The early embryology of Aulocara elliotti (Thomas) (Orthoptera: Acrididae) with studies on the effects of maternal age and environment of the developmental rate of the egg
by Margaretha Harders Wessel

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
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Abstract:
The early embryology of Aulocara elliotti was investigated using histological methods. A staging criteria was formulated for this period. The female pronucleus was observed about 1/5 the egg length from the posterior part of the egg, while the first cleavage division of the zygote nucleus was observed later in the same vicinity.

Chromatin was eliminated during the second cleavage division. Cleavage nuclei were first noticed in the posterior periplasm and only later were present in the anterior periplasm although they were never as numerous there. Cell membranes were not observed in the presumptive serosa before differentiation of the embryonic rudiment. One nucleolus was observed in presumptive serosal cells.

. Comparisons of the developmental rates of eggs from females of different ages and reared at different densities were made and it was found that eggs from females reared at one pair per cage developed fastest when laid during the middle of the fecund period. Eggs from females reared at a density of six pairs per cage developed fastest when laid during the early part of the fecund period and thereafter the rate of development declined steadily.

The incorporation of tritiated uridine and thymidine during early development was determined with autoradiographic methods. Tritiated uridine was first incorporated into RNA during blastema formation, Tritiated thymidine was incorporated into DNA during the entire time period (6 days). 'A posterior-anterior gradient of the incorporated 3H-thymidine was observed, A large number of eggs developed abnormally after being exposed to the isotope in Ringer’s solution, No conclusions therefore, could be drawn concerning maternal effects (age, density) on RNA and DNA synthetic patterns.

THE EARLY EMBRYOLOGY OF *AULOCARA ELLIOTTI* (THOMAS) (ORTHOPTERA: ACRIDIDAE) WITH STUDIES ON THE EFFECTS OF MATERNAL AGE AND ENVIRONMENT ON THE DEVELOPMENTAL RATE OF THE EGG

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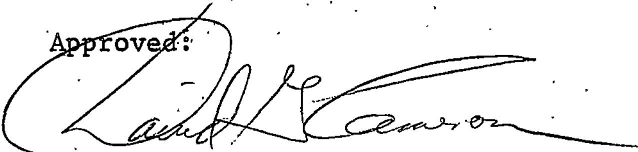

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TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENT	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT	x
INTRODUCTION	1
MATERIALS AND METHODS	7
Biological Material	7
Descriptive and Maternal Effects	8
Autoradiography	9
EARLY EMBRYOLOGY: DESCRIPTIVE	12
Events of Oogenesis and Egg Deposition	12
Definition of Terms	15
Results	17
External Appearance of the Egg	17
Staging Criteria	18
The Newly-Laid Egg	22
Membranes	22
Nucleus	24
Yolk	28
Temporal Pattern of Development	31
Discussion	54
EARLY EMBRYOLOGY: EXPERIMENTAL	73
Introduction	73
Results	74
Maternal Influence	74
Delayed Oviposition	83
Autoradiography	83
Tritiated uridine	83
Tritiated thymidine	85

TABLE OF CONTENTS
(Continued)

	Page
Abnormalities	85
Discussion	90
SUMMARY	99
APPENDIX A	103
APPENDIX B	104
APPENDIX C	105
LITERATURE CITED	106

LIST OF TABLES

Table Page

I. Reproductive data for 1970 81

II. Reproductive data for 1971 82

LIST OF FIGURES

Figure | Page

1. Egg pod and newly-laid egg of *A. elliotti* 14

2. Diagram of stages A to I of the early embryology of *A. elliotti* 20

3. A longitudinal section of the chorion of a newly-laid egg of *A. elliotti* 23

4. A longitudinal section of the chorionic cap of a newly-laid egg of *A. elliotti* 25

5. A longitudinal section of the periphery of a newly-laid egg of *A. elliotti* 26

6. Basophilic droplets and second cleavage division in a longitudinal secion of a six-hour egg of *A. elliotti* 27

7. The female pronucleus in a longitudinal section of a newly-laid egg of *A. elliotti* 29

8. Polar body in a newly-laid egg of *A. elliotti* 30

9. Chromatin elimination during second cleavage division in a longitudinal section of an egg of *A. elliotti* 32

10. Interphase cleavage nucleus in the anterior part of the egg of *A. elliotti* 33

11. Interphase nucleus in the anterior part of the egg of *A. elliotti* 34

12. Peripheral nuclei at the posterior end of the egg of *A. elliotti* 36

13. Periplasm of the anterior end of the egg of *A. elliotti* . . 37

14. Blastema nucleus at the periphery of a three-day-old egg of *A. elliotti* 38

15. Beaded blastema nucleus at periphery of a four-day-old egg of *A. elliotti* 39

LIST OF FIGURES
(Continued)

Figure | Page

16. Doughnut-shaped nuclei in posterior part of a three-day-old egg of *A. elliotti* 40

17. Mitosis at a 90° angle to the periphery of a three-day-old egg of *A. elliotti* which produces a daughter blastema nucleus and a secondary vitellophag 42

18. Mitosis tangentially to the periphery of a one-day-old egg of *A. elliotti* which produces two blastema nuclei 43

19. Internal nuclear aggregate in a five-day-old egg of *A. elliotti* 45

20. Peripheral nucleus with distinct nucleolus in a four-day-old egg of *A. elliotti* 46

21. Cuboidal cells of the embryonic rudiment in posterior end of a six-day-old egg of *A. elliotti* 47

22. Nuclei in mosaic germ disc in the posterior end of an eight-day-old egg of *A. elliotti* 50

23. Germ band and serosal cells in the posterior part of a ten-day-old egg of *A. elliotti* 52

24. Embryo, amnion and serosal cells in a twelve-day-old egg of *A. elliotti* 53

25. Displaced periplasm in egg of *A. elliotti* used for autoradiography 66

26. Internal aggregation of nuclei in a five-day-old egg of *A. elliotti* 70

27. Developmental stage reached by known-age eggs of *A. elliotti* during early development 76

28. Developmental stage reached by known-age eggs of *A. elliotti* during early development 76

LIST OF FIGURES
(Continued)

Figure | Page

29. Developmental stage reached by known-age eggs of *A. elliotti* during early development 77

30. Developmental stage reached by known-age eggs of *A. elliotti* during early development 77

31. Developmental stage reached by known-age eggs of *A. elliotti* during early development 78

32. Developmental stage reached by known-age eggs of *A. elliotti* during early development 78

33. Composite graph of Figs. 27-32 79

34. Longitudinal section of a five-hour-old egg of *A. elliotti* 84

35. Longitudinal section of a three-day-old egg of *A. elliotti* 86

36. Longitudinal section of posterior part of a six-day-old egg of *A. elliotti* 87

37. Anterior part of the same egg as shown in Fig. 36 88

38. Displaced periplasm in posterior part of a four-day-old egg of *A. elliotti* 89

39. Developmental stage reached by known-age eggs of *A. elliotti* exposed to a solution of ^{3}H-uridine in insect Ringer's 91

40. Developmental stage reached by known-age eggs of *A. elliotti* exposed to a solution of ^{3}H-thymidine in insect Ringer's 91

ABSTRACT

The early embryology of *Aulocara elliotti* was investigated using histological methods. A staging criteria was formulated for this period. The female pronucleus was observed about 1/5 the egg length from the posterior part of the egg, while the first cleavage division of the zygote nucleus was observed later in the same vicinity. Chromatin was eliminated during the second cleavage division. Cleavage nuclei were first noticed in the posterior periplasm and only later were present in the anterior periplasm although they were never as numerous there. Cell membranes were not observed in the presumptive serosa before differentiation of the embryonic rudiment. One nucleolus was observed in presumptive serosal cells.

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INTRODUCTION

The importance of the Acrididae has been recognized throughout the history of mankind in accounts of grasshopper plagues devastating crops and rangelands. A voluminous literature has been published concerning aspects of the ecology, morphology, physiology and behavior of both nymphal and adult acridids. Some of the more readily observable characteristics of the development of the egg such as water and temperature requirements, incidence and duration of diapause, and external morphogenesis of the embryo also have been investigated in a number of species. The research on the Acrididae has been reviewed by Uvarov (1966), Chapman (1969) and Hemming and Taylor (1972). In 1961, Roonwal compiled the Bibliographia Acrididiorum with supplements in 1961 and 1968, citing the literature to the Acrididae.

Few descriptive studies have been done on the histology of early embryogenesis of the Acrididae. Roonwal (1936) published an extensive monograph on the early development of the African migratory locust, *Locusta migratoria* (R & F), and in 1937 he published his studies on the organogensis of that same species. These works were reviewed by Johannsen and Butt (1941) in "Embryology of Insects and Myriapods." Slifer and King (1934), in a short paper, discussed the early embryology of the differential grasshopper *Melanoplus differentialis* (Thomas), a species widely distributed in the United States, and in 1963, Van Horn (Visscher) completed detailed investigations of the

histology of organogenesis in *Aulocara elliotti* (Thomas), a species indigenous to the western United States and Canada.

At Montana State University in the 1950's, *A. elliotti*, one of the ten most important economic pests of rangelands (Anderson, 1961), was selected for intensive studies in order to try to gain an understanding of the factors underlying the wide fluctuations in population numbers observed in this species. In 1952 Anderson and Wright included data on *Aulocara elliotti* in their investigations of behavior and damage of Montana grasshoppers and Anderson (1961, 1962, 1964, 1972) reported on relationships between grasshoppers and vegetation.

Studies on the viability of newly-hatched nymphs of *A. elliotti* under conditions of stress were published by Hastings and Pepper (1964), while the structure and performance of a specific adult population in the field was investigated by Mussgnug (1972). Bromenshenk (in progress) is investigating the communication and behavioral characteristics between individuals of a population of *A. elliotti* while Hastings (1971) studied the fecundity of females mated to males of a different population. The least understood aspect of the biology of *A. elliotti* appeared to be that of the embryology and, therefore, a series of investigations were conducted dealing with different aspects of embryonic development.

The size and weight of the egg, the numbers of eggs produced, the morphological and physiological features of the diapause egg, as

well as developmental respiratory patterns were described by Roemhild (1961, 1965a, b, 1967, 1968). He hypothesized that hormone depletion may be a contributing factor in diapause initiation and concluded that the diapause itself was maintained by the differences in pH values present in compartments of the eggs formed by embryonic membranes.

Biochemical and physiological aspects of development were investigated to determine metabolic patterns before, during and following diapause. Svoboda (1964) and Svoboda, Pepper and Baker (1966) reported on the lipids in the egg during development, while Bunde (1965) and Bunde and Pepper (1968) described the biosynthesis and occurrence of free amino acids during embryogenesis. The effects of temperature on oxygen consumption in the egg were studied by Laine (1966). Horvath (1967) examined the development of muscles in the embryo of *A. elliotti*. Leopold (1967) used histochemical methods to study postembryonic ovarian development and oogenesis in *A. elliotti*. Quickenden (1969, 1970) and Quickenden and Roemhild (1969) investigated the occurrence of carbohydrates in eggs and their relationship to maternal age and density, while Robinson (1970) recorded the distribution, rate of synthesis and characterization of proteins in eggs of *A. elliotti*. Urban (1970) followed the ontogeny of six hydrolytic enzymes during embryogenesis, using histochemical and electrophoretic techniques.

Van Horn (Visscher), (1963, 1966a) reported on the histology and morphogenesis of embryonic development of *Aulocara elliotti* from the

time of germ disc formation until hatching and established the staging criteria for the embryogenesis of this species. Using these criteria, comparisons of the growth and variability of embryos from a single wild population in two different years were made. The effects of maternal aging on the pattern of embryonic development was studied (Van Horn, 1966b).

Investigations were begun to describe the fecundity, viability and developmental rate of embryos obtained from single pairs of adults from different wild populations exposed to varying environmental conditions. It was found that photoperiod, temperature, aging and crowding of the parental generation brought about marked changes in the rate of development of the embryonic offspring. Young females from some populations produced eggs with high incidence of sterility in their first egg pods (Visscher, 1971).

Alteration of embryonic growth in the progeny by diverse environmental factors, as well as aging, suggested that the stimuli were probably acting upon the maternal neuroendocrine system and, in turn, altering the kinds or amounts of materials incorporated into the egg system.

Gland volume changes were observed during the post-diapause development of *A. elliotti* (Van Horn, 1968) and the influence of endogenous hormones upon embryonic development was suggested. The possibility that exogenous hormonal or other growth factors from the mother

could play an important role in the regulation of the rate of embryonic development was also hypothesized. Experiments using applications of analogues of juvenile hormone revealed that embryonic morphogenesis of *A. elliotti* was profoundly altered (Visscher, 1972) and histological analysis demonstrated that endocrine gland changes accompanied these morphological effects. These results supported the hypothesis that maternally-contributed growth factors may determine the rate and pattern of early embryonic development of *A. elliotti* and, thereby, be of great importance to the population success of this species.

Before experimental studies could be undertaken to demonstrate such a mechanism, a basic understanding of the events of early embryonic development in this species had to be obtained. The descriptive studies reported in this thesis, therefore, were undertaken to gain understanding of the developmental processes occurring during early embryogenesis of *A. elliotti* and to establish a basis for experimental analysis of early development in this species.

The scope of this thesis encompasses the following:

1. Examination and description of the early embryogenesis of *A. elliotti* using histological methods.
2. Creation of a staging criteria for early embryonic development.
3. Determination of the developmental rates of eggs from crowded and uncrowded parents, during the early stages

of development.

4. Comparison of developmental rates from young, middle-aged and old females.

5. Determination of patterns of RNA synthesis and DNA synthesis in early eggs to learn whether these are affected by maternal factors.

6. Establishment of the beginning of new RNA synthesis in early eggs.

MATERIALS AND METHODS

Biological Material

Fourth and fifth instar nymphs of *A. elliotti* were collected from a field near Billings, Montana in early June of 1970 and 1971. These nymphs were reared in a greenhouse insectary at Montana State University in clear lucite cylindrical cages (11" high, 8½" diameter) and placed on a removable pan filled with soil from the collection site according to methods of Visscher (1971). A vial with water and fresh western wheatgrass (*Agropyrum smithii*, Rydberg) was provided every other day.

In 1971 the temperature regime fluctuated diurnally from 75°F to 85°F. Due to mechanical failures, a larger range of temperature was experienced during 1970 (60-104°F). Neither photoperiod nor humidity was regulated and therefore correspond approximately to the local conditions in the insectary.

In 1970 twelve pairs of adults were reared with one pair per cage (designated hereafter as "single") and 24 pairs of adults were maintained under crowded conditions with six pairs per cage ("crowded"). In 1971, 20 pairs of adults were reared with one pair per cage and only two cages of crowded pairs, six pairs per cage, were maintained. Only adult males were replaced when they died.

Egg pods were collected each morning at nine o'clock and at other appropriate times when needed, by sifting the soil from the cage pans.

Egg pods were stored upright in plaster of Paris blocks according to methods of Visscher (1971), kept in an incubator at 25°C constant temperature and watered every other day.

At scheduled intervals the egg pods were removed from the incubator to obtain eggs of a known period of development after which the ootheca was removed with watchmaker forceps.

Descriptive and Maternal Effects

Eggs were fixed for 12-20 hours in a solution of 85 volumes dioxane saturated with picric acid, 10 volumes of 40% formalin and five volumes of concentrated formic acid. The methods of Anderson (1964), a modification of Griffiths and Carter (1958), were used with minor changes throughout this study in the preparation of serial sections. After one hour of fixation the chorion was pricked with a glass needle or removed completely with watchmaker forceps. Following 12 hours of fixation the eggs were washed in three changes of dioxan, dehydrated in Cellosolve (Sargent) for four changes of at least two hours each and placed in a 2% solution of celloidin in Cellosolve at 30°C overnight. Three changes of benzene, for a total of 15 minutes, were used for clearing. To prepare the eggs for embedding, they were placed in a solution of equal volumes of benzene and a paraffin-ceresin mixture. Because excessive heat during the embedding procedures altered the structural components of the egg, causing the yolk to be powdery and refractile, a low melting point mixture of ceresin wax and paraplast

was used. The blocks were stored at 4°C until they were ready to be sectioned and the face of the block was cut to expose the egg. The block was then placed in a 5% Tergitol 7 - ethane-diol (J. T. Baker Chemical Co.) solution for 12 hours and soaked in distilled water for an additional 24 hours or more to facilitate sectioning.

Serial sections 5-8 μ thick were cut with an AO Spencer rotary microtome. The ribbons were attached to the slides with albumin, except those for autoradiography, dried overnight and then stained with Harris' and Delafield's hematoxylin and eosin Y. Slides were permanently mounted with Adams Histoclad and viewed with a Zeiss binocular microscope (ocular 12.5, objectives neofluar 10, 16, 40 and Apo 100). Photographs were taken with a Zeiss 35 mm camera and Pan-X film.

A large number of other fixatives, dehydrating, infiltrating, embedding and wetting agents were tried, including the cupric-phenol methods of Slifer and King (1933) and Roonwal (1935) but none proved to be satisfactory.

Egg structures in each individual section were recorded on specially prepared figures (Appendix A) and important structures were photographed.

Autoradiography

Eggs to be used for qualitative autoradiographic experiments were incubated at 25°C until the desired age was reached after which they

were removed from the ootheca. Originally it was planned to inject the eggs with Ringer's solution and tritiated uridine and thymidine through a micro-injection apparatus but the high turgor pressure of the egg and the fragility of the shell made this impossible, even after a period of desiccation. The eggs were, therefore, exposed to a radioactive solution after a short period of desiccation according to methods used by Bunde (1965) with *A. elliotti* eggs. The absorption solution consisted of either tritiated Thymidine (methyl - H3) or tritiated Uridine (-5-H3) in Ringer's solution to indicate DNA or RNA synthesis, respectively. Both radioisotipes were obtained from Amersham/Searle and the vials contained 250 μCi in a 10% aqueous solution with a specific activity of 27 Ci/mmol (^{3}H-T) and 24 Ci/mmol (^{3}H-U). A 1.5 ml solution containing a concentration of 100 μCi of tritiated materials /10 ml of Ringer's solution was pipetted into five depressions of tissue culture dishes (Linbro multi-dish-disposo trays, Limbro Chemical Co.) containing six depressions each. One depression in each dish was used as a control and contained only Ringer's solution.

Ten eggs were selected for each of the six compartments in the series on the basis of maternal factors involved (age, density). The eggs were incubated for periods of four hours, 12 hours, 24 hours, 48 hours, 72 hours and 96 hours. On completion of the exposure period the eggs were prepared for sectioning as described previously. The sections were placed on precleaned slides on boiled distilled water

instead of albumin and picric acid was removed from the tissues by passing them through two changes of ethyl alcohol, 70% and 50%, for five minutes each.

Liquid nuclear track emulsion (Kodak NTB 2) according to methods of Prescott (1964) was used to coat the slides after which they were air dried and stored in black slide boxes for the required exposure time (thymidine 10 days; uridine 20 days). Silvergrain reduction above background concentration was taken as evidence of isotope presence.

Slides were developed with D-11 Kodak developer, rinsed in distilled water and fixed in Kodak fixer. They were rinsed in running tap water for 20 minutes, rinsed in distilled water and stained with Harris' hematoxylin and eosin Y. All of the autoradiographic procedures were carried out in complete darkness to minimize background.

EARLY EMBRYOLOGY: DESCRIPTIVE

Events of Oogenesis and Egg Deposition

Eggs of *A. elliotti* are produced by two ovaries, each having an average of five ovarioles (Leopold, 1967). The ovary is of the panoistic type, characteristic of the more primitive orders, such as the Orthoptera. The panoistic type ovary, unlike the meroistic type, does not have specialized "nurse cells" and, therefore, the follicular epithelial cells of the vitellarium are thought to constitute the only trophic tissue for the developing oocytes.

According to Leopold, the ovariole is divided into a germarium and a vitellarium as early as the second nymphal instar. At this stage, the nuclei of the oocytes within the vitellarium are in the post-pachytene stage of the first maturation division, and will remain in that state until after they leave the vitellarium.

Prefollicular tissue surrounds the primary oocytes in the vitellarium beginning at the second instar. In the fifth instar, cells can be recognized in this tissue and it differentiates further into the definitive follicular epithelium soon after the adult molt. At this time the primary oocyte have increased greatly in size, primarily due to an increase in cytoplasm. Yolk deposition commences two to three days after the adult molt in the ultimate and penultimate oocytes and, accompanying vitellogenesis, the oocytes enlarge rapidly in size. At the time of laying they contained large amounts of yolk characteristic

of eggs of the Acrididae, rendering them most difficult to section. Leopold observed that the follicular epithelium laid down the chorion upon completion of yolk deposition.

At the posterior end of each follicle, established according to the orientation of the female, there are cells which appear to be different from the remainder of the follicle cells, being somewhat larger and mostly of a columnar shape. These are thought to produce the specialized chorionic cap which later overlies the hydropyle connection with the developing embryo. In most grasshopper species the chorion has a sculptured appearance characteristic of the species (Tuck and Smith, 1939) reflecting the arrangement of follicular cells of the ovary. In *A. elliotti* the chorion appears to be smooth with the exception of the chorionic cap when magnified 6X-50X.

During oviposition, the eggs are enveloped by a moist, frothy material called spumaline, said to be secreted by the accessory glands (Johannsen and Butt, 1941). Soil particles adhere to the outside layer of the spumaline and this mixture hardens into a protective egg pod (Fig. 1).

Each ovariole seems to synchronize the release of the most mature egg, possibly by muscle contraction; therefore, the eggs are laid in groups, the egg pods containing from 0-12 eggs, with an average of eight eggs per pod (Van Horn, 1966a). Occasionally pods are laid without any eggs, perhaps because the female was disturbed during laying.

Figure 1. Egg pod and newly-laid egg of *A. elliotti*.

Definition of Terms

Blastema
- a layer of peripheral nuclei not separated by cell walls (Rempel and Church, 1969b).
- designates an association of embryonic cells with a definable functional state (Krause and Sander, 1962).
- stage at which the nucleate periplasm is still without cell membranes. The egg at this stage becomes a syncytium (Weissman in Ando, 1962).

Blastoderm
- peripheral cell layer surrounding the yolk, which differentiates into the embryonic and extra-embryonic regions (usually follows blastema stage). It appears that in *A. elliotti* differentiation in the embryonic area occurs before the rest of the blastema forms cell membranes.

Uniform Blastoderm
- the cells are evenly distributed over the entire surface (Anderson, 1972).

Differentiating Blastoderm
- a differential concentration of cells in a localized area at the surface, with a more attenuated distribution elsewhere (Anderson, 1972).

Cleavage Center
- cleavage of the zygote occurs in this region.
- the first, identifiable center of control (Counce, 1961).

Alternative Term: Furchungszentrum (Krause, 1938a).

Cleavage Energid	- cleavage nucleus and its associated island of cytoplasm (Krause, 1953).
Embryonic Germ Anlage	- part of blastoderm destined to become embryo after differentiation of blastoderm into embryonic and extraembryonic areas. Alternative Term: Embryonic Primordium
Endoplasmic Reticulum	- a network of internal cytoplasm in which deutoplasmic components are suspended (Krause and Sander, 1962). Alternative Term: Protoplasmic or Cytoplasmic Reticulum (Johannsen and Butt, 1941).
Periplasm	- a yolk free cytoplasmic layer at the periphery of the egg. Alternative Terms: Keimhautblastema (Patten); Cortical Layer, Cortex (Counce, 1961).
Percentage Egg Length (n% E. L.)	- the length of each egg is translated into 100 units, 0 representing the posterior pole and 100 the anterior. Specific locations are given as n% E. L. (Counce, 1973).
Vitellophags	- nuclei with encompassing islands of cytoplasm which lie within the yolk mass and act as agents of yolk digestion (Anderson, 1972).