The embryogenesis and embryonic variability of Aulocara elliotti Thomas (Acrididae, Orthoptera)
by Saralee Neumann Van Horn

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Entomology
Montana State University

Abstract:
The variability of A. elliotti embryos from laboratory reared populations of two successive years was investigated according to stage criteria formulated for this species. Statistically significant differences were found between these two populations with regard to stage-length, age-length, and age-stage relationships. In addition, comparisons were made between laboratory reared and wild population embryos, and between embryos from the same wild population from different years. Statistically significant differences were found in almost all instances with regard to length-stage relationships in the various populations. Experiments were conducted to test the requirements for diapause aversion and termination in this species.

An histological study of the embryogenesis, with emphasis on the neuroendocrine system, revealed the presence of several structures thought to be new to Acrididian literature. These included paired peripheral ganglia in the labrum, mandibles, and maxillae, and a single ganglion of paired labial origin located in the hypopharynx. Investigation showed that the embryological origin of the ventral head glands was lateral hypoderm between the bases of the mandibular and maxillary appendages (formerly thought to be the site of corpora allata origin).

The corpora allata appeared to arise from hypoderm near the basal dorsal wall of the anterior tentorial invagination. Labial glands (salivary glands) arose from lateral hypoderm between the maxillary and labial segments. All of these incretory organs and the peripheral ' ganglia arose prior to blastokinesis in A. elliotti as well as in five other Acrididian species examined. The differentiation of giant paired cells in the region of the protocerebral-optie lobe junctions and in the metathoraeic ganglia was first observed at Stage 19 when most embryos of A. elliotti entered the diapause state. The giant metathoraeic cells were found to he present and constant in their position throughout the embryology and persist in the adult, as well as in the embryos of seven other Acrididian species. The development and maturation of tissues and organs at all stages during the embryogenesis was described.

Histological comparisons of diapause A. elliotti embryos of young and advanced ages revealed that deposition of fat body and increase in pleuropodial cell size continued during diapause. Study of embryos retarded in their external stages of development in relation to their ages showed that certain internal differentiation processes continued when external growth and mitotic activity had ceased.

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Entomology

Approved:

Head, Major Department

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MONTANA STATE COLLEGE
Bozeman, Montana

June, 1963

ACKNOWLEDGMENTS

To my Advisor and Chairman of my committee, Dr. James H. Pepper, I want to acknowledge my indebtedness and offer my deep gratitude for his interest, confidence, and assistance throughout the course of this study. I want to thank Dr. Pepper, Dr. Harold Watling, Dr. P. D. Skaar, and Dr. David Blackmore for their advice and help in preparation of the manuscript. My gratefulness is offered to the members of the Zoology-Entomology Department at Montana State College, especially Mr. Ellsworth Hastings, who gave assistance many times during this study. I wish to thank Dr. Charles Mode and Dr. Glen Ingraham for help with the statistical treatment of the data.

My gratitude is offered to the Anti-Locust Center in London, particularly Dr. P. Hunter-Jones, for sending embryonic specimens of Locusta migratoria and Schistocerca gregaria, to the United States Department of Agriculture Bureau of Entomology, Bozeman, Montana, especially Mr. R. Connin and Mr. Frank Cowan, for embryos of Melanoplus bruneri, Melanoplus bivittatus, Melanoplus sanguinipes, and Camnula pellucida, and to Dr. Carl Rettenmeyer at Kansas State University at Manhattan for obtaining embryos of Melanoplus differentialis for this study.

My sincere appreciation is offered to Dr. Guenter Schmalz, Chairman of the Department of Modern Languages at Montana State College, for all his help with the German translations and to Dr. Orazio Guisti, Department of Modern Languages, Arizona State College, Flagstaff,

Arizona, for his help with the Italian manuscripts.

I want to acknowledge the fine work of Mr. Don Fritts, who helped with the photomicrographs.

And finally, I would like to thank the members of my family for their patience and understanding while this work was in progress.

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ABSTRACT

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An histological study of the embryogenesis, with emphasis on the neuroendocrine system, revealed the presence of several structures thought to be new to Acrididian literature. These included paired peripheral ganglia in the labrum, mandibles, and maxillae, and a single ganglion of paired labial origin located in the hypopharynx. Investigation showed that the embryological origin of the ventral head glands was lateral hypoderm between the bases of the mandibular and maxillary appendages (formerly thought to be the site of corpora allata origin). The corpora allata appeared to arise from hypoderm near the basal dorsal wall of the anterior tentorial invagination. Labial glands (salivary glands) arose from lateral hypoderm between the maxillary and labial segments. All of these incretory organs and the peripheral ganglia arose prior to blastokinesis in A. elliotti as well as in five other Acrididian species examined. The differentiation of giant paired cells in the region of the protocerebral-optic lobe junctions and in the metathoracic ganglia was first observed at Stage 19 when most embryos of A. elliotti entered the diapause state. The giant metathoracic cells were found to be present and constant in their position throughout the embryology and persist in the adult, as well as in the embryos of seven other Acrididian species. The development and maturation of tissues and organs at all stages during the embryogenesis was described.

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GENERAL INTRODUCTION

Few subjects in the field of entomology have engendered more interest than the Acrididae. The titles of researches concerning this family make up a large volume, the Bibliographia Acrididiorum (Roonwal, 1961).

Characteristically, the Acrididae show dramatic fluctuations in population size. The explanations for these fluctuations are diverse and often conflicting. Some authors propose strict environmental regulation, while others deny any environmental role in population changes. To maintain that current environment plays no role in regulating population size would be to reject evolutionary theory as developed since Darwin's time. There is increasing evidence, however, that animal populations are auto-regulative in size, by means of a series of physiological changes induced by the stress of crowding.

In some mammals the self-limiting mechanism is thought to operate in the following manner: At a certain population level, density stress triggers changes in hormonal secretions. These changes, in turn, adversely affect the reproductive capacity of the population causing its decline (Christian, 1957).

There is evidence which favors the hypothesis that an auto-regulative mechanism may be operative in insect populations. A number of workers have demonstrated that reproductive processes in insects are dependent upon endocrine activity (Wigglesworth, 1936;

Weed, 1936; Johansson, 1955; Scharrer and von Harnack, 1956, 1958). Albrecht et al. (1958) and Albrecht (1959) demonstrated that crowding had a profound effect upon egg production in locusts as well as upon the morphology of their progeny. The importance of various environmental factors, such as photo-period, food conditions, temperature, and social conditions in determining the hormone activity of endocrine glands in insects has been emphasized by DeWilde (1961). It was shown by Hodgson and Geldiay (1959) and by Highnam (1962) histologically that stimulation of the nervous system brought about changes in the release of neurosecretory products. Highnam further showed with locusts that reproductive processes were influenced by these changes. It appears that environmental factors exert their influence via the neurosecretory cells of the insect brain which regulate the activity of subordinated glands.

In Montana numbers of Aulocara elliotti Thomas have been observed to vary greatly and yet extensive data failed to reveal any direct environmental cause for these fluctuations which was of any predictive value. If fluctuations are due in part to a hormonally mediated auto-regulative mechanism, one might expect to find differences in the development of embryos from populations at various stages of the cycle. Therefore, the following study was begun to establish the limits of natural variability in different populations and to investigate, for comparative purposes, the embryological morphogenesis of this species, in particular, the neuroendocrine system.

EXTERNAL MORPHOGENESIS OF A. ELLIOTTI EMBRYOS

Introduction

The eggs of A. elliotti are laid in oothecae which contain an average of eight eggs. The fixed newly laid eggs measure about 4.5 to 5.5 mm. in length and about 1.5 mm. in width at their widest point and are bright yellow. Like other Orthopteran eggs they are rich in yolk, meroblastic, undergo superficial cleavage, and are of the centrolethical type peculiar to the Arthropods.

The morphogenesis of the embryos of the Acrididae may be divided into several periods for convenience of study. In the first, the pre-diapause, the embryo is formed and grows with its head toward the hydropyle at the posterior end of the egg. At the end of this first period many species, including A. elliotti, Melanoplus differentialis Thomas, and Camnula pellucida Scudder, enter a quiescent second period called the diapause during which metabolism is greatly depressed and mitotic activity is greatly reduced. The third period ensues after diapause termination and therefore, can be called post-diapause. During this phase the embryo undergoes a revolution around the posterior pole of the egg and after this is completed, development continues uninterrupted until the definitive embryo is ready to-hatch.

Other species of grasshopper embryos enter the-diapause period shortly before they are ready to hatch, for example, Melanoplus bivittatus Say, Melanoplus sanguinipes F., Melanoplus packardii Scudder, and Melanoplus bruneri Scudder. Some other species may possess no diapause period in their embryology, but undergo revolution without delay when

they mature to that stage. Locusta migratoria migratorioides R. and F. and Schistocerca gregaria Forskål., belong to this latter type. In Montana, species such as A. elliotti, possessing an obligatory diapause overwinter in the egg stage, hatch in the spring, mature and reproduce in the summer, thus having but one generation per year.

Materials and Methods

To obtain known-age eggs for the study of A. elliotti embryogenesis, adult grasshoppers were collected from a wild population in the vicinity of Maudlow, Montana in late May and early June of 1959. Three pairs of grasshoppers were placed in each of a series of screened cages, three inches wide, eight inches long and ten inches high. Fine sifted soil was provided for oviposition in removable pans attached to the unscreened bottoms of the cages. These cages were arranged on tables in a greenhouse in which inside temperatures fluctuated diurnally, sometimes reaching extremes later found to be unfavorable for maintaining the grasshopper population.

The eggs were collected at 24 hour intervals and fresh Western Wheatgrass, Agropyron smithii Rydb., the preferred food plant of A. elliotti (Anderson, 1962) was supplied at this time. Replacement animals were obtained from the same field population for use in the event of mortality in the oviposition cages. Egg pods were obtained first on July 19, oviposition increased in frequency during August, and the last pod was collected September 20.

The following summer an additional series of known age embryos

was obtained for comparative purposes using somewhat different techniques. A mesh cover was installed on the greenhouse roof to modify inside temperatures and more spacious oviposition cages were utilized. Cylinders, 20 inches high made of clear plastic, were attached to a circular plywood bottom, one square foot in area, which was elevated on four inch wooden legs. Holes were bored around the periphery of this bottom to provide ventilation and allow the insertion of vials in which food was placed. A nine inch hole in the center of this base contained a removable oviposition pan filled with sifted soil, and the top of the cage was covered with a piece of cheese cloth. Collection of eggs and feeding of adults was accomplished in the same way as the previous year.

Each of the daily collections of egg pods was placed in a plastic vial on moist filter paper, labeled and incubated at a constant temperature of 25° C. From these known age pods, embryos representing successive 24 hour intervals of growth were obtained. Two pods of the same age were removed from incubation each day, the eggs separated from the pods, fixed in hot Bouin's solution (approximately 40° C) for one hour, cooled and stored overnight in the same solution. These eggs then were rinsed several times with and stored in 70 percent ethyl alcohol. The daily fixation of two pods of embryos was continued through the ninetieth day to obtain representative embryos at 24 hour intervals long after they had entered the diapause condition. All of the remaining egg pods then were incubated at 5° C. Single pods were taken out and returned to 25° C after 43, 46, and 63 days of cold exposure, but these conditions were found to

be inadequate to break the diapause state. After 111 days at 5° C, additional pods were returned to 25° C and daily fixation of two egg pods was resumed. Some difficulty still was encountered in getting many of the embryos to break diapause, but a sufficient number of rotated animals finally was obtained to work out a progressive series of post-diapause stages. These embryos were fixed and stored in the same manner as the pre-diapause series.

All specimens were removed from the egg membranes, and individuals which appeared to represent distinct morphological stages were drawn free-hand with the aid of an ocular grid in a binocular dissecting microscope and centimeter graph paper. Because of the extreme variation in the rate of development of different individuals, morphological differentiation and maturation could not always be related to the age of the embryo or its length. This was in agreement with the findings of Slifer (1932) with M. differentialis. When the representative series of drawings was completed it was found that some of the embryos drawn were at the extremes of the range of lengths for their stage. Therefore, a drawing of an embryo intermediate in length was included so that the series would more nearly represent the growth stages of one embryo. While all of the drawings do not necessarily represent the mean length of the embryos in a given stage, the lengths portrayed fall within the range of the stage. Embryos in Stages 1 through 21 were drawn at a magnification of 60 X and those in Stages 22 through 27 at a magnification of 30 X. The 60 X drawings then were photographically reduced to make them equivalent to

those drawn at 30 X.

Embryonic Staging

A number of workers have published papers which describe and illustrate external morphogenesis of Acrididian embryos. Among these are Wheeler (1893), Xiphidium ensiferum Scudd., Slifer (1932), M. differentialis, Nelsen (1934), M. differentialis, Roonwal (1936b), L. migratoria migratorioides, Jannone (1940) from Roonwal (1961), Dociostaurus maroccanus Thnb., Steele (1941), Austroicetes cruciata Sauss., Jhingran (1947), S. gregaria, Bodenheimer and Shulov (1951), D. maroccanus, Shulov and Pener (1959), L. migratoria migratorioides, Carlson (1959), Chortophaga viridifasciata DeG., and Riegert (1961), M. bilituratus Wlk. (now sanguinipes F.).

Two approaches have been used to portray the external development of Acrididian embryos. One approach, used by Slifer (1932) and Riegert (1961), defines a stage in terms of embryonic age, each stage corresponding to a given day of incubation under known temperature conditions. Salt (1949) also followed this plan in his description of the development of three species of Melanoplus: M. bivittatus, M. mexicanus (now sanguinipes F.), and M. packardii. Illustrations were not included in Salt's paper. The other approach, used by Steele (1941) and Shulov and Pener (1959), defined stage on the basis of readily distinguished morphological differences, irrespective of the ages of the embryos. Because of the wide variation in morphological differentiation encountered in embryos of A. elliotti of any given age, it was decided to use the second method.

The various authors differ markedly in their designation of the stages

through which Acrididian embryos develop. Slifer (1932), for example, divided morphogenesis into 24 stages, with one stage used to illustrate rotation in the egg. The development of S. gregaria was divided into 18 stages, one portraying blastokinesis, by Jhingran (1947). Shulov and Pener (1959) used 23 stages, three of which concerned rotation. Matthée's treatment (1951) of the embryology of Locustana pardaline Wlk. (only partially illustrated) divided the development into 30 stages to the diapause. Salt's description (1949) of three species divided development into 24 daily intervals beginning with the fourth day, and designated two intervals for revolution processes. Steele's account of A. cruciata divided development into 16 stages, two of which concerned rotation. Riegert used 17 daily intervals with the non-diapause form of M. bilituratus; one daily interval was said to be required for revolution.

In the present study the morphogenesis of A. elliotti embryos has been divided into 27 stages with four stages representing the revolution around the posterior pole of the egg. The differentiation of the external genitalia is sufficiently advanced by the 19th Stage that the sexes can be separated. Therefore, two drawings have been made of Stages 19, 24, 25, 26, and 27. However, the genitalia were not visible during rotation of the embryo and therefore, only one drawing was made of the embryo in Stages 20, 21, 22, and 23.

The early developmental stages of A. elliotti are seen to be very similar to those described for M. differentialis and L. migratoria. However, close comparison of later stages of the three species reveals

characteristic differences in general appearance which are not confined to any single morphological structure. The illustrations and descriptions pertaining to the embryonic stages of A. elliotti are presented on Plates I through VIII, drawn from the ventral aspect of the embryo. A discussion of the differentiation of external genitalia follows the Plates.

Plate I

Embryonic Stages of *A. elliotti*

Stage 1. The embryo is present as a germinal disc lying on the surface of the yolk directly under the hydropyle region at the posterior end of the egg.

Stage 2. There is an elongation of the embryo toward the anterior end of the egg.

Stage 3. The protocorm equals the protocephalon in length.

Stage 4. The protocorm is at least twice the length of the protocephalon.

Stage 5. There is evidence of invagination in the region of the stomodeum between the two lobes of the protocephalon.

Stage 6. Segmentation begins to appear along the edges of the protocorm.

Stage 7. The labrum is visible above the stomodeal invagination; the antennae appear as evaginations lateral to the stomodeum. Protognathal and thoracic segments are distinct. There is indication of segmentation of the first abdominal segments.

Stage 8. The thoracic appendages are larger than those of the mouthparts. Each thoracic segment is distinctly separated across the sternum. The antennal evaginations are bulbous turning medially below the labrum. There is evidence of further abdominal segmentation beginning.

Stage 9. The proctodeal invagination appears. The thoracic appendages show evidence of segmentation.

Stage 10. The abdomen is completely segmented. There is evidence of segmentation of the maxillary and labial appendages.

Stage 11. The thoracic appendages turn medially. Labial palpae now lie lateral to the inner ligular lobes. Abdominal segmentation is distinct across the sternal region.

Stage 12. The thoracic appendages now have distinct tarsal, tibial, and femoral divisions.

Plate II

Embryonic Stages of *A. elliotti*

Stage 13. Paired labial mouthparts now lie medial to the maxillary palpae. The vertex and frons of the head have enlarged so that the optic regions appear to be caudal to them.

Stage 14. The abdominal appendages are distinct. The mesothoracic and metathoracic legs are turned medially so that the tarsae lie parallel to each other on the sternum.

Stage 15. The metathoracic legs are bending toward the head at the tibial femoral joint. Three segments are visible on the labial palpae. The labrum has concave curvature along its lateral edges.

Stage 16. The tarsae of all legs lie in a parallel position on the sternum.

Stage 17. The labro-clypeal suture begins to appear as a notch on the dorso-lateral margins of the labrum. The mesothoracic and metathoracic legs are both drawn upward.

Stage 18. The first evidence of red pigmentation occurs along the lateral margins of the eyes. All tibial-tarsal joints are drawn toward the head with the prothoracic and mesothoracic tibia and tarsae parallel.

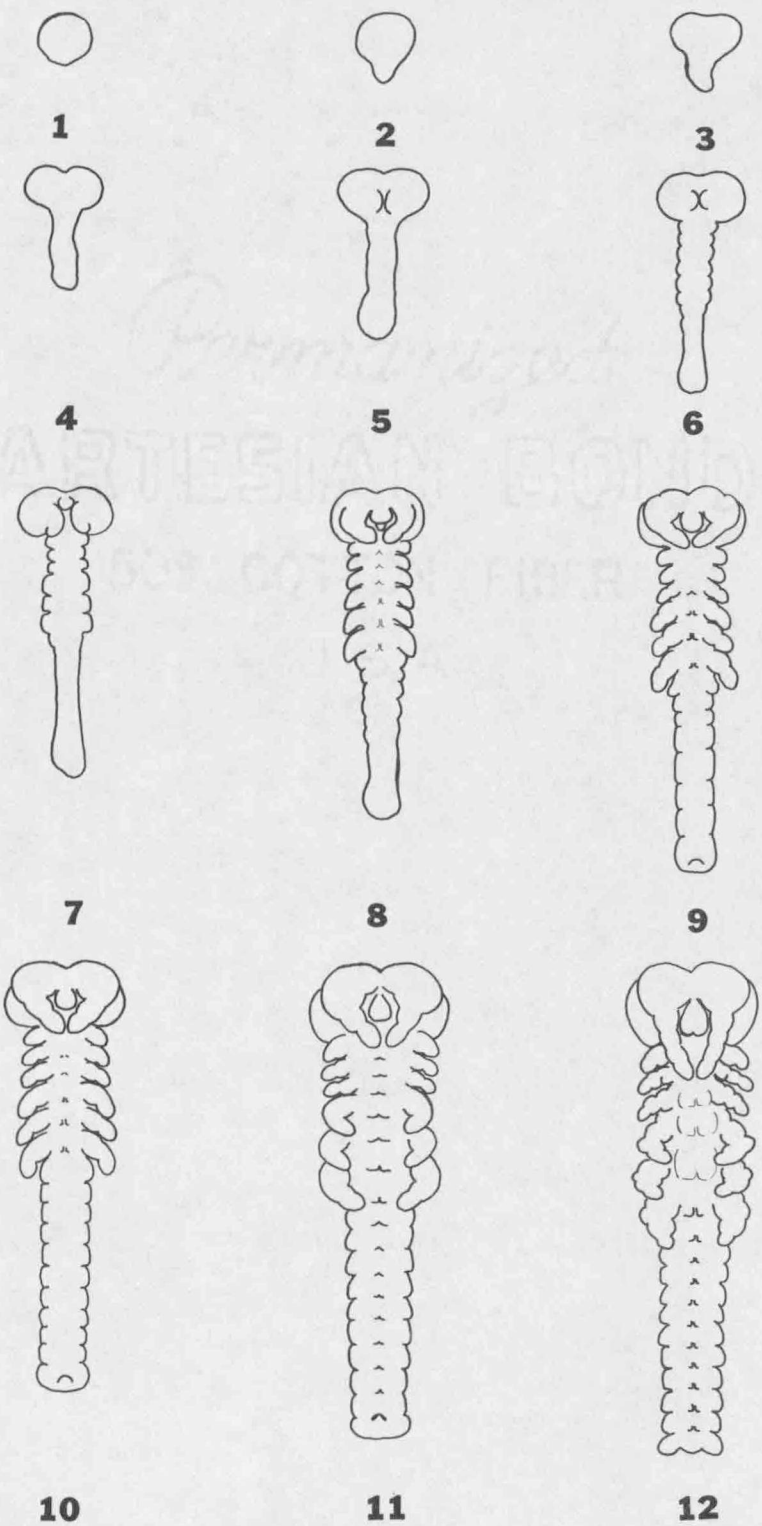
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Plate III

Embryonic Stages of *A. elliotti*

Stage 19. Male (right) and female (left). Distal tibial spines are visible on the metathoracic legs. This is the stage at which the majority of embryos enter diapause and at which the sexes first can be easily differentiated.

Stage 20. The embryo begins revolution. The head and mouthparts are turned around the posterior pole of the egg and are visible on the dorsal surface of the yolk.

Stage 21. The prothoracic and mesothoracic segments now are turned and are visible dorsally.

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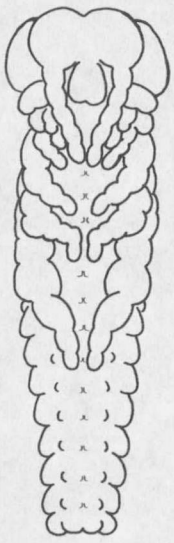

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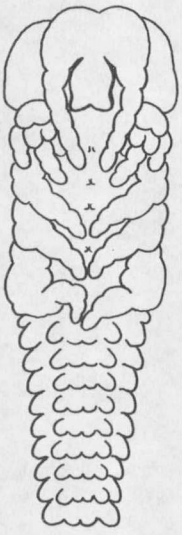

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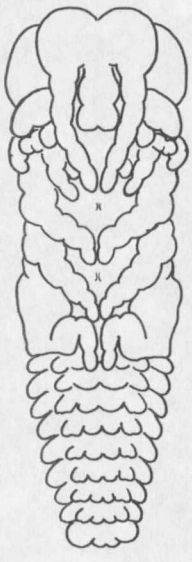

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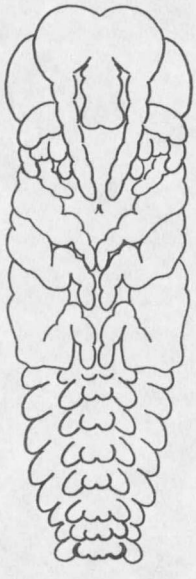

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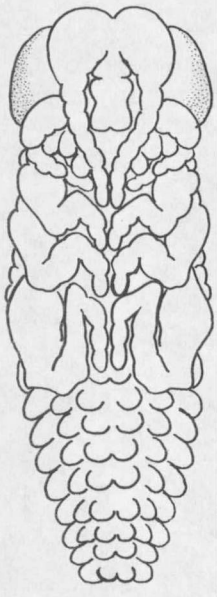

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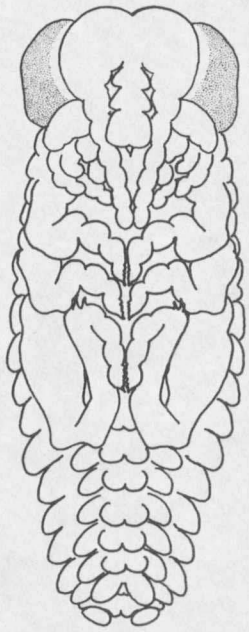
Plate IV

Embryonic Stages of *A. elliotti*

Stage 22. All three thoracic segments now are turned around the posterior pole of the egg and are visible dorsally.

Stage 23. The whole abdomen now is turned; revolution is completed. The embryo is approximately 2/3 the length of the egg. Labial ligula are visible posterior to the tip of the labrum.



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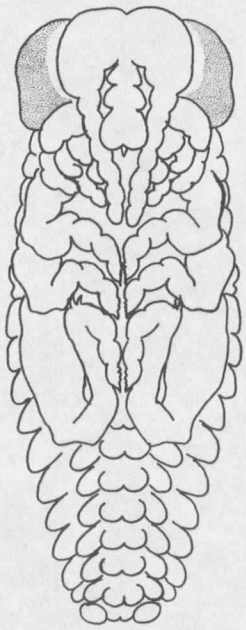

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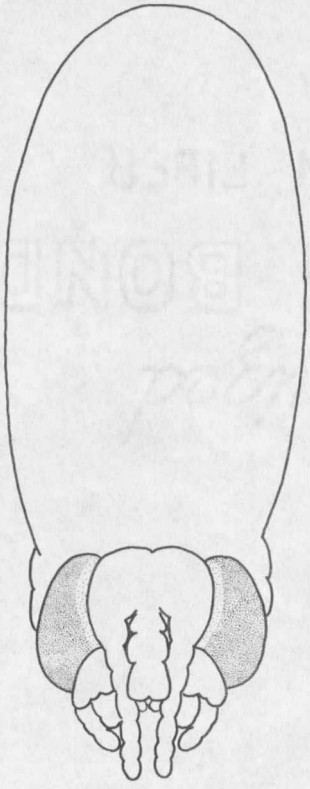

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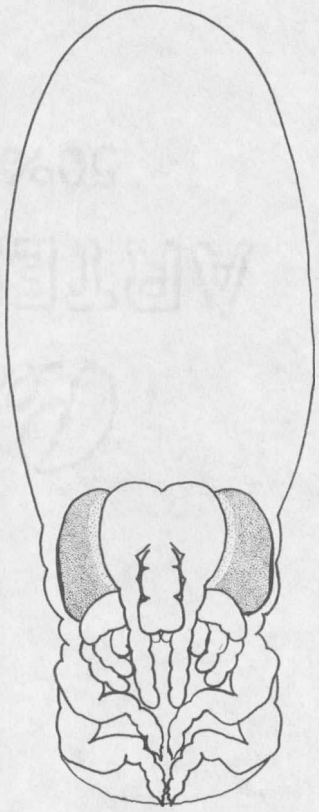

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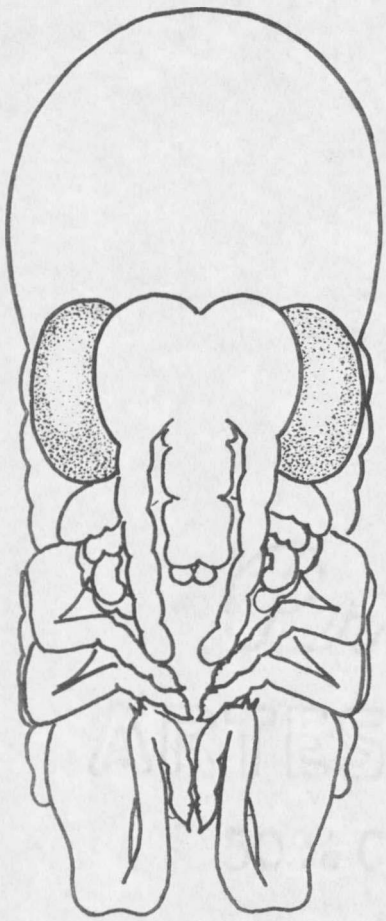
Plate V

Embryonic Stages of *A. elliotti*

Stage 24. Male (right) and female (left). The rudiments of teeth are visible on the posterior edges of the mandibles. Only a small portion of unengulfed yolk remains at the anterior end of the egg above the embryo.



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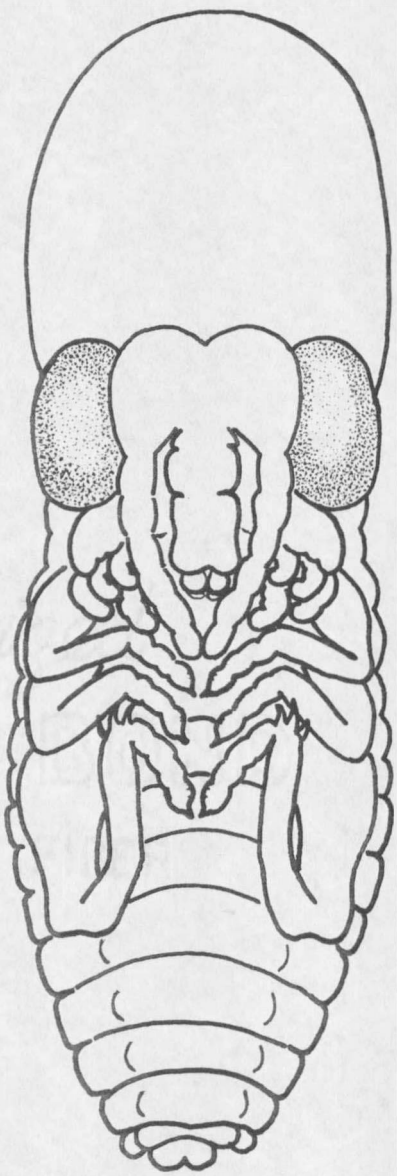

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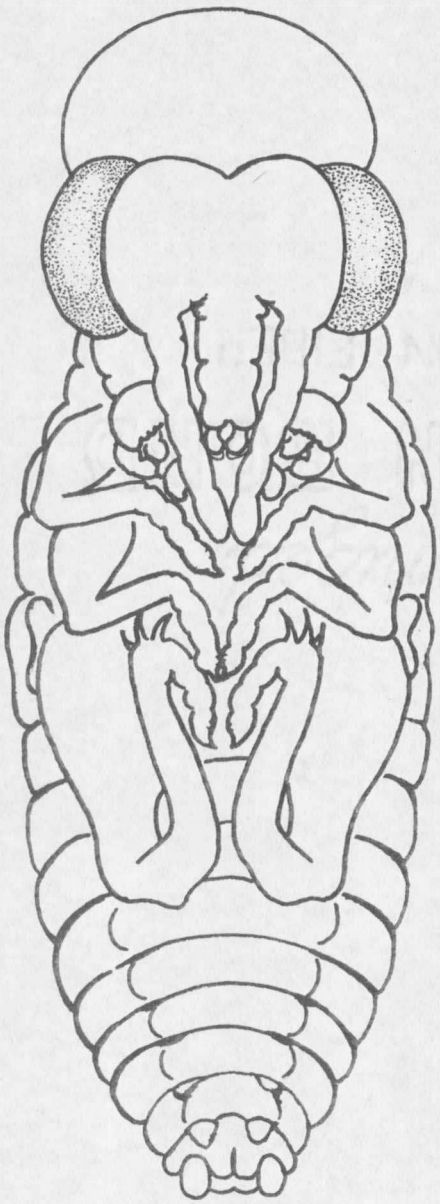
Plate VI.

Embryonic Stages of *A. elliotti*

Stage 25. Male (right) and female (left). Dorsal closure is completed.



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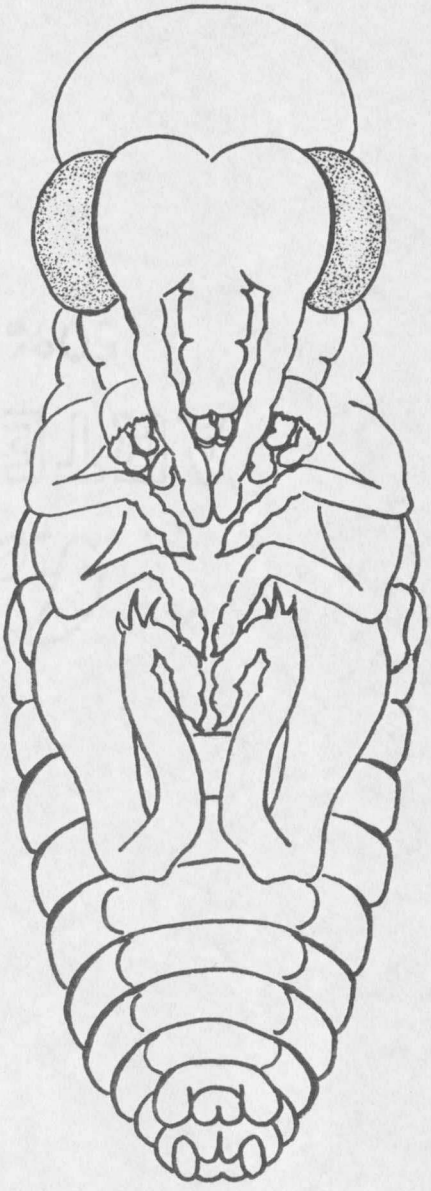

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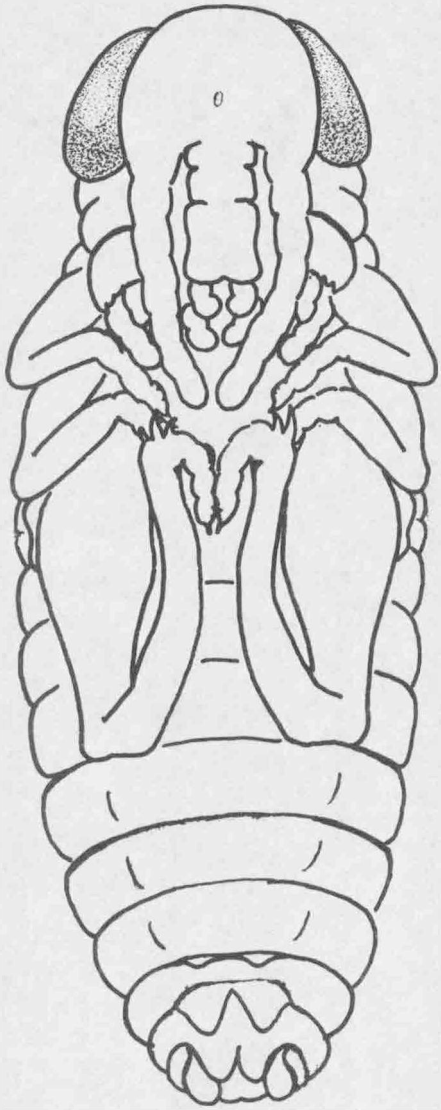
Plate VII

Embryonic Stages of A. elliotti

Stage 26. Male (right) and female (left). The mandibular teeth now are sclerotized and pigmented dark yellow-brown. Spines are visible along the medial edges of the metathoracic tibia.



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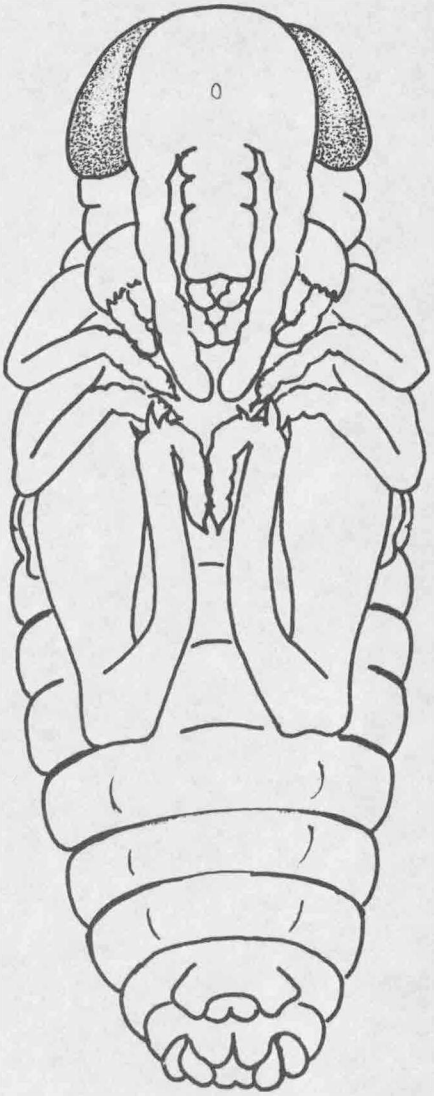

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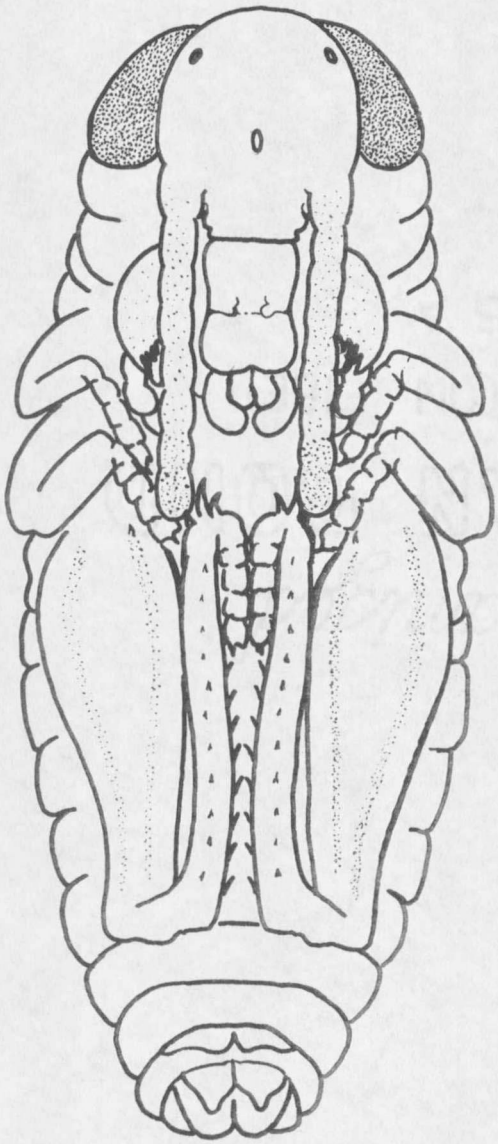
Plate VIII

Embryonic Stages of *A. elliotti*

Stage 27. Male (right) and female (left). This stage represents the definitive embryo. Integumental pigmentation is completed. Tibial and tarsal claws and spines are pigmented on all legs.



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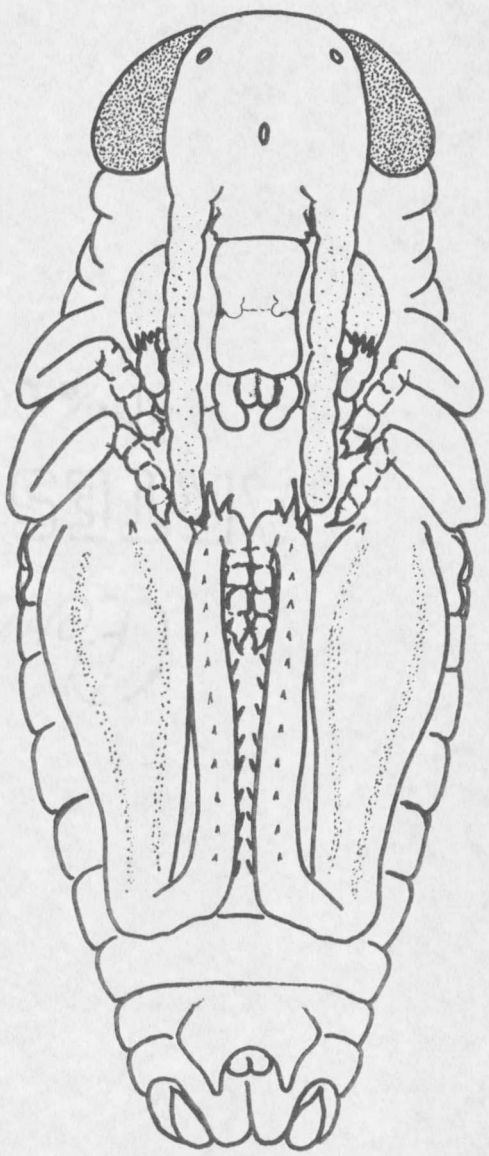

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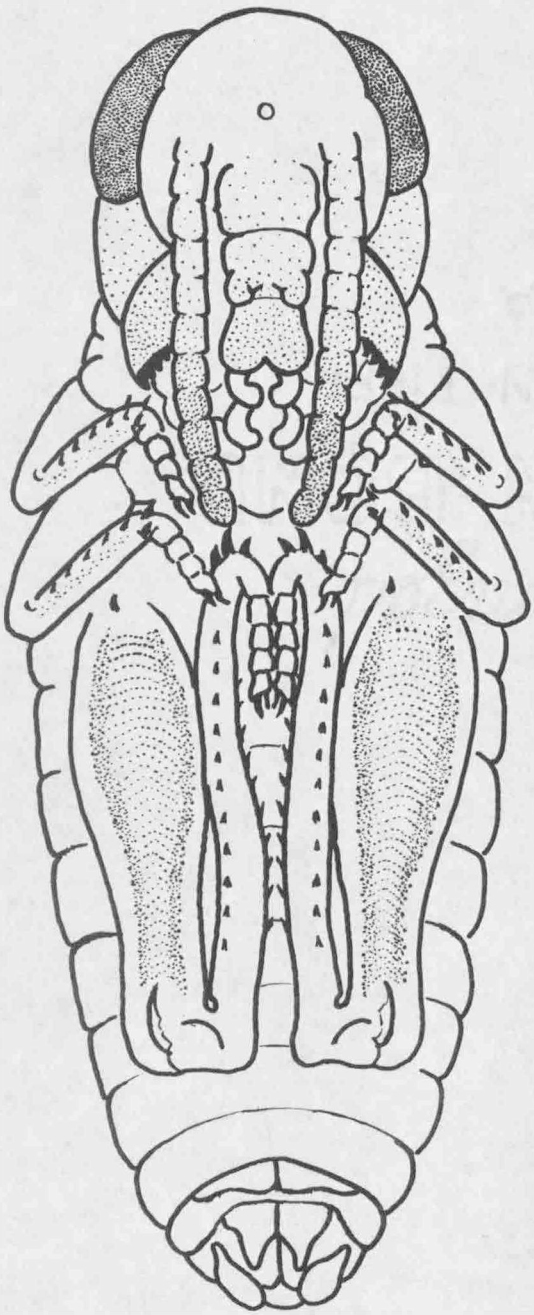
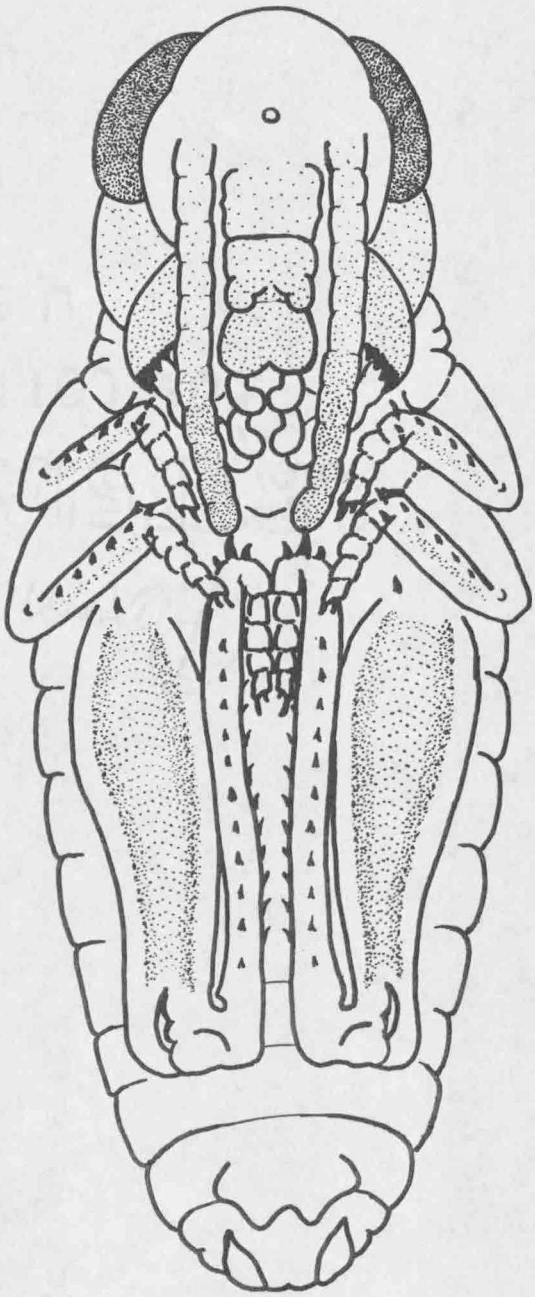


27



27

Differentiation of the External Genitalia

Each of Stages 19, 24, 25, 26, and 27 have been illustrated with the drawings of two embryos to show the progressive development of the external genitalia of both sexes of A. elliotti.

At Stage 19 the female embryo is easily distinguished from the male of the same stage by the distinct involution of the abdominal appendages of the tenth segment. At this stage these tenth appendages of the female already are many times smaller than those of the eighth and ninth, while in the male they are equal in size to those of the ninth. In both sexes the eleventh abdominal appendages are distinct and eventually form the cerci.

In the post-revolution female embryo, Stage 24, there has been a loss of all abdominal appendages except those of the eighth and ninth segments, whereas in the male only those of the ninth and tenth remain distinct.

At the time of dorsal closure, a decided change has occurred in the form of the genitalia of both sexes. In the female, Stage 25, the appendages of the ninth segment are pointed posteriorly; those of the eighth segment appear only as very slight protuberances. These two sets of appendages will form the ovipositor blades of the adult female animal. In the male, the appendages of the ninth segment have fused to form a rudimentary genital plate, and the tenth appendages, which have begun to involute, are visible behind the posterior curvature of the genital plate.

Stage 26 embryos demonstrate growth in the genital appendages of both

the eighth and ninth segments of the female, and further development of the genital plate of the male. Further involution of the tenth appendages in the male has occurred.

In the definitive embryo, Stage 27, there is some advance in the development of the eighth appendages in the female which now appear as small, divided plates. The appendages of the ninth are at least three times the length of those of the eighth and are acutely pointed. The tenth appendages have completely disappeared in the definitive male embryo, and the genital plate has assumed a somewhat cup-shaped contour, curving dorsally. The posterior margin of the genital plate is still distinctly concave.

When compared with the drawings of the sex differentiation of M. differentialis, Nelsen (1931) and Else (1934), the pre-revolution embryos of A. elliotti appear to be slightly more advanced in their genital development. Stage 19 male and female A. elliotti embryos appear to be intermediate in their development between the pre- and post-revolution embryos of M. differentialis. At hatching, however, the genital appendages of the two species appear to be quite comparable in their differentiation.

EMBRYONIC VARIABILITY IN LABORATORY REARED AND WILD POPULATIONS OF A. ELLIOTTI

Introduction

The great variation observed in the rates of development of embryonic grasshoppers at constant temperatures has been reported by many authors. Parker (1930) gave data for the incubation periods of Melanoplus mexicanus Sauss. (now sanguinipes F.) eggs at various temperatures as follows: At 22° C the incubation period ranged from 36 to 213 days, at 27° C from 23 to 32 days, and at 37° C from 23 to 92 days. He also found that the amount of variability and total incubation time was reduced if newly laid eggs were exposed to varying durations of temperatures at 0° C. For example, after 30 to 240 days at 0° C when eggs were transferred to 27° C, the incubation periods requred for hatching ranged from 15 to 26 days. Parker concluded that exposure to cold accelerates the developmental rate when embryos are returned to higher temperatures.

Slifer (1932) described the variation in the rates of development of Melanoplus differentialis Thos. embryos in terms of the number of days ahead or behind the mean stage of development at a given day. Compared to the mean, development was found to be retarded as many as ten days, but only advanced two days in embryos of this species.

Pener and Shulov (1960) were able to show considerable developmental variation during different years in embryos of Calliptamus palaestinenesis Bdhmr. reared under various temperature and humidity conditions. These authors, in a 1961 paper, reported the following durations of incubation

for different species:

Portions of Table I, p. 145, from Shulov and Pener (1961) "The duration of noninterrupted development of eggs in some Acrididae at 27° ± 1°."

	Mean	Range (Min-Max)
Nomadacris septemfasciata (Serv.)*	32 days	27 - 40 days
Acrotylus insubricus (Scopoli)*	23.4 days	22 - 26 days
Pareuprepocinemis syriacus (Br.)*	43.6 days	42 - 57 days
Anacridium aegyptium (L)*	35.2 days	33 - 46 days
Schistocerca gregaria (Forsk.)	17.63 days	15 - 21 days
Locusta migratoria migratorioides (R. and F.)	16 - 17 days	14 - 18 days

*Preliminary figures

Roonwal (1937) in his account of the embryology of L. migratoria migratorioides reported that embryos of that species developed in 13 days if reared at 33° C. LeBerre (1951) found that eggs of non-diapause strains of L. migratoria gallica hatched within periods varying from 13 - 140 days at temperatures of 33° C and 16° C respectively. Faure (1932) incubated eggs of Locustana pardalina Walker to obtain information on developmental rates at different temperatures and found a minimum incubation time of eight days at 31° C. He observed delayed hatching of healthy embryos of this species kept for 19 months with favorable temperature and moisture conditions. Salt (1949) described an average developmental period of 24 days in the three species of Melanoplus which

he studied, but he stated by way of clarification, "Hatching does not necessarily take place as soon as the embryo reaches the 24 day stage. It appears in most cases that a stimulus to hatch may be necessary; if so, its nature is not known." Riegert (1961) working with a non-diapause strain of Melanoplus bilituratus Wlk. selected from laboratory animals reared through 12 generations observed a, "rigid schedule of development", in this species. Only a three day range in the hatching time was reported in a population of 2000 eggs.

Studies describing variability in the stages of grasshopper embryos from field collected eggs are less numerous than those reporting variability in hatching rates and incubation requirements of laboratory reared embryos. Steele (1941) in studies with Austroicetes cruciata Sauss. reported a variation of three stages in the embryos from eggs of two different field populations in South Australia. Popov (1959) studied the incubation durations of field populations of L. migratoria migratorioides eggs from three different localities in Africa. The duration of incubation ranged from 21 to 33 days with an average of 27 days. Popov indicated that in a small number of pods from one population several eggs failed to develop although they appeared turgid and healthy. "These eggs had grown little in comparison with newly laid ones, but had not yet undergone blastokineses." One pod of these under-developed embryos was enclosed in a cylinder of soil and kept 86 days from the time of laying. Following this extended incubation period, examination disclosed that these eggs were still in the arrested condition of development.

The addition of greater amounts of water was thought to finally have induced their continued development and most embryos hatched 102 days after laying. Popov further suggested that, "the presence of a small number of under-developed eggs in an otherwise normal clutch in which all the eggs were presumably subject to nearly identical conditions, suggests a genetic effect", although environmental conditions were also thought to be important in influencing arrested development. In this regard, Mattheé (1951) concluded from his studies with L. pardalina that the duration of incubation time was in large measure determined by the stage at which the egg is in contact with water.

In a recent study, Ashall and Ellis (1962) reported hatching of S. gregaria eggs from nine egg fields occurred within 13 to 16 days after laying. These authors found that 3.2 percent of the healthy eggs failed to hatch, as compared with the 3.8 percent which they indicated Stower et al. (1958) found. Ashall and Ellis stated, "It is probable that there is some variation in the number of apparently healthy eggs failing to hatch on different egg fields, but it is unlikely that this would be very large." However, various workers have reported that large percentages of certain collections of embryos may fail to hatch. Kelly and Middlekauff (1962) in a study on Dissosteira spurcata Sauss. observed a total hatch of only 59.3 percent from a collection of 400 eggs. They explained this by saying that many embryos were not able to accomplish the intermediate molt and died while in this process.

Data of Hastings and Pepper (personal communication) show that the

wide variability in percentages of hatch found with embryos of A. elliotti is statistically related to the population site. In addition, considerable variability was observed in the earliest date of hatch and also in the range of hatching dates from single pods from the various populations.

Hastings and Pepper, unpublished data.

Year	Population site	Range of hatching dates	% hatched
1960	Putnam Lake	9 - 27 days	87
1960	Smith River	9 - 25 days	78
1960	Kuhr	7 - 16 days	30
1960	Ogden	13 - 39 days	76

Within a single pod the hatching dates ranged from ten to 30 days at 25° C. Failure of embryos to accomplish the intermediate or embryonic molt also was observed in many individuals of this species.

Moore (1948) investigated the variability in developmental stages attained in embryos of three grasshopper species collected in the fall from field populations. His conclusions with regard to the development of each of these species were as follows: "Camnula pellucida Scudd. evidently enters a very definite diapause in the fall at the 50 percent stage just before blastokinesis (60 percent stage), unless development is stopped by adverse weather conditions before this stage is reached." The stage of development at which eggs of Melanoplus bivittatus Say enter the winter was quite variable within a population from one location to another and from year to year. Concerning Melanoplus mexicanus Sauss. he reported, "Embryos were found in stages of development varying from mere embryonic discs (ten percent stage), to practically fully developed

embryos (80 percent stage) in the fall.....Differences in development were greater between locations than between years in the collections examined." Moore reported only slight variation in the stage of development within a single pod in these three species, which is in agreement with Slifer's findings with M. differentialis, but differs from the findings of Popov with L. migratoria. It should be pointed out that M. bivittatus and M. mexicanus, as well as some strains of M. differentialis, do not possess an obligatory diapause, such as that observed in C. pellucida and A. elliotti.

Materials and Methods

In order to obtain data for the study of embryonic variability of A. elliotti all embryos from the laboratory reared populations were removed from their egg membranes and classified according to the previously established stage criteria. With the aid of an ocular grid in a binocular dissecting microscope, the body length measurements of all specimens were recorded. To allow comparison identical fixation and measuring procedures, as described previously, were used to obtain the data for both 1959 and 1960 populations. Embryos in Stage 19 which had begun to undergo revolution and those lying in other curled positions were not measured, although their stage of development was noted. This accounts for some discontinuity in the Tables containing stage and length data.

At intervals during the fall and winter of 1959, 1960, and 1961 wild population A. elliotti embryos were obtained from the field areas designated in the previous summer as collection sites. Sections of top soil

were dug out, broken apart and sifted through a coarse wire screen which retained the egg pods. The pods were placed in covered vessels filled with moist soil, surrounded by ice in an insulated container and transferred to the laboratory. Refrigeration was maintained to prevent development until pods could be removed and the eggs fixed and stored according to the previously described procedures.

Subsequently the membranes of all the wild population eggs were removed, and the stage, body length, and sex (where possible) of the embryos recorded by the same methods used with the laboratory reared populations.

Variability in the Pre-Diapause Laboratory Reared Populations

Three types of data were compiled for the 1959 and 1960 pre-diapause laboratory reared populations; length and stage, length and age, and stage and age. The data contained in Tables I and II represent the number of individuals classified into each of the stage categories in the daily age samples from the 1959 and 1960 populations. The wide variation in the stages of development of individuals within the various age samples illustrates the difficulty one might encounter in attempting to compare embryos of a given age even when they are reared under constant conditions. The range of stages found in many of the age samples in the 1959 population is greater than the range found in the 1960 population. Two individuals from the 35 day and one from the 43 day sample in the 1959 population developed to Stage 20 and were apparently undergoing revolution without a diapause period. In the 1960 population, Table II, one embryo,

TABLE I

Age-Stage Data for the Pre-Diapause 1959 Reared Population of A. elliotti Embryos