stage of development in relation to their period of incubation. Although, at 23-26°C., development may be resumed by the embryos of G. pennsylvanicus this is very irregular, further indicating the occurrence of diapause in all eggs.

The eggs of G. pennsylvanicus immediately after cold treatment were found to contain embryos in stage IVa. This ~~may~~ perhaps mean that when eggs in stages IIIa or IV of development were subjected to cold treatment, the embryos immediately resumed their development to reach stage IVa, the diapause stage before actually entering diapause. Thus it may be presumed that during the period of cold treatment there is, at most, only very slight development to bring the embryos into a diapause condition.

# VI. DIAPAUSE IN THE EGGS OF *GRYLLUS PENNSYLVANICUS*.

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## 1. Introduction

Experiments with the eggs of two non-diapause species of Gryllus, viz., G. veletis and G. assimilis, have shown that these when incubated at 28°C. or at 23-26°C., begin hatching on the twelfth day or on the seventeenth to eighteenth day of incubation respectively, and that further hatching continues only for a day or two in either case. The hatching period (i.e., the difference in time between the hatching of the first and last eggs in a batch) thus varies only within two to three days. The eggs laid by the females of G. pennsylvanicus, however, behave very differently in this respect, owing to an apparently obligatory diapause in the embryo.

Diapause is a physiological adaptation which assists in the preservation of a species during unfavourable climatic conditions. The literature on the various aspects of diapause in insects is voluminous and has recently been reviewed by Andrewartha (1952) and Lees (1955, 1956). In the Gryllinae diapause may occur in the egg stage, in the late nymphal instars, or not at all, according to the species. There appear to be only three species of field cricket which are known to enter diapause in the egg stage. These are "Acheta" commodus[1], which is Australian, and G. firmus Scudder and G. pennsylvanicus from North America; the North American species presumably enter diapause to resist the rigours of cold.

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1. See footnote, page 55.

In G. pennsylvanicus this is almost certainly the case, for the species is found where winters are very severe and is one of the common species in Canada. It has been extensively studied in the Province of Quebec where cold weather may extend over six months of the year and where temperatures below -15°C. may persist continuously for several weeks.

G. pennsylvanicus has but one generation a year and the eggs are laid in soil, probably from late July to early October, but mainly during August and September. Hatching occurs during the following May and the nymphs become adult from about the second or third week of July onward. Egg-laying begins within a week or so and after the oviposition period the females die. In the laboratory, field-collected females behave more or less similarly and by the second week of October most of these females are dead. A few which may continue to live a little longer have their abdomens greatly swollen due to the presence of large number of eggs in their ovaries, but further oviposition does not occur and ultimately they die also.

This part of thesis is concerned with laboratory experiments to confirm the occurrence of obligatory diapause in the eggs of G. pennsylvanicus and to discover the role of exposure to low temperatures in terminating this diapause.

## 2. Materials and methods

Adult G. pennsylvanicus were collected locally in the vicinity of Ste Anne de Bellevue, Quebec, from the last week of July to

the first week of September and kept in glass cages at room temperature. Eggs, laid in moist sand, were normally collected at the intervals of three to four days. A large number of eggs laid by the females reared in the laboratory, but originating from Vermont, Prince Edward Island and New Brunswick stock were also collected in a similar manner. In this way about 17,000 eggs were obtained between 31st July and 5th October. All eggs collected between 31st July and 29th September were incubated at 28°C. until 3rd October, and then either transfered to 23-26°C. or exposed to different temperatures.

Most of the eggs were left undisturbed in the moist sand in which they were laid, but five batches of eggs (all laid by Quebec females) after incubating for 4, 9, 18, 22 and 25 days at 28°C. were removed from the sand. Lots of 25 eggs from each batch were counted out, and each lot kept in a 500 ml. jar on a moist filter paper (9 cm. diameter) lying on 30 c.c. of sand moistened with 10 ml. of water. About 2,000 eggs in all were so treated. Two replicates of five lots of 25 eggs, one from each batch, together with most of the undisturbed eggs, were transfered from 28°C. to 23-26°C. (room temperature) on 3rd October. The remaining lots of 25 were placed in a cold room at 6-7°C. for varying periods. In some cases the eggs were subjected to alternating periods of cold and warm temperatures. For each of these different temperature treatments five lots of 25 eggs were used. After the treatment the eggs were again incubated at 23-26°C. until they hatched.

Jars, containing eggs incubated at 23-26°C., were opened on alternate days and the hatched nymphs were removed and counted. Each week a few drops of water was added to the sand to ~~avoid~~ prevent its drying out. Whenever mould appeared in the jars, the eggs were transfered to new jars. When mould was noticed in the jars kept at 6-7°C., the new jars were kept at the same temperature for 24 hours before transfering the eggs to them.

In this way it was hoped to determine the effect on egg development of constant high temperature, exposure to low temperature for different periods, and alternation of high and low temperatures, with a view to confirming that diapause is obligatory in this species and discovering the optimum conditions necessary for terminating this diapause.

3. Results

Constant incubation at 23-28°C.: In the present series of experiments, when the incubation temperature was 23-28°C. it was observed that the egg batches laid earliest by field-collected females (from 31st July to 4th August) required the longest period of incubation before the first hatching began ('pre-hatching period'), namely about 80 days (the first hatching took place on the 19th October). Those collected later, from 4th August onward, had a more or less progressively shortened pre-hatching period, some of the eggs from the batches laid from 9th to 11th September requiring the minimum pre-hatching period, namely 28 days. After 11th September, however, the pre-hatching period of the egg batches progressively increased again, 38 days being required in case of

the first to hatch of those laid from 30th September to 5th October (Fig. 15). It is notable that the eggs laid on different dates during August started hatching almost simultaneously, viz., between the 17th and 19th October and that those which were laid during the first fortnight of September began hatching earlier but also within a few days of each other, namely between 6th and 10th October. Comparable behaviour was also shown by eggs from batches laid during the latter half of September but these did not begin to hatch until 22nd to 26th October; that is, eggs of these batches were the last to start hatching.

It was also observed that eggs laid by field-collected females and incubated at the same temperature (23-28°C.) did not all hatch after similar incubation periods either within or between batches. In some egg batches hatching continued over a period of as much as 112 days (eggs laid from 9th to 11th September). The "intensity" of diapause, as defined by Andrewartha (1952) and Lees (1955)[1], thus varied greatly in different eggs, some hatching 28 days after oviposition, while others emerged after 140 days of incubation although they were laid at the same time (September 9th to 11th, see Fig. 15).

On the basis of the hatchings of 6,543 nymphs from the eggs of females laid during the period 31st July to 5th October, it was found that, at a temperature between 23-28°C., almost all

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[1] The duration of the incubation period, at temperatures suitable for development but without diapause-breaking stimuli, provides a measure of the 'intensity' of diapause in the eggs.

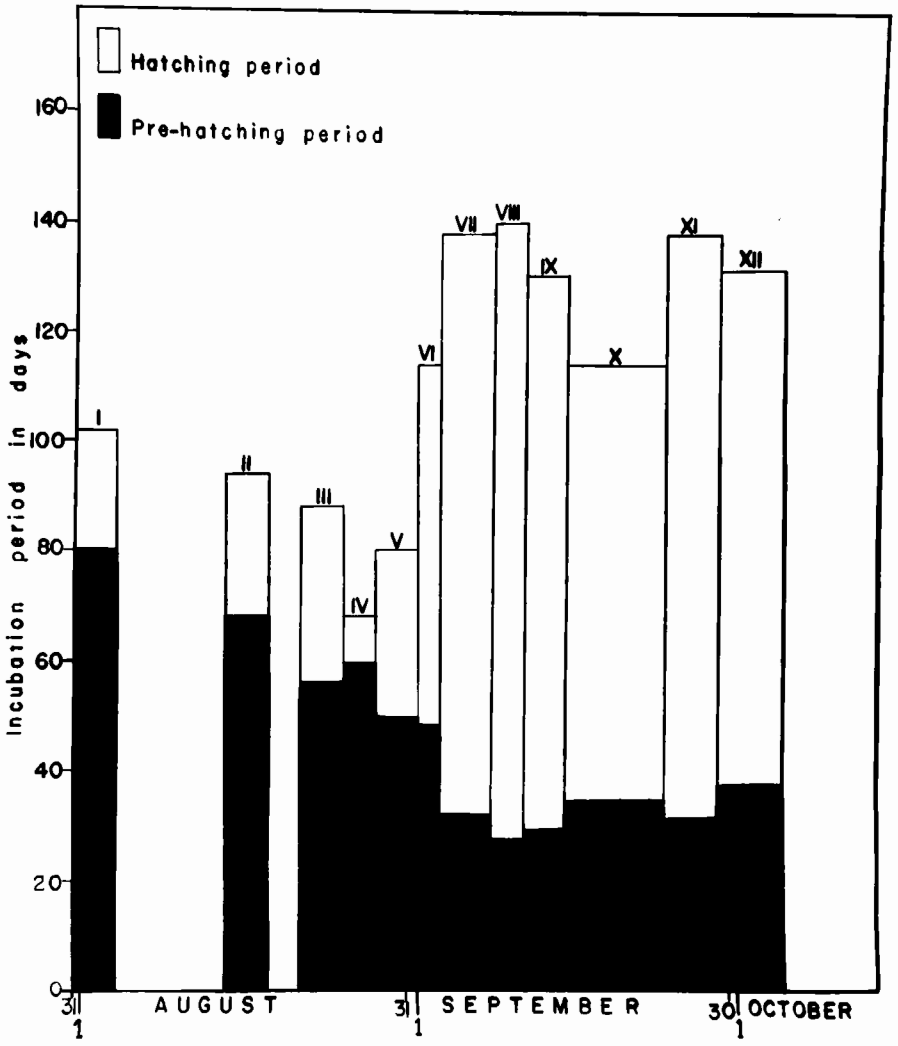
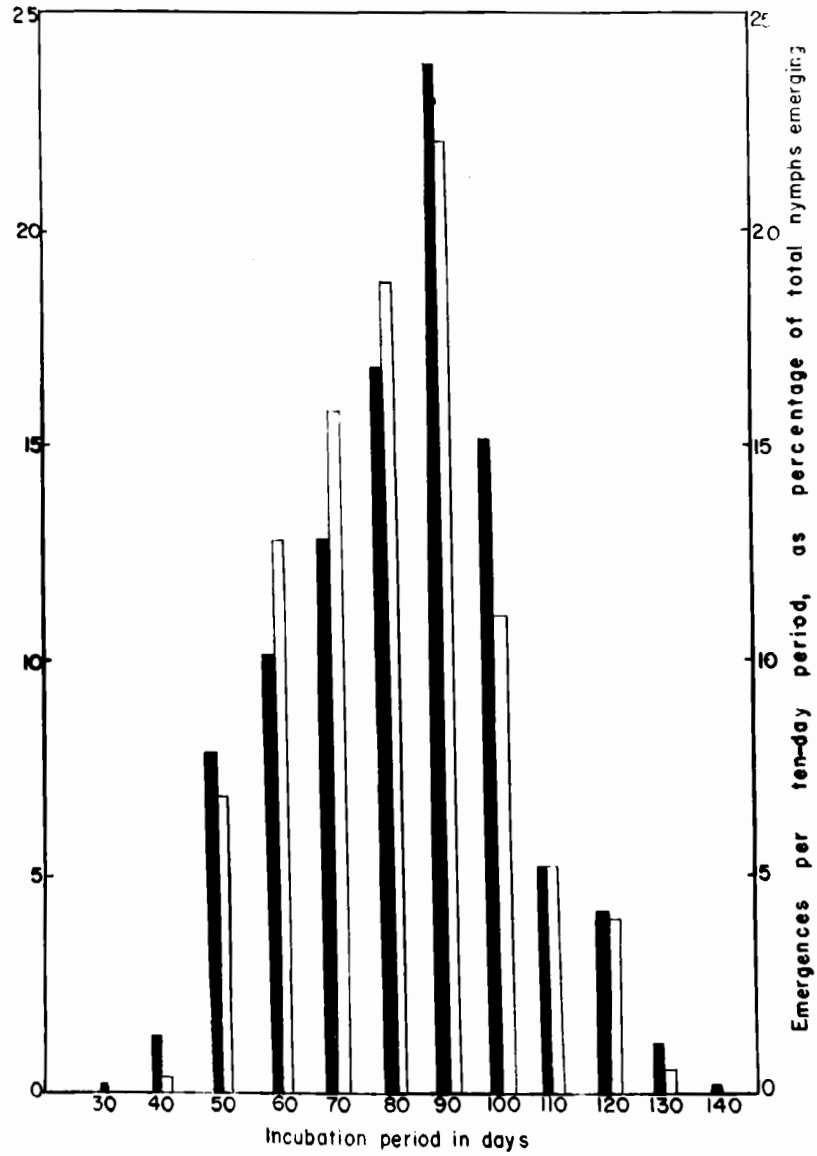



Fig. 15. Histograms showing the incubation period at 23-28°C. of 6,543 eggs laid on different dates by field collected females. The "pre-hatching" period is interval of time between oviposition and the hatching of the first nymph; the "hatching" period is the interval between the hatching of the first and last nymphs of a batch of eggs. Dates of laying, no. of eggs laid in parenthesis: I, 31 July-4 August (136); II, 14-18 August (211); III, 21-25 August (221); IV, 26-28 August (271); V, 29 August- 1 September (419); VI, 2-3 September (431); VII, 4-8 September (991); VIII, 9-11 September (887); IX, 12-15 September (878); X, 16-24 September (893); XI, 25-29 September (613); XII, 30 September-5 October (472).

emergence occurred between 50 and 110 days after the eggs were laid. The maximum amount of hatching (emergence of about 24 per cent of the nymphs obtained) took place within a 10-day incubation period of 81-90 days (Fig. 16). Only about 0.2 per cent of the nymphs emerged after less than 30 or more than 130 days of incubation. The number of nymphs obtained represented about half of the eggs incubated. Eggs laid by females from Vermont, Prince Edward Island and New Brunswick stocks produced, 232, 186 and 297 nymphs respectively and the results agreed very closely with those shown by the progeny of Quebec females. About the same proportion of the eggs hatched as in the case of Quebec stock.

Two replicates of 25 eggs each from five batches transfered to filter paper (see above) were incubated in a similar manner to the undisturbed eggs just referred to and it was again found that the peak period of hatching was similar; about 22 per cent of the nymphs emerged after 81-90 days of incubation (Fig. 16). Only 125 (50 per cent) of the eggs hatched.

Exposure to 6-7°C. after incubation at 28°C.: In order to determine the effect of exposure to low temperature on embryonic diapause, seven lots of 25 eggs from each of the five batches, initially incubated at 28°C. for 4, 9, 18, 22 and 25 days respectively and subsequently transfered to moist filter paper, were exposed to 6-7°C. for varying periods and then finally incubated at 23-26°C. In estimating the extent to which diapause had been broken, the following was assumed: that when the percentage of the nymph emergence within 16 days of the the final




■ Undisturbed eggs (total 6,543 nymphs)

□ Eggs transfered from moist sand to filter paper

Fig. 16. Histograms showing the percentage of total nymph-emergence after different periods of incubation at 23-28°C. Data from 6,543 nymphs hatching from undisturbed eggs and 125 nymphs hatching from eggs transfered to filter paper in batches of 25 (see text). Fifty per cent of the eggs hatched.

incubation (the majority of diapause-terminated eggs hatch within 14-16 days at 23-26°C., see Tables IV-VIII) was below 30, little or no effect was demonstrated; that when it was between 30 and 50, only slight breaking of diapause was indicated; that when the percentage was between 50 and 79, termination of diapause was only partial; but that when the percentage was 80 or more, the breaking of diapause was virtually complete.

Exposure to nine days of cold had no influence on the diapause as only two per cent of the eggs hatched within sixteen days of the final incubation which was continued for 81-109 days (Table IV). Similarly exposure to cold had little influence even when it was extended for 16, 23, and 30 days. Nevertheless it was observed that in these cases the final incubation periods were progressively shortened to 74-95, 67-84 and 44-84 days respectively, and that some of the eggs which had an initial incubation at 28°C. for 25 days showed slight breaking of diapause. Exposure to cold for 60 days, however, had a different influence on the eggs according to their different initial incubation periods at 28°C. The eggs receiving four and nine days of initial incubation showed only slight and partial breaking of diapause respectively, while the eggs which had 18-25 days of initial incubation showed complete termination of diapause, 100 per cent of the nymphs emerging within sixteen days of the final incubation (Table IV). When the cold treatment was extended for 91 days or 122 days the eggs became virtually completely free from diapause and more than

Table IV

Showing the effect of exposure to 6-7°C. on termination of egg diapause (Quebec population)

| Duration of cold treatment in days | Initial incubation period at 28°C. in days | Final incubation period required at 23-26°C. in days | | Percentage of viable eggs hatching within 16 days after cold treatment |
|---|---|---|---|---|
| | | Minimum | Maximum | |
| 9 | 25 | 17 | 81 | 0 |
| | 22 | 18 | 81 | 0 |
| | 18 | 16 | 86 | 0 |
| | 9 | 28 | 88 | 8 |
| | 4 | 28 | 109 | 0 |
| 16 | 25 | 13 | 74 | 41 |
| | 22 | 13 | 77 | 7 |
| | 18 | 21 | 84 | 0 |
| | 9 | 18 | 95 | 0 |
| | 4 | 35 | 91 | 0 |
| 23 | 25 | 16 | 67 | 30 |
| | 22 | 16 | 67 | 6 |
| | 18 | 16 | 77 | 6 |
| | 9 | 32 | 67 | 0 |
| | 4 | 32 | 84 | 0 |
| 30 | 25 | 14 | 60 | 43 |
| | 22 | 14 | 56 | 14 |
| | 18 | 14 | 44 | 20 |
| | 9 | 18 | 79 | 0 |
| | 4 | 23 | 84 | 0 |
| 60 | 25 | 14 | 16 | 100 |
| | 22 | 14 | 16 | 100 |
| | 18 | 14 | 16 | 100 |
| | 9 | 14 | 56 | 56 |
| | 4 | 14 | 49 | 42 |
| 91 | 25 | 13 | 15 | 100 |
| | 22 | 14 | 16 | 100 |
| | 18 | 13 | 15 | 100 |
| | 9 | 14 | 20 | 95 |
| | 4 | 14 | 16 | 100 |
| 122 | 25 | 14 | 16 | 100 |
| | 22 | 14 | 16 | 100 |
| | 18 | 13 | 17 | 95 |
| | 9 | 14 | 20 | 90 |
| | 4 | 14 | 16 | 100 |

95 per cent of the hatching was completed within sixteen days of the final incubation which varied only between fifteen and twenty days.

Fluctuating cold and warm temperatures: To determine the influence of fluctuating temperatures, eggs from Quebec females were exposed to cold and warm temperatures for varying periods (Table V). One batch of 25 eggs was first incubated at 28°C. for 22 days, then kept for seven days each at 6-7°C., 23-26°C., 6-7°C. and 23-26°C., followed by 14 days at -5 to -6°C. and a further 14 days at 6-7°C., and finally incubated at 23-26°C. On the fourteenth day of the final incubation the eggs began to hatch; and within three days hatching was completed. A second batch of 25 eggs was similarly treated except that, a 7-day treatment at 6-7°C. followed by a reduced period of 7 days at -5 to -6°C. was substituted for 14-day period at -5 to -6°C. The effect of this treatment was more or less the same as in the case of the first batch, namely, that the diapause termination was virtually complete; hatching in this case did not actually begin until the sixteenth day of the final incubation but was again completed within three days thereafter.

Third and fourth batches of eggs were given the same treatment as the first and second respectively except that the initial incubation at 28°C. was reduced to eighteen days. Diapause termination was again complete or virtually complete in both instances. Hatching began on the eighth and fourteenth day of

Table V

Showing the effect of exposure to fluctuating temperatures on termination of egg diapause (Quebec population)

| Treatment at fluctuating temperatures | Initial incubation period at 28°C. in days | Final incubation period required at 23-26°C. in days | | Percentage of viable eggs hatching within 16 days after cold treatment |
|---|---|---|---|---|
| | | Minimum | Maximum | |
| Treatment I | 22 | 14 | 16 | 100 |
| | 18 | 8 | 16 | 100 |
| | 9 | 16 | 16 | 100 |
| | 4 | 14 | 60 | 40 |
| Treatment II | 22 | 16 | 18 | 80 |
| | 18 | 14 | 17 | 91 |
| | 9 | 16 | 16 | 100 |
| | 4 | 16 | 56 | 30 |

Treatment I: 7 consecutive days each at 6-7°C., 23-26°C., 6-7°C. and 23-26°C. followed by 14 days each at -5 to -6°C. and 6-7°C.

Treatment II: 7 consecutive days each at 6-7°C., 23-26°C., 6-7°C., 23-26°C., 6-7°C., and -5 to -6°C., followed by 14 days at 6-7°C.

the final incubation and continued for eight and three further days respectively.

A fifth and sixth batch of eggs were also given the same treatment as those of the first and second respectively, except that the initial incubation period at 28°C. was shortened to nine days. In these two experiments the commencement of hatching was delayed until the sixteenth day, as in the second experiment, but was completed all on one day. Diapause was thus completely broken by these experiments also.

Seventh and eighth batches of eggs were incubated at 28°C. for only four days, but the treatments were otherwise the same as in the first and second experiments. The effect of this treatment differed materially from the others, for, although the eggs began hatching on the fourteenth or sixteenth day, hatching continued for 60 and 56 days respectively. Termination of diapause was, in fact, only slightly indicated since most of the eggs showed delayed hatching.

Five batches of eggs from the New Brunswick population and three batches from each of the Vermont and Prince Edward Island populations were compared with the above. After incubating for 9-38 days at 28°C. these were kept at 6-7°C. for 83 days and then each batch was divided approximately into two halves. One half of each batch was incubated at 23-26°C. while the other half, after keeping for seven days at -5 to -6°C., was incubated at the same temperature as the first half. The eggs belonging to the half

batches not receiving freezing treatment started hatching on the thirteenth day of the final incubation, and the eggs of many of these showed complete termination of diapause. Others, however, showed only a partial effect (Table VI). The eggs of the half batches which had received freezing treatment mostly began to hatch on the fourteenth day of the final incubation and there was virtually complete termination of diapause in all cases (Table VII).

The effect of cold on the percentage of egg hatching: As already indicated, only about 50 per cent of the eggs of G. pennsylvanicus hatched when incubated continuously at 23-28°C. without any cold treatment. The incubation period required by those eggs which hatched under these conditions varied between between 28 and 140 days, but tended towards the longer period. It was therefore decided to investigate the effect of cold treatment on the percentage of eggs hatching.

A total of 875 eggs initially incubated at 28°C. were subjected to 6-7°C. for varying periods before their final incubation at 23-26°C.; 125 for 9 days; 125 for 16 days; 125 for 23 days; 125 for 30 days; 125 for 60 days; 125 for 91 days; and 125 for 122 days. The figures of 50 per cent hatching for eggs receiving no cold treatment were taken from ~~the~~ previous results. Nine to 23 days of cold treatment effected a slightly better percentage of hatching than no cold treatment, but this beneficial effect was more pronounced when the treatment was prolonged for a month. In the latter case the percentage of the eggs which

Table VI

Showing the effect of exposure to 6-7°C. for 83 days on termination of egg diapause in Non-Quebec populations.

| Population | Initial incubation period at 28°C. in days | Final incubation period required at 23-26°C. in days | | Percentage of viable eggs hatching within 16 days after cold treatment |
|---|---|---|---|---|
| | | Minimum | Maximum | |
| New Brunswick | 38 | 13 | 25 | 97 |
| | 33 | | 15 | 100 |
| | 29 | | 16 | 100 |
| | 22 | | 27 | 57 |
| | 9 | | 39 | 90 |
| Vermont | 29 | 13 | 15 | 100 |
| | 22 | | 27 | 89 |
| | 9 | | 27 | 78 |
| Prince Edward Island | 29 | 13 | 13 | 100 |
| | 22 | | 19 | 98 |
| | 9 | | 36 | 65 |

Table VII

Showing the effect of exposure to 6-7°C. for 83 days followed by -5 to -6°C. for 7 days on termination of egg diapause in Non-Quebec population.

| Population | Initial incubation period at 28°C. in days | Final incubation period required at 23-26°C. in days | | Percentage of viable eggs hatching within 16 days of cold treatment |
|---|---|---|---|---|
| | | Minimum | Maximum | |
| New Brunswick | 38 | 14 | 27 | 97 |
| | 33 | 13 | 17 | 96 |
| | 29 | 14 | 20 | 91 |
| | 22 | 14 | 17 | 99 |
| | 9 | 14 | 41 | 96 |
| Vermont | 29 | 15 | 20 | 97 |
| | 22 | 14 | 25 | 92 |
| | 9 | 14 | 39 | 92 |
| Prince Edward Island | 29 | 14 | 20 | 95 |
| | 22 | 14 | 25 | 81 |
| | 9 | 14 | 39 | 90 |

hatched increased to about 65 per cent. This percentage continued to rise as the period of cold treatment was increased and it was maximal (nearly 85 per cent) when the treatment was prolonged for 122 days. Treatment was not prolonged beyond this period. The results are indicated in Fig. 17.

## 4. Discussion

The term diapause was first coined by Wheeler (1893) to describe the post-anatrepsis[1] stage in the morphogenesis of the embryo of the long-horned grasshopper, Xiphidium [now Conocephalus] when the embryo is more or less stationary. Physiological diapause in the eggs of the Orthoptera occurs at three different stages in the morphological development of the embryo in different species. These stages may be called the pre-anatrepsis, post-anatrepsis and pre-hatching stages. In Austroicetes cruciata and Homoeogryllus japonicus the embryo is pear-shaped (Andrewartha, 1952; Umeya,1950), while in Gryllulus [Acheta] mitratus it is dumbbell-shaped at the time of occurrence of diapause (Umeya, l.c.); all these are pre-anatrepsis stages. In a few orthopterous insects diapause is known to occur shortly before hatching, as in Melanoplus mexicanus mexicanus [M. bilituratus] (Parker, 1930), M. bivittatus (Moore, 1948), M. packardi (Salt, 1949), M. flavidus flavidus, and M. foedus fluviatilis (George,1950). But in most of the Orthoptera so far studied diapause occurs in the post-anatrepsis stage (i.e., in the stage described by Wheeler) for

[1] Anatrepsis is completed when the embryo reaches the ventral surface of the yolk with its head directed towards the posterior pole of the egg; when it sinks into the yolk it is in post-anatrepsis stage.

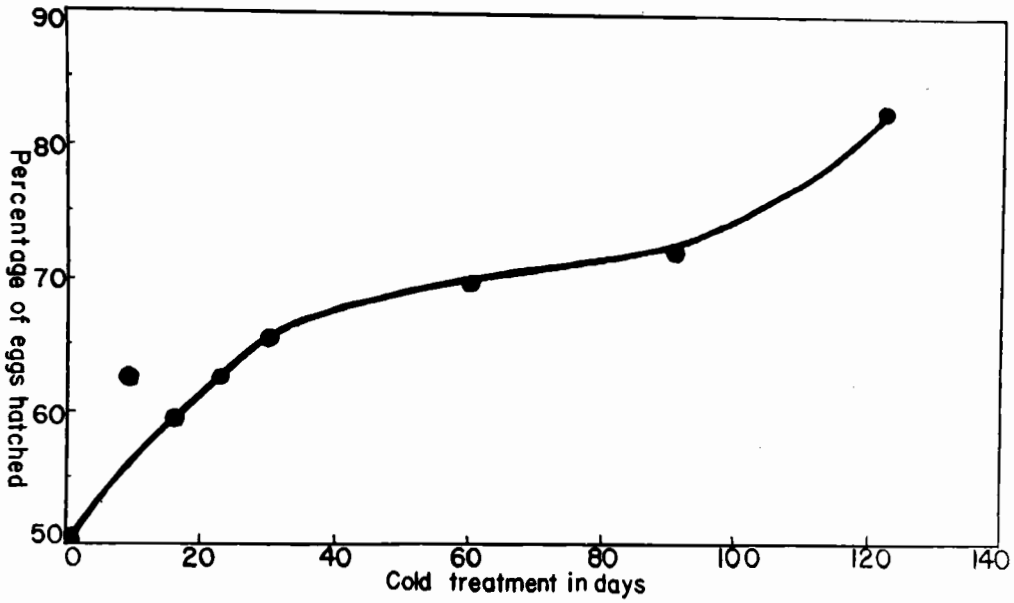



Fig. 17. Effect of exposure to 6-7°C. for different periods on the percentage of hatching of eggs previously incubated at 28°C. Number of eggs used 1,000.

example, in Dichromorpha viridis, Chloealtis conspersa, Circotettix verrucullatus, Hesperotettix [viridis] pratensis, H. [v.] viridis, Melanoplus differentialis and M. femur-rubrum (Carothers, 1923), in Aeropus [now Gomphocerus] sibiricus (Beĭ-Bienko, 1928), Chorthippus parallelus (Sansom et el., 1935), Camnula pellucida (Moore, 1948), Melanoplus keeleri luridus, M. scudderi scudderi (George, 1950), Locustana pardalina (Matthée, 1951), and in Locusta migratoria gallica (Le Berre, 1952). In "Acheta" commodus (Hogan, 1960a) and Gryllus pennsylvanicus (see Section V), diapause also occurs in the post-anatrepsis stage. All the species mentioned above have more or less strictly univoltine life cycle, which might perhaps imply that all the orthopterous species which enter diapause may have a univoltine life cycle and that they occur in those geographical regions where there is a single period of extreme climate and where this condition continues for a large part of the year. This suggestion is supported by the study of the life cycle of G. pennsylvanicus.

In G. pennsylvanicus diapause occurs after about four days of incubation (see Section V). Diapause-terminated eggs require further incubation for about fourteen ( ±1) days to complete their development, although a few eggs may take a day or two longer. Thus in the strict sense the true developmental period lasts about twenty days (four days pre-diapause and about sixteen days post-diapause). This means that the eggs of G. pennsylvanicus have more or less the same developmental period as those of other

species of Gryllus having non-diapause eggs, such as G. veletis, G. rubens and G. assimilis (see Sections IV and V) whose eggs hatch at 23-26°C. within seventeen to twenty days of the commencement of incubation. In G. pennsylvanicus, however, not a single egg actually hatched within twenty days of oviposition when incubated at 23-26°C. or at 28°C. The minimum pariod of incubation has been found (in a few cases only) to be 28 days and it may thus be concluded that the females of this species do not lay non-diapause egg. In other words, diapause in the eggs of G. pennsylvanicus is obligatory. This fact is further supported by the study of the morphological development of the embryo (see Section V),

In some cases the total incubation period of G. pennsylvanicus was found to be as long as 140 days at 23-28°C. Such a prolongation of the required incubation period indicates an increase in the 'intensity' of diapause as defined by Andrewartha (1952) and Lees (1955) (see footnote on page 99) and thus eggs which hatch only after a long period may be said to show a high diapause intensity. By plotting a graph (Fig. 18) on the basis of percentage emergence of nymphs from all eggs incubated at 23-28°C. (see page 99) and presuming that the eggs which hatch on or before twenty days of incubation show minimal diapause intensity (zero on an arbitrary scale of "diapause intensity"), and those which hatch after 140 days show maximal intensity (100 on the arbitrary scale), the diapause intensity shown by different numbers of eggs can be

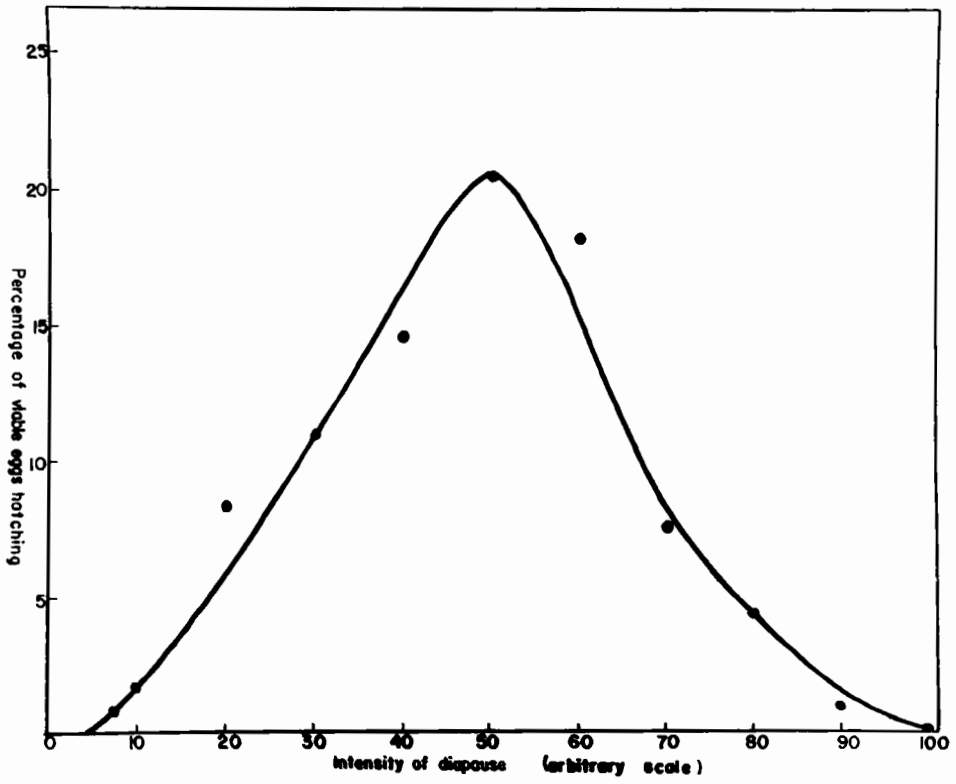



Fig. 18. Degree of diapause "intensity" shown by different percentage of eggs incubated continuously at 23-28°C. Zero on the arbitrary scale represents hatching on or before 20 days of incubation (i.e., minimal diapause intensity); 100 represents hatching on or after 140 days (maximum diapause intensity).

indicated. The graph shows that the maximum number of eggs exhibit a diapause intensity about midway between the two extremes (50 on the arbitrary scale). A normal distribution is apparent.

Cold treatment of the eggs effects a reduction in the intensity of diapause. As the period of cold treatment increases the diapause intensity decreases until the cold treatment is given for 90 days or more, when it becomes minimal (Fig. 19)(about zero on an arbitrary scale of diapause intensity). In calculating the effect of cold treatment it has been presumed that the eggs prior to cold treatment have been incubated for at least four days and that 140 days incubation before hatching means maximum intensity (100 on the arbitrary scale).

It is interesting to note that when eggs were incubated at 28°C. for varying periods from four to 38 days prior to cold treatment all hatched after approximately the same interval of time subsequent to the termination of diapause. This shows that all eggs were at more or less the same stage of development at the time of cold treatment, irrespective of the duration of prior incubation. This fact is further supported by a study of the embryos before and after the cold treatment (see Section V). It may be concluded that the preliminary incubation for four to 38 days at 28°C. has no detrimental effect on the development of the embryo.

Eggs laid by the young females (eggs laid early in a season) begin hatching after a longer time than those laid by middle-aged females (eggs laid late in a season). Thus the first batches of

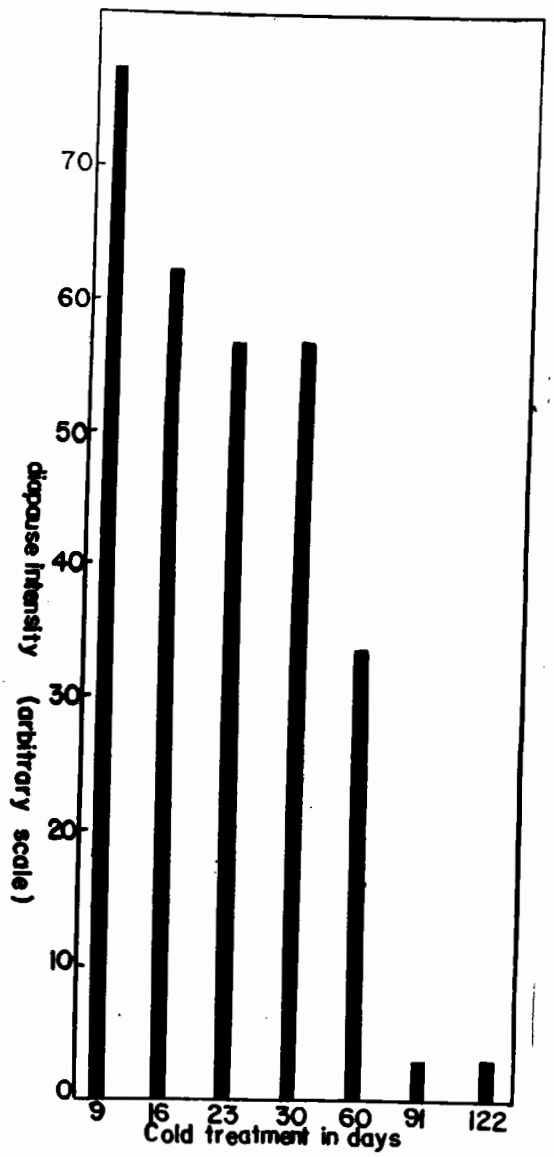



Fig. 19. Histograms showing the effect of exposure to 6-7°C. for different periods on the intensity of diapause of eggs previously incubated continuously at 28°C. The arbitrary scale of diapause intensity is as in fig. 18.

eggs were found to begin hatching after 80 days of incubation, while eggs laid after 40 days from the first laying started hatching after only 28 days. Cook, in a personal communication quoted by Burdick (1937), observed a similar phenomenon in Melanoplus differentialis. This implies that the general physiological condition of the mother affects the eggs. The same has been found to be the case in a variety of insects: in Bombyx mori (Kogre, 1933), in Phlebotomus papatassi (Roubaud, 1928; 1935), in Spalangia drosophilae (Simmonds, 1948), in Locustana pardalina (Matthée, 1951), and in Lucilia sericata (Cragg and Cole, 1952).

The observed behaviour of the eggs of G. pennsylvanicus (laid by field-collected females) in the laboratory must be of very great significance in the life of the cricket, if similar behaviour is shown by the eggs in the field. In nature some females become mature by the middle of July and start laying eggs by the end of the month. Even if high temperatures are maintained, such eggs would not be ready to hatch until the middle of October, but by that time the temperature would have dropped so that few, if any, eggs would have completed their development and hatching would not occur. If the eggs of young females behaved like those of the middle-aged ones, they would have hatched by the end of August or by the beginning of September and the young nymphs would die due to winter cold. To avoid this untimely hatching, the eggs of the young females have this long pre-hatching period. In fact whatever the date of oviposition by the females, hatching would

not begin before the first week of October, by which time the temperature would have declined sufficiently and no hatching would occur before the onset of winter.

The eggs which were kept for 83 days at 6-7°C. did not show complete termination of diapause, but when they were exposed for 91 days at this temperature or 83 days at 6-7°C. and for seven days at -5 to -6°C., the termination of diapause was almost complete. Therefore to terminate diapause, the eggs must be exposed to cold conditions for at least 90 days. This long period is also an adaptation to the insect's environment. Sometimes it may happen in nature that the temperature may go down as low as 6°C. for a week or so before the actual onset of winter, after which it may again become warm enough to allow the continued development of the eggs. Thus, if only a short exposure to cold were sufficient all the eggs might hatch prematurely and ultimately all young nymphs would die of exposure when winter cold finally set in. To avoid such a disaster, therefore, the eggs have developed the necessity for a long cold treatment to terminate diapause.

The effect,of the alternate warm and cold temperatures for 56 days, on the eggs is more or less similar to that of the continuous cold for 60 days; since under both conditions the termination of diapause was achieved in some eggs. It may also be pointed out that the exposure to warm temperatures (23-26°C.) during alternating warm and cold treatments, did not affect the

post-diapause incubation period which remained about the same, namely 14-16 days, showing that this kind of temperature fluctuation does not have any demonstrable effect on the resumption of morphogenesis.

Eggs which do not receive, or which receive insufficient cold treatment show a very small percentage of emergence within three days from the start of hatching, but diapause-terminated eggs complete about 95 per cent of their hatching within three days. This is again an adaptation to the environmental conditions. In this way the great majority of nymphs will mature at about the same time, so as to be ready to copulate and lay eggs at the appropriate time for winter diapause. Similar behaviour over a very wide geographical range can thus, to a large extent, be explained.

The behaviour of the eggs discussed above thus clearly indicates that the different faculties developed by them enable the species to overcome the rigours of cold at every stage of embryonic development. The critical temperatures, however, have not yet been determined.

Browning's (1952) observations on "Acheta" commodus, confirmed by Hogan (1960 a),that as the incubation temperature is raised an increasing percentage of the eggs develops without interruption and that there is a strong tendency for diapause to be averted at high temperatures, do not hold good for the eggs of G. pennsylvanicus in which not a single egg was found to develop

without interruption, even although some were incubated at 28°C. for more than two months (Fig. 15). Lees (1955) has also propounded a general rule that high temperatures tend to avert diapause while low temperatures favour arrest of growth. At least the first part of the general rule is not applicable to the eggs of G. pennsylvanicus.

Diapause has sometimes been regarded as a state of blocked or inhibited development which must be 'broken' by the stimulus of cold. In the eggs of G. pennsylvanicus morphogenesis becomes blocked at least for a time, and to break this blocking exposure to cold for at least 90 days is necessary. This means that during the period of cold some kind of physiological change is induced in the egg, so that when it is incubated at an adequate temperature the embryo will resume regular morphogenesis as in the eggs of non-diapause species of Gryllus. If the period of cold treatment is insufficient the physiological effect is incomplete and therefore there is no regular resumption of morphogenesis.

The only visible physiological effect of the cold treatment which was observed was a change in the consistency of the yolk which became more or less semi-fluid. The fluid nature of the yolk may allow its easier assimilation by the growing embryo and thus hasten growth with the result that all eggs hatch at more or less the same time. Embryos unexposed to cold are presumably only able to assimilate the yolk slowly, probably depending on their individual capacities, so that some are able to complete their

development earlier than others, although never so rapidly as those which have been exposed to cold. This might explain the irregular hatching of the eggs without cold treatment.

The eggs of Lymatria dispar enter diapause when the embryo is almost ready to hatch and its gut is loaded with yolk (Tuleschkov, 1935). In these eggs, when they are incubated at 25°C. after cold treatment, the yolk is liquefied and absorbed and hatching takes place. Hodson and Weinman (1945) have also observed, in Malacosoma disstria, that before hatching of the eggs occurs the yolk in the gut of the embryo must be liquefied and absorbed. Similar changes in the yolk of the eggs of Austroicetes cruciata have been observed by Andrewartha (1943) and he (1952) is of opinion that these visible changes in the yolk indicate that diapause may have set in because, at the critical stage in the life cycle, the yolk proved intractable and could not be moblized for ~~the~~ use in the next stage of development. Kevan (1944) in the larva of Diatraea [now Zaediatraea] lineolata and Lees (1953) in Metatetranychus [now Panonychus] ulmi have shown that unsuitability or a failure of the food supply led to the onset of diapause. It may, therefore be suggested that the non-availability of suitable food may be one of the causes of occurrence of diapause in G. pennsylvanicus also.

It is unlikely that cold is directly responsible for the difference in consistency of the yolk and it is also very improbable that the constitution of the yolk is alone responsible for diapause in the embryo of G. pennsylvanicus since Bigelow

(1960a) from the results obtained by hybridization experiments with non-diapause species, has concluded that diapause in this species seems to be determined by the genetic constitution of the embryo and not by cytoplasmic or yolk constituents of themselves. It may well be, however, that the constitution of the yolk may be affected by the embryo, itself influenced by exposure to cold, and there is some intraction between the two.

# VII. EFFECT OF COLD ON PRE- AND POST-DIAPAUSE EGGS OF *GRYLLUS PENNSYLVANICUS*

# VII. EFFECT OF COLD ON PRE- AND POST-DIAPAUSE EGGS OF GRYLLUS PENNSYLVANICUS

## 1. Introduction

It has been shown that when diapause eggs are exposed to 6-7°C. the "intensity" of diapause is reduced and that exposure for three months or more lowers this intensity almost to nil (see Section VI). In most species of insects that have been studied (Parker, 1930; Burdick, 1937; Andrewartha, 1942; Muroga, 1951; and Le Berre, 1953) it has been found that exposure to low temperatures at the appropriate stage in the egg's development (when the embryo is in diapause) results in the egg being able to complete its development promptly when subsequently incubated at some appropriate higher temperature. Until very recently, however, no systematic study has been made to show the effect of cold treatment on pre- and post-diapause eggs of any orthopterous insect. Since the bulk of this thesis was prepared, however, Hogan (1960b) has published some such observations on pre-diapause eggs of "Acheta" commodus in Australia. This section deals with experiments to determine the effect of exposure to low temperature (6-7°C.) on such eggs in G. pennsylvanicus. The main objects of the study were to discover, firstly, the extent to which development takes place in the embryo of pre-diapause eggs during exposure to cold and the effect of such exposure on the intensity of diapause in these eggs; and secondly, to determine if cold treatment has any detrimental effect on post-diapause eggs and whether any development occurs during such periods of exposure.

## 2. Materials and methods

Eggs were collected and experiments were performed in the manner described previously (see Section VI). Freshly collected eggs were divided into five groups of 75 each and incubated initially at 22-23°C. for different periods ranging from one to five days. Each group was then exposed to 6-7°C. for three months, after which they were finally incubated at 23-26°C. Another batch of 300 eggs was initially incubated at 22-23°C. for seven days (by which time all had entered diapause) and was then exposed to 6-7°C. for three months to terminate diapause. The post-diapause eggs were then divided into four groups of 75 each, each of which was again incubated at 23-26°C. for a different period - namely for 3, 6, 9, and 12 days respectively. All were then exposed to 6-7°C. for one further month and finally incubated at 23-26°C.

## 3. Results

Table VIII shows the effect of exposure to 6-7°C. on pre-diapause eggs. The eggs which were initially incubated at 22-23°C. for one day only did not hatch after their final incubation; all died. As the period of initial incubation at 22-23°C. was increased, however, the percentage which eventually hatched after the final incubation also increased. The percentage of eggs which hatched after an initial incubation at 22-23°C. for 2, 3, 4, and 5 days was 30, 36, 44, and 52 respectively. The eggs began hatching on the thirteenth to sixteenth day of

their final incubation and the hatching continued until 26th day. Most of the eggs in any batch, however, hatched within three days from the start of hatching indicating thereby that the termination of diapause was almost complete.

Table IX shows the effect of exposure to 6-7°C. on the post-diapause eggs. The eggs which were incubated at 23-26°C. for three days after the termination of diapause and before the second cold treatment began hatching on the fourteenth day of the final incubation at 23-26°C. As the immediate post-diapause incubation was increased from 3 to 12 days the final pre-hatching period was reduced by a comparable length of time. Thus when the immediate post-diapause incubation at 23-26°C. was 6, 9, and 12 days before the second cold treatment, the final pre-hatching period at 23-26°C. was shortened to 10, 8 and 5 days respectively. In this way it was shown that all four groups of eggs required incubation at 23-26°C. for a total seventeen days irrespective of the time at which they were subjected a cold treatment. The greatest percentage hatching, namely 75, was shown by eggs which were given six-day post-diapause incubation at 23-26°C. before the second cold treatment, but those incubated for only three days showed 65 per cent of hatching. Eggs incubated for nine and twelve days showed a minimum percentage hatching - only about 50 per cent. Six-day post-diapause incubated eggs hatched over a period of seven days while in the other cases the overall hatching period was only two to three days.

Table VIII. Showing the effect of 6-7°C. on pre-diapause eggs of G. pennsylvanicus.

| Incubation period in days | | Percentage of hatching | Percentage of hatching of viable eggs in first three days |
|---|---|---|---|
| Initial | Final | | |
| 1 | 30 | 0 | 0 |
| 2 | 16-26 | 30 | 83 |
| 3 | 14-17 | 36 | 100 |
| 4 | 14-15 | 44 | 100 |
| 5 | 13-20 | 52 | 98 |

Table IX. Showing the effect of 6-7°C. on post-diapause eggs of G. pennsylvanicus.

| Incubation period after cold treatment in days | | Percentage of hatching |
|---|---|---|
| After initial cold treatment | After final cold treatment | |
| 3 | 14 | 65 |
| 6 | 10 | 75 |
| 9 | 8 | 50 |
| 12 | 5 | 50 |

## 4. Discussion

Pre-diapause eggs, after initial incubation at 22-23°C. for only a day (i.e., before distinct embryos are formed - see Sections IV and V) and subsequent exposure to 6-7°C. for three months, are not resistant and are killed by the latter temperature. After longer initial incubation period at 22-23° C. (i.e., when the embryos have assumed a definite shape) they are able to withstand cold to a greater degree, becoming more and more resistant as they grow. When the embryos are only two days old only 30 per cent survive, but when they attain five days of age their survival rate increases to 52 per cent. Even when the embryos are in a pre-diapause condition, therefore, they may become almost completely free from diapause on being exposed to 6-7°C. for three months.

The effect of cold on pre-diapause eggs of orthopterous insects has not been studied systematically and there are only a few casual records dealing with this aspect. Parker (1930) noted that when freshly laid eggs of Melanoplus "mexicanus" [i.e., M. bilituratus] were chilled for 240 days at 0°C., the post-diapause incubation period was reduced from 46 days to 11 days, thus indicating that the exposure to cold of pre-diapause eggs of this species helps to bring about termination of diapause. Church and Salt (1952) have pointed out that, in late autumn, cold weathercombined with dryness aften stops development in the eggs of Melanoplus bivittatus well before diapause stage. In such eggs

the potential for diapause is broken in the same way as is diapause in mature diapause eggs. This means that the winter cold terminates diapause in pre-diapause eggs in a similar manner to that in diapause eggs. They refer to such eggs as "diapause averted" eggs.

Browning (1952) found that if the eggs of "Acheta" commodus were held at 13°C. for several weeks shortly after their oviposition and then incubated at 26°C., they invariably developed without interruption; he refers to this as the completion of 'diapause development'. Thus, according to Browning, the exposure, of the pre-diapause eggs to low temperatures, terminates diapause. He is further of the opinion that "A." commodus is unusual in this respect, because, in most of the species in which diapause occurs, low temperature is most influential after the embryo has entered diapause (Parker, 1930; Burdick, 1937; Andrewartha, 1943).

Browning's observations have been criticised by Hogan (1960a) who remarks that completion of diapause in this way would be most unusual and that such a phenomenon is contrary to the general rule. On the basis of his own experiments he presumes that at lower temperatures all eggs enter diapause. His suggestion that the low-temperature treatment of pre-diapause eggs does not terminate diapause is not, however, supported by his own experiments. There is always a threshold for growth below which development cannot proceed. Thus, when Hogan kept eggs of "A." commodus at 12.8°C. he did not observe any resumption of development beyond the

diapause stage even after three months. A temperature of 12.8°C., however, is probably not a suitable one for egg development. Had he kept eggs which had been exposed to 12.8°C. at a suitable incubation temperature it seems likely that they would have developed in a comparable manner to what was found in the case of G. pennsylvanicus unless "A." commodus behaves very differently from the former. In fact, very recently, Hogan (1960b) kept cold-treated pre-diapause eggs at a suitable incubation temperature (26.7°C.) and found that the diapause was then broken. To maintain his previous interpretation he states, "Such an exposure so weakens the tendency of the eggs to enter diapause that it is readily averted when they are transfered to a suitable incubation temperature..... Actually low temperature may be said to eliminate diapause from such eggs rather than to terminate it".

Hogan (1960a) is not correct in assuming that both low and high temperatures are required for the complete termination of diapause. In his more recent studies (1960b) he modifies his interpretation and states, "Diapause is not finally eliminated until the eggs are incubated at a higher temperature." In fact only low temperature is required for termination of diapause while high temperature is necessary for the resumption of development.

Hogan (1960a) also believes that there is no clear evidence to show that failure to enter diapause by cold-treated pre-diapause eggs results from the same processes as those bringing about the termination of diapause. In his subsequent publication (1960b) he writes, "If diapause development is completed in the pre-diapause

stages, then there should be a marked similarity between the relative effectiveness of different temperatures for the elimination of diapause from pre-diapause eggs, and for the termination of diapause in eggs that have actually entered diapause". He does not, however, discuss this point further. In fact, there is a marked similarity between the elimination of diapause from pre-diapause eggs and the termination of diapause in eggs which have actually entered diapause. In both kinds of eggs, low temperature treatment has exactly the same effect: it renders the yolk assimilable by the embryo (see page 121) and it is quite reasonable to expect that cold treatment would have same effect on the yolk whether the embryo were in the pre-diapause or diapause stage. If cold stimulates the embryo to affect the yolk, rather ~~from~~ than affecting the yolk directly, however, the condition of the embryo might help in difference. The similar behaviour of the pre-diapause and diapause embryos after the cold treatment, nevertheless, further support my contention that the physiological effect of the cold treatment is to make the yolk assimilable (see page 121). When the yolk becomes assimilable the embryos develop in the normal manner resulting in the termination of diapause. Table VIII clearly shows that almost all pre-diapause eggs after treatment hatched within three days just as was the case in diapause eggs which had become diapause free (see Section VI), i.e., there was almost complete termination of diapause. Browning (1952) also thinks that there is no reason to

believe that the processes which occur in pre-diapause eggs during exposure to cold, resulting in their competence to continue their development without interruption, differ fundamentally from those occurring in other species when exposed to cold after diapause has become manifest.

It may therefore be concluded that the eggs of G. pennsylvanicus may receive the required cold treatment to terminate diapause at any time after the second day of incubation, but to ensure a greater percentage of hatching they should be so treated when the embryos are well advanced. This conclusion may perhaps be applicable to all insects whose eggs enter diapause. Lees (1955) points out that in many insects, and in Orthoptera in particular, the temperature treatment to end diapause should synchronize with some definite stage in the morphological or physiological development of the embryo, known as the period of sensitivity. However, this general principal is not applicable in case of G. pennsylvanicus in which such a period of sensitivity extends from the Stage I to IVa (see Sections IV and V).

Pre-diapause eggs, after cold treatment for three months, when studied were found to contain embryos only in the diapause stage (morphological Stage IVa - see Section V), and a few were without embryos. This means that the pre-diapause embryos continue developing during cold treatment until they reach the diapause stage. It has already been pointed out (see page 93) that when eggs containing embryos in stages IIIa or IV are kept at 6-7°C., they resume

development and reach Stage IVa which is the true diapause stage. The present investigation conclusively supports my previous suggestion in this regard (see page 93). Hogan (1960a) has also shown that pre-diapause eggs, when given cold treatment, reach the diapause stage and then stop further development.

Generally 6-7°C. might be supposed to be so low that no development is likely, but present observations show that the embryos of G. pennsylvanicus can in fact continue to develop even at such a low temperature until they reach the diapause stage although they do not continue to do so beyond this point. This kind of development is found only in those insects which undergo diapause in the egg stage. In the diapause stage the embryos can probably live for a longer time at low temperatures which would probably kill pre-diapause stages if they were to remain at these temperatures for long. Further the embryos in diapause stage can resist much lower temperatures than are required to terminate diapause. G. pennsylvanicus has presumably developed the characteristic of continuing to develop at low temperatures in order to protect themselves from unexpected inclement weather conditions. The diapause eggs of G. pennsylvanicus are very hardy for they can withstand -5 to -6°C. for three months at least (unpublished observations).

Andrewartha (1952) has coined the term 'diapause development' for the processes going on during the cold treatment of diapause eggs to terminate diapause. Present studies clearly indicate that

the processes which go on during the cold treatment in pre-diapause and diapause eggs of G. pennsylvanicus are exactly similar. But 'diapause development' means development during diapause only and does not include development during the pre-diapause phase, therefore the use of the term is not quite appropriate and, it is suggested, may not be used in the present context.

Post-diapause eggs of G. pennsylvanicus can also withstand exposure to low temperatures but the percentage hatching after exposure to 6-7°C. was slightly reduced - from 72 (see page 111) to 60. Thus it may be presumed that cold has a slightly detrimental effect on the post-diapause embryos. There is probably no development during post-diapause cold treatment since most of the eggs required about seventeen days of post-diapause incubation instead of the normal fourteen days required in the absence of the second cold treatment (see Section VI). This suggests that the embryos need about three days to recover from post-diapause cold treatment before resuming development.

# VIII. RESPIRATORY METABOLISM DURING EMBRYOGENESIS OF *GRYLLUS VELETIS* AND *G. PENNSYLVANICUS*

# VIII. RESPIRATORY METABOLISM DURING EMBRYOGENESIS OF GRYLLUS VELETIS AND G. PENNSYLVANICUS

## 1. Introduction

Eggs of field crickets of the subfamily Gryllinae can be grouped into three classes according to the type of development: In Class One are those eggs that develop normally if incubated at an adequate temperature, e.g., Gryllus veletis (see page 88). In Class two are those eggs which require exposure to cold before they can continue normal development, and which, if they do not receive such treatment, develop very irregularly, their incubation period varying between wide limits; such eggs are known as diapause eggs, e.g., in G. pennsylvanicus, in which development takes from 28 to 140 days at 23-28°C. (see page 99). The eggs of the Third Class are mid-way between the other two classes, normally requiring low temperature exposure but hatching normally if maintained at high temperatures, e.g., in "Acheta" commodus (Hogan, 1960a).

It has been shown that the eggs of "A." commodus and G. pennsylvanicus enter morphological diapause after an incubation of about three and four days respectively (Hogan, 1960a; Page 81), but by studying respiratory metabolism, the exact time of the onset of physiological diapause can also be determined, since Bodine (1929) and others have shown that when the embryos enter diapause respiratory metabolism drops. The rate of metabolism may, in fact, be used as a test for the presence or absence of diapause in the eggs. Thus a study of respiratory metabolism is of great significance.

Amongst the Orthoptera respiratory metabolism has been described only for grasshopper embryos, for example, Chortophaga, Romalea, Cercotettix (Bodine,1929), and Melanoplus differentialis (Bodine, 1929; Burkholder,1934; Boell, 1935; Bodine and Boell, 1936). The metabolism of developing eggs in the other groups of insects (Coleoptera, Lepidoptera, Hemiptera, Diptera etc.) has also been studied, e.g., in Leptinotarsa, Crioceris, Anasa, Cotinis, Popillia (Fink, 1925), Tropaea luna,Platysamia (Semia) cecropia, Pyrausta ainsliei, Anasa tristis (Melvin, 1928), Oncopeltis fasciatus (Argo, 1939), Rhodnius prolixus (Tuft,1949), Drosophila and Musca (Smith and Kleiber, 1950), and Popillia japonica (Ludwig and Wugmeister, 1955).

No study of the respiratory metabolism of developing eggs of any of the three classes, however, has hitherto been published and an account of the respiratory metabolism and respiratory quotients of developing eggs of G. veletis, a non-diapause species, and G. pennsylvanicus, a diapause species, is presented here.

## 2. Materials and methods

Adult females and males of G. veletis were collected from the field in the vicinity of Ste Anne de Bellevue, Quebec at the beginning of June, 1960. The crickets were kept in large candy jars and provided with moist sand for oviposition. The sand containing the eggs laid by these females was collected after about twelve-hours, and the eggs used were thus not more than about twelve hours old at the beginning of the experiments. The eggs were then sieved out in water, washed with distilled water,

stored in jam jars on moist filter paper and incubated at 27°C. At this temperature most of the eggs hatched on the fourteenth day of incubation. The eggs of one and the same batch were used for all the experiments for the entire period of thirteen days. Before starting the experiments the eggs were thoroughly mixed so as to ensure random distribution. Determinations were made on the rate of oxygen consumption and carbon dioxide production for each day of embryonic development starting from immediately after their collection on the first day.

Adults of G. pennsylvanicus were also collected locally at the beginning of August and the eggs were obtained in the manner described above. To study the respiratory metabolism of the pre-diapause and diapause embryos, the eggs collected on one and the same day were used, and for observing the respiratory metabolism of the post-diapause embryos, the eggs were collected earlier and were given a cold treatment at 6-7°C. for three months to terminate diapause. All the eggs were incubated at 28°C. and at this temperature they hatched on the twelfth day of the post-diapause incubation. In the case of the post-diapause eggs the experiment was started about two hours after the completion of their cold treatment, i.e., on the first day of incubation at 28°C. During this period the eggs were washed thoroughly to remove any mould, and then the excess of moisture was removed by placing the eggs on dry filter paper.

Oxygen consumption was measured at 27.2°C.,using a constant volume Warburg manometer according to the procedure outlined by Umbreit,Burmes and Stauffer (1957). For these determinations 100 eggs and 0.5 ml. distilled water were placed in the main chamber of each of four manometer vessels. The central well of the first two vessels contained 0.2 ml. of 15 per cent KOH for the absorption of carbon dioxide. A small piece of filter paper was placed in the KOH to increase its surface area. Vessels 3 and 4 contained filter paper moistened with 0.2 ml. of distilled water in place of KOH. A vessel containing only distilled water served as a thermobarometer.

The vessels were fastened to their respective manometers, placed in the water bath at 27.2°C. After allowing 15 minutes for temperature equilibrium the manometer taps were closed and the zero reading was taken. The readings of all five manometers were recorded at intervals of one hour for five hours. The average oxygen consumption for each interval as well as for the entire experimental period was determined from the readings of the first two manometers after correcting for thermobarometer changes. The average oxygen consumption for each experimental period was used in calculating the carbon dioxide production in each of the two manometers of the second set. Respiratory quotients were calculated from the values obtained from these two determinations ($CO_2$ production/$O_2$ consumption).

Every day ten eggs were examined from the lots used, and the stage of the embryo was determined by a comparison with the illustrations given previously (Figs. 9 and 10).

### 3. Experimental Results

The rate of oxygen consumption, as micro-liters of oxygen per 100 eggs of G. veletis per hour, for each day of embryonic development is shown in figure 20. The rate of oxygen consumption in newly laid eggs is low. On the second day the oxygen consumption rose abruptly and continued to increase almost steadily until the third day. Thereafter there was a further sudden increase in oxygen consumption, and the increase continuing for two days, reaching a maximum average value of 24.31. On the sixth day the rise in the consumption was low and this situation lasted for two days, the average value being only 25.62. From the eighth day oxygen consumption again increased and continued to increase more or less steadily for three days. On the eleventh day the rise in the oxygen consumption was again slow. On the twelfth day the consumption again increased suddenly reaching an average value of 48.26 which was the maximum for the entire course of development. On the thirteenth day the consumption fell to an average value of 42.18. Four per cent of the eggs hatched in the manometer vessels.

Figure 21 shows the respiratory quotient curve of G. veletis. The respiratory quotient in the first two days of development rose slowly, and on the third day it increased rapidly and reached

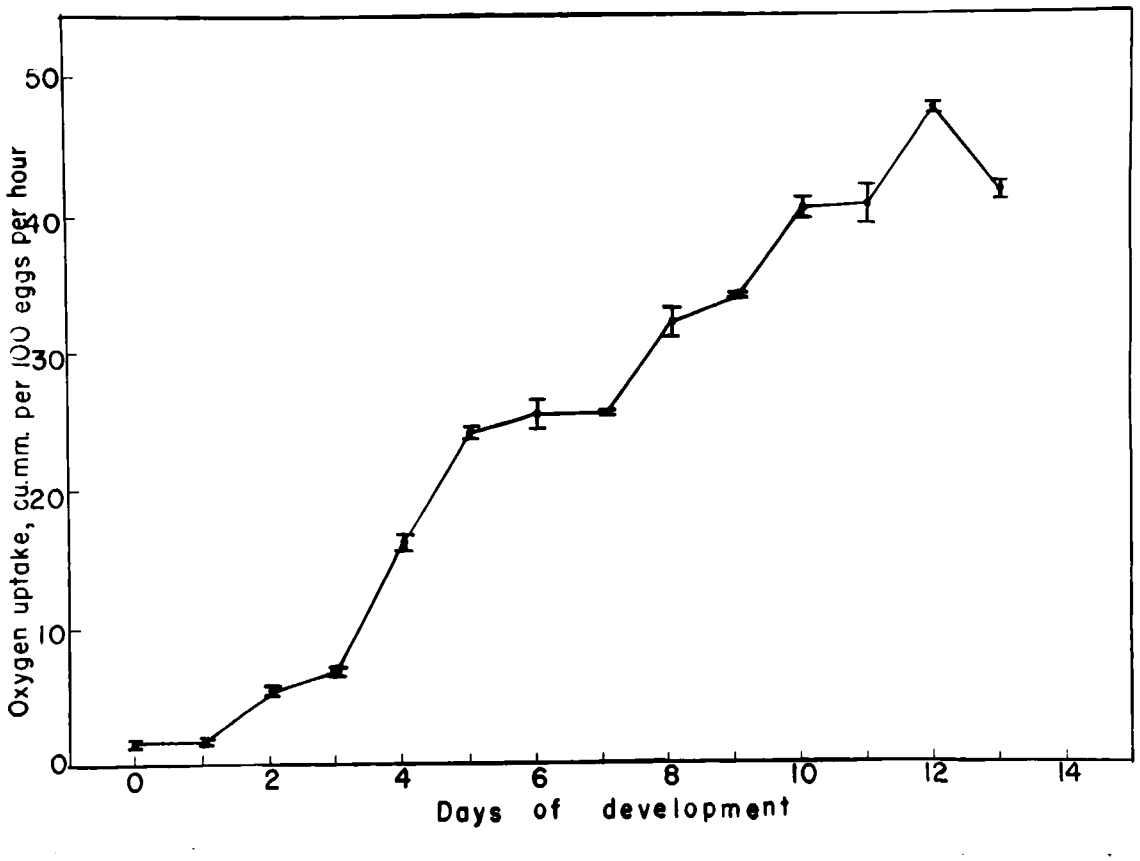



Fig. 20. Oxygen consumption of the eggs of G. veletis during embryonic development.

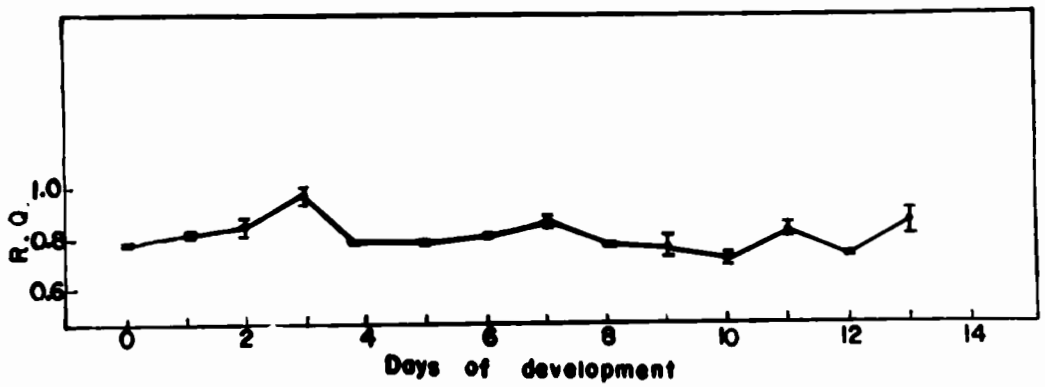



Fig. 21. Respiratory quotient of the eggs of G. veletis during embryonic development.

the maximum value. It then fell abruptly from an initially high value of 0.975 to 0.79 on the fourth day. It continued to fall until the fifth day. Following this dip a gradual increase in the quotient again occured until, on the seventh day. Then a sudden fall occured and the value dropped on the eighth day, and this value was then maintained without significant variation for a day, after which the value fell further to 0.705, on the tenth day. This was the minimum value reached during the entire course of development. Then the quotient again rose on the eleventh day, falling again on the twelfth day. On the thirteenth day a rise was again recorded.

Figure 22 shows the respiratory metabolism curve of the developing eggs of G. pennsylvanicus. The oxygen consumption for the first two days was low and then it rose abruptly and continued rising steadily for three days reaching an average value of 18.43, this being the maximum value during the pre-diapause period. After this high value the rate of oxygen consumption fell abruptly and continued falling for two days. After the seventh day the fall in the rate of oxygen consumption was slow and this drop continued until the tenth day reaching an average value of 2.47. During the next ten days the rate of oxygen consumption remained around this value varying only between 2.02 to 2.62. After the twentieth day the oxygen consumption began to rise slowly and on the thirtieth day it reached an average value of 3.85. On the thirtieth day a few eggs showed slight development beyond the diapause stage and the first hatching was noted on the fortieth day of incubation.

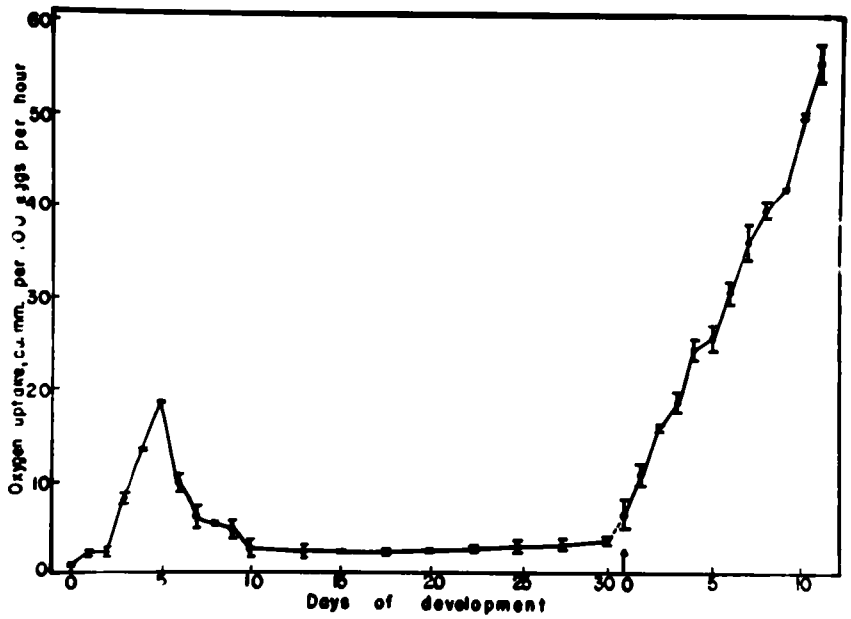



Fig. 22. Oxygen consumption of the eggs of G. pennsylvanicus during embryonic development. Pre-diapause period 0-5 days; diapause period 6-30 days; post-diapause period from arrow to 12 days.

The oxygen consumption of the post-diapause eggs of G. pennsylvanicus was higher than that of the diapause eggs. It continued rising almost steadily throughout the entire period of post-diapause development and an average value of 56.29 was reached on the eleventh day. Most of the eggs hatched on the twelfth day.

Figure 23 shows the respiratory quotient curve of G. pennsylvanicus. Newly laid eggs showed a respiratory quotient near unity and as the development proceeded the quotient fell, and on the fourth day it reached a value of 0.65, which was the minimum value during the pre-diapause period. Then the value began to rise and on the sixth day it reached 0.94 which continued until seventh day. From this high value a drop occured in the quotient and on the tenth day the value became 0.23. During the next twenty days the value of the quotient was very variable. In the case of post-diapause development the value of the respiratory quotient was more or less constant and it was around 0.7 varying between 0.634 and 0.732.

## 4. Discussion

During the entire period of embryonic development of G. veletis the respiratory metabolism continued to rise. It fell only at the time of hatching. A similar fall in the oxygen consumption has been noted by Tuft (1949) in Rhodnius prolixus, another hemimetabolous insect. The rate of increase of oxygen consumption was not the same during the entire period of development, for during certain stages of development it was only slight, as happened at the time of katatrepsis.

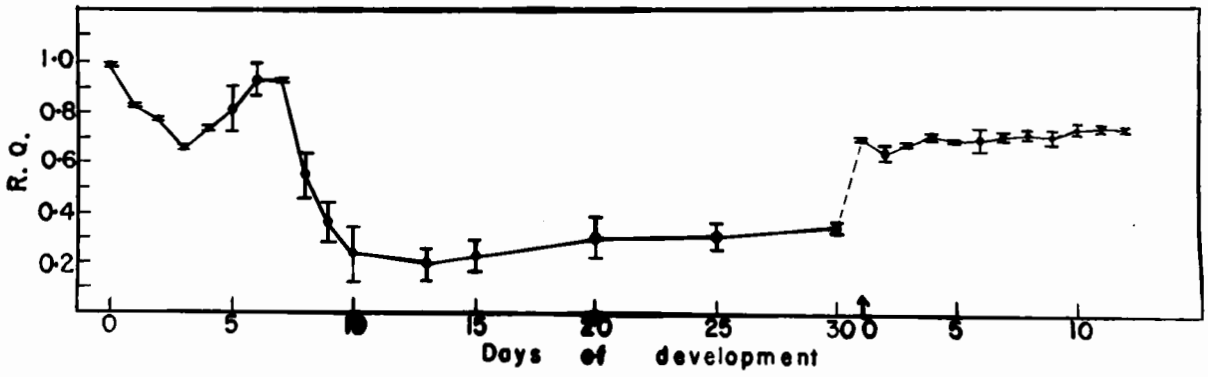



Fig. 23. Respiratory quotient of the eggs of G. pennsylvanicus during embryonic development. Pre-diapause period 0-5 days; diapause period 6-30 days; post-diapause period from arrow to 12 days.

During the embryonic development of G. pennsylvanicus the oxygen consumption also rose and continued rising until the fifth day. Then it began to fall. This means that ~~that~~ the embryos entered diapause at this time. The rate of uptake continued falling until the tenth day when the value reached one-ninth of the maximum pre-diapause value, and this fall was maintained for a further period of ten days. This shows that there was no further development in the embryos for at least fifteen days. The present study, therefore, supports the contention that every embryo enters diapause and remains in a diapause condition for a period of at least fifteen days. It also implies that in G. pennsylvanicus diapause is obligatory as has been pointed out already (see Sections V and VI). A similar kind of fall in respiratory metabolism has been noted in the embryos of Melanoplus differentialis (Bodine, 1929; Boell, 1935), Bombyx (Ashbel, 1930; 1932) and Lymantria dispar (Tuleschkov, 1935), although in these insects the fall takes place at different stages of development since their embryos enter diapause at different stages.

It has been shown previously (see page 92) that the embryos of G. pennsylvanicus enter morphological diapause after about 100 hours of incubation at 23-26°C. In this experiment the eggs were incubated at 28°C. and therefore it may be presumed that the eggs entered morphological diapause a little earlier - say, after 96 hours. From the studies of the respiratory metabolism it becomes clear that the embryos take one day longer to enter physiological diapause, and on the tenth day they enter a complete diapause

condition. As growth is resumed by the post-diapause embryos the oxygen consumption rises, however, the rate of increase is not the same throughout the entire post-diapause development. Further the embryos take about three days to reach that maximum rate of oxygen consumption which they experienced during pre-diapause state.

The respiratory quotient curve for the embryos of G. pennsylvanicus is very similar to that for the embryos of Melanoplus differentialis during the pre- and post-diapause stages (Boell, 1935). It is therefore probable in the case of G. pennsylvanicus, that during the first day of development (as in M. differentialis also) the chief metabolite for combustion is carbohydrate, that during the post-diapause period it is fat, and that fat is the chief metabolite during embryogenesis.

# IX. CONCLUSIONS

## IX. CONCLUSIONS

A comparative study of the sound-producing organs of Gryllus rubens, G. assimilis, G. veletis and G. pennsylvanicus reveals that the size of tegmen, harp,resonator and file have little or no taxonomic value. The tooth number and shape in the file, however, are useful additional characters for the identification of males of different species of Gryllus. There is some overlap in tooth number between G. pennsylvanicus and G. veletis, but even in these morphologically very similar species the majority of male specimens can be distinguished by the number and shape of the stridulatory teeth. There is also some small difference in the venation, particularly of the harp, at least in populations from the same area (Randell, unpublished), but it is not yet known if such difference is constant for the two species.

The stridulatory organs of both the tegmina in each species are equally efficient. The removal of the lateral or apical parts of either or both tegmina does not affect the modulation quality of the song; it only lowers the intensity. At the time of stridulation only about one-third of the file is engaged by the scraper and it is the inward movement of the tegmina that produces the song. The kind of song depends on the elevation of the tegmina. A pulse is produced when the tegmina move inward, followed by a non-pulse period due to outward movement of the tegmina. Each pulse consists of a number of 'spikes' (seen in audiospectrographs) each of which is formed by a tooth being struck.

The structure of the female and male reproductive organs of G. pennsylvanicus and G. veletis is described in brief, but the two species show no significant differences either in morphology or development. In this thesis, however, the shape of the male phallic structures has not been exhaustively studied. Certain differences in their structure do exist, at least in populations from the same area (Randell, unpublished), but it is not yet known if such differences are constant for the two species. Similarly the length of the ovipositor differs between the two species (Alexander and Bigelow, 1960), but not absolutely.

The rudiments of the ovipositor valves arise, one pair on each of the eighth and ninth sterna, in the third instar; those of the eighth do not divide and directly develop into the anterior ovipositor valves; those of the ninth divide to form the lateral and inner pairs of ovipositor valves. In the fifth instar a pair of lateral pouches are formed in the intersegmental membrane behind the seventh sternum, which later fuse to form the subgenital plate.

The oviducts are present in the first instar and extend up to the posterior margin of the seventh sternum, each terminating in a hollow ampulla. In the sixth instar the common oviduct develops as a thickening on the ventral surface of the dorsal wall of the subgenital plate, and the spermatheca as an invagination in the eighth sternum.

The male gonopore has a pair of lateral lips and a ventral fold, the latter remains upturned, covering the lateral lips and gonopore, and occupies the cavity of the spermatophore chamber. When the spermatophore is present in the genital cavity, the ventral fold is extruded and supports the spermatophore ventrally. The lateral lips become released and thus support the spermatophore from the sides.

In the sixth instar the intersegmental membrane behind the ninth sternum develops a pair of penis lobes in the dorsal part and a median sclerotization in the ventral part. The penis lobes do not divide but fuse together in the middle line, and from their junction evaginates the dorsal prong; the spermatophore guide is formed from the mesial margins of the penis lobes and the part of the membrane lying between them, while the bifid lateral prongs from the sides of the guide. The ventral sclerotization forms the ventral fold.

The vasa deferentia are present in the first instar extending up to the posterior margin of the ninth sternum each terminating in a hollow ampulla. The left ampulla overgrows the right one and becomes looped. The ejaculatory duct develops in the third instar in the intersegmental membrane behind the ninth sternum and later extends and fuses with the ampullae of the vasa deferentia. The accessory glands arise from the posterior fused part of the ampullae of the vasa deferentia.

It is conclusively shown that the ovipositor of the female and the phallic organs of the male arise as new structures in the post-embryonic stages and have nothing in common with the embryonic abdominal appendages which are resorbed long before hatching.

The embryo of G. assimilis arises at the posterior pole of the egg after about 36 hours of incubation at 23-26°C. A brief account of the morphological development of the embryo is given and the characteristic features of the various stages of the living embryo, when inside the egg, are also described.

Blastokinesis has been studied in the living egg. Anatrepsis begins on the third day of incubation and becomes completed in about 21 hours, while katatrepsis starts on the seventh day and takes about one and a half day to reach completion. The movement of the posterior part of the abdomen is a most interesting feature of katatrepsis.

The amnion and serosa are formed by the end of the first day of incubation, the development of the serosal cuticle begins during the second day and by the end of the fourth day the cuticle becomes sufficiently thick to permit separation from the chorion. The ental membrane arises from the sides of the trough-like dorsum of the embryo on the fifth day of incubation.

In G. pennsylvanicus development is arrested when the embryos are in any one of the three stages completed in 92-100 hours of incubation at 23-26°C. but the stage at which it enters diapause

is not attained until about 100 hours. Further development of the embryo beyond this "diapause stage" may occur under conditions of constant temperature but it is very irregular. Eggs which have completed diapause resume regular development similar to that of non-diapause species. There may be slight development during the period of cold treatment, probably to bring embryos of earlier stages of development into the diapause condition.

The morphological development of the embryos of G. pennsylvanicus is more or less similar to those of G. veletis, G. assimilis and G. rubens, provided they are given cold treatment for a minimum period of three months at 6-7°C.

The eggs of the four species of Gryllus are so similar morphologically that it is not possible to distinguish those of one species from those of another. The embryos also are almost indistinguishable; only at a very late stage can those of G. pennsylvanicus be separated from those of G. veletis and the other species by the presence of a brown band at the base of the post-pedicel of the antenna and by the orange coloured cerci.

G. pennsylvanicus in nature has a univoltine life cycle. In the laboratory its eggs enter diapause after about four days of incubation at 28°C. when the embryos are in the post-anatrepsis stage. The diapause is obligatory, but the so-called 'intensity' of diapause varies greatly in different eggs. Some hatch 28 days after oviposition while others require 140 days when incubated continuously at 23-28°C., under these conditions about 50 per cent

of the eggs hatch. A period of cold treatment increases the percentage of hatching, the increase being greater as this period is lengthened.

In eggs exposed to 6-7°C. for varying periods after incubating for periods of four to thirty-eight days at 28°C., the effect of the cold treatment is to reduce the 'intensity' of diapause. Cold treatment for 90 days or more causes virtually complete termination of diapause. Such eggs require further incubation at 23-26°C. for about fourteen to sixteen days to complete their development. Fluctuating warm and cold temperature on the eggs has a more or less similar effect to that produced by more stable temperature regimes and does not have any demonstrable effect on the resumption of morphogenesis during the period of treatment.

Eggs laid by young females (i.e., early in a season) have a longer pre-hatching period than those laid by middle-aged females (i.e., late in a season). The longer pre-hatching period needed by the former thus minimizes the chances of their hatching before the onset of winter and the resultant winter-killing of the nymphs which are not cold-hardy.

The behavior of the eggs, as observed in the laboratory, is presumably of great significance in the life of the cricket in the field and the different faculties, developed by the eggs, enable the species to overcome the rigours of cold at every stage of embryonic development. One physiological effect of the cold treatment (which may be indirect) appears to be that the yolk

becomes semi-fluid, thus possibly allowing its easier assimilation by the embryos so that growth is speeded up with the result that almost all eggs hatch within a relatively short period of time. It is suggested that the non-availability of suitable food for the embryo may be one of the causes (though not necessarily the primary cause) of diapause in G. pennsylvanicus.

Pre-diapause eggs of G. pennsylvanicus, after being incubated for one to five days at 22-23°C. and then exposed to 6-7°C. for three months show differences in the percentage which hatch. Eggs incubated at 22-23°C. for one day only do not hatch and die, while the percentage of hatching of other eggs varies from 30 to 52 depending on the length of ~~the~~ initial exposure to 22-23°C. Most of the emergence is completed between thirteenth and sixteenth day of final incubation. Thus cold treatment of pre-diapause eggs for a period similar to that required by eggs which had already entered diapause terminates diapause almost completely, provided the embryos have passed the earliest stage of development. Pre-diapause eggs continue developing at 6-7°C. until they reach diapause stage. The physiological effect of the cold treatment on pre-diapause eggs is similar to that on diapause eggs, namely, the yolk becomes assimilable. It has been suggested that the term 'diapause development' coined by Andrewartha (1952) to explain the effect of cold on diapause eggs is misleading and may not be used for G. pennsylvanicus eggs.

Post-diapause eggs can withstand exposure to 6-7°C., but as the percentage of hatching is slightly reduced, the treatment appears to be detrimental to some extent. It has been suggested that the post-diapause embryos take about three days to become normal before resuming development.

The rate of oxygen consumption rises as the embryos of G. veletis grow and reaches a high value of 48 cu. mm. per 100 eggs per hour shortly before hatching; at the time of hatching the rate falls to a value of 42.

During the embryonic development of G. pennsylvanicus the rate of respiration continues rising until the fifth day, after which the rate falls off to about one-ninth on the tenth day. This value is maintained for a further period of ten days. In the post-diapause embryos the rate of oxygen consumption rises continuously throughout the entire period of development. The studies reveal that the diapause in G. pennsylvanicus is obligatory and that the embryos enter physiological diapause one day later than the morphological diapause and enter a complete diapause condition after a further period of five days. The post-diapause embryos take three days to reach the maximum rate of respiration of the pre-diapause period.

In conclusion, therefore, it may be stated that, although there are some minor morphological differences between post-embryonic G. pennsylvanicus and G. veletis, they have not so far proved very reliable for separating the two species. Differences in the morphology, biology and physiology of the embryos, however, show that the two are abundantly distinct.

# X. SUMMARY

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1. The size of harp, resonator and file of the stridulatory organs have little or no taxonomic value, but tooth number and shape in the file can be used in the identification of different species of Gryllus.

2. A comparative study of the sound-producing organs of G. pennsylvanicus and G. veletis reveals that there is a distinct difference in the means and the range of variation in the tooth number in the file between the two species, G. pennsylvanicus having more teeth on the average. Unfortunately the overlap in tooth number is so great that this character is of very limited value in distinguishing between them. The shape of the individual teeth, however, is very slightly different in the two species. Due to the overlap in tooth number the two species are very close in song character.

3. The stridulatory organs of both tegmina are equally efficient in all species of Gryllus studied. At the time of stridulation only about one-third of the file is engaged by the scraper and it is the inward movement of the tegmina that produces the sound. The kind of song depends on the elevation of the tegmina.

4. The structure and development of the reproductive organs of G. pennsylvanicus and G. veletis are virtually, if not absolutely, identical. The ovipositor of the female and the phallic organs of the male arise as new structures in the third and sixth instar respectively and are not formed from the embryonic appendages, which are resorbed long before hatching. The subgenital

plate of the female arises as a paired structure in the intersegmental membrane between the seventh and eighth sterna. The common oviduct of the female and the ejaculatory duct of the male also develop in the third and sixth instar respectively.

5. It is not possible to distinguish the eggs of G. pennsylvanicus from those of G. veletis. The embryos are also almost without characters which may help in distinguishing one species from the other; only at a very late stage can the embryos of G. pennsylvanicus be separated from those of G. veletis by the presence of a brown band at the base of the postpedicel of the antenna and the orange coloured cerci in the former species.

6. The embryo of G. assimilis (a species allied to G. pennsylvanicus and G. veletis and having a regular development) arises at the posterior pole of the egg. Anatrepsis and katatrepsis are completed in about 21 and 36 hours respectively. The amnion and serosa are formed on the first day while the serosal cuticle and ental membrane arise on the second and fifth day of incubation respectively.

7. The eggs of G. veletis continue regular development and hatch after about seventeen to twenty days when incubated at 23-26°C. In G. pennsylvanicus development is arrested after about four days at 23-26°C., i.e., they enter diapause in the post-anatrepsis stage. After remaining in this state for some time, some of the embryos may resume further development, but this is very irregular and the 'intensity' of diapause varies greatly in

different eggs. Some eggs hatch 28 days after oviposition while others hatch only after 140 days of incubation although laid at the same time.

8. However, when diapause eggs are exposed to 6-7°C. for three months or more and then finally incubated at 23-26°C. all hatch after about fourteen days. Thus the active developmental period (about 18 days in all) is more or less the same as that of the eggs of G. veletis. Pre-diapause eggs will continue to develop at 6-7°C. until they reach the diapause stage, after which their behaviour is similar to that of diapause eggs.

9. One physiological effect of cold treatment appears to be that the yolk becomes semi-fluid thus allowing its easier assimilation by the embryo. Non-availability of suitable food may thus be one of the causes of diapause.

10. Respiratory metabolism during embryogenesis in G. veletis continues rising until hatching time, i.e., it does not fall during development except just before hatching. In G. pennsylvanicus respiratory metabolism falls after the embryo has completed about one-fourth of its development (i.e., after the fifth day of incubation at 28°C.), continues dropping for some time (to about one-ninth of its former value by the tenth day), and remains at this low level for a further period of about ten days. During post-diapause development the oxygen consumption rises again continuously.

11. Although G. pennsylvanicus and G. veletis in the post-embryonic stages are so similar morphologically that it is not possible to separate them satisfactorily from each other, the characters of the late embryos may be used in distinguishing between them. In morphological development the embryos of the two species are quite distinct: those of G. pennsylvanicus cease further development soon after sinking into the yolk, while the embryos of G. veletis continue development without a break.

12. Further, eggs of G. veletis, laid at the same time, all hatch within three days of one another, whereas those of G. pennsylvanicus, also laid at the same time, always hatch over a longer period than three days, unless they are given a cold treatment for three months or more, after which the hatching period is reduced to about three days also.

13. In conclusion, it may be said that present studies reveal that the morphological, biological and physiological characters of the embryos appear to be the only characters so far discovered which are of practical value in separating the two species, G. pennsylvanicus and G. veletis from each other. Certain post-embryonic morphological and behavioural differences exist but they are too variable to be considered reliable at least until much more work is done in this field.

# XI. REFERENCES

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