Studies on the eggs of the grasshopper species Aulocara elliotti Thos.
by George R Roemhild

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology at Montana State College
Montana State University

Abstract:
Various aspects of the biology and physiology of the egg of the grasshopper species, Aulocara elliotti Thos., have been investigated. Eggs from three different populations were used for these studies.

Mean weight for all the eggs was 15.4 milligrams. Mean weights for the eggs from the three populations were 16.0, 14.5 and 14.2 milligrams. The mean number of eggs per pod was 8.1 for all pods collected. The mean number of eggs per pod for the three populations were 8.9, 8.3 and 7.1 There was a significant difference in the weights of the eggs from the three populations when adjustments were made for the number of eggs per pod. Respiration of eggs from the three different populations was studied in relation to effects of moisture and temperature. It was found that eggs of this species respire through the entire cuticle. Isolated embryos and yolk respired at a higher rate than did the egg from which they came if the egg was in diapause but respired at a lower rate than the egg from which they came if the egg was not in diapause. The respiration rate of eggs was measured from 5°C to 45°C and characteristic responses to temperature were noted. Reducing materials were measured in the egg during development and were shown to be higher during diapause than during other periods of development. Physiological characteristics of diapause eggs were studied and related to structures within the egg. Studies on respiration show that neither an inhibitor nor lack of water are likely to be factors in maintaining or terminating diapause in this species.

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ABSTRACT

Various aspects of the biology and physiology of the egg of the grasshopper species, Aulocara elliotti Thos., have been investigated. Eggs from three different populations were used for these studies. Mean weight for all the eggs was 15.4 milligrams. Mean weights for the eggs from the three populations were 16.0, 14.5 and 14.2 milligrams. The mean number of eggs per pod was 8.1 for all pods collected. The mean number of eggs per pod for the three populations were 8.9, 8.3 and 7.1 There was a significant difference in the weights of the eggs from the three populations when adjustments were made for the number of eggs per pod. Respiration of eggs from the three different populations was studied in relation to effects of moisture and temperature. It was found that eggs of this species respire through the entire cuticle. Isolated embryos and yolk respired at a higher rate than did the egg from which they came if the egg was in diapause but respired at a lower rate than the egg from which they came if the egg was not in diapause. The respiration rate of eggs was measured from 5°C to 45°C and characteristic responses to temperature were noted. Reducing materials were measured in the egg during development and were shown to be higher during diapause than during other periods of development. Physiological characteristics of diapause eggs were studied and related to structures within the egg. Studies on respiration show that neither an inhibitor nor lack of water are likely to be factors in maintaining or terminating diapause in this species.

INTRODUCTION

The big-headed grasshopper, Aulocara elliotti Thos., is one of the most abundant grasshoppers on Montana rangelands and is non-migratory in habit. The distribution and food habits of this species have been described by Pfadt (1949), Criddle (1933), and Anderson and Wright (1952). There are no published studies concerning the egg stage of A. elliotti.

The studies reported in this paper had as their objective the elucidation of various aspects of the biology of the egg stage of this species. It was desirable to obtain information on the physical characteristics of the eggs such as size, weight, and number of eggs per pod. It was likewise desirable to study the physiological characteristics of the egg stage such as respiratory activity during development, characteristics of diapause, and water absorption. Three populations were used for comparison to determine the extent of variation in the factors measured.

Most of the studies concerned with grasshopper eggs have been confined to single physiological systems and have been carried out on species which do not undergo an obligatory diapause as does A. elliotti.

Tuck and Smith (1939) have described the chorionic sculpturing of the eggs of forty-eight species of mid-western grasshoppers but A. elliotti was not included. Only three of the species they described were devoid of chorionic sculpturing, the condition which exists in A. elliotti.

Several studies have been made on the role of water in the development of grasshopper eggs. Parker (1930) has investigated the effects of both temperature and moisture on the eggs of Camnula pellucida Scudder and Melanoplus mexicanus mexicanus Saussure and has shown that different levels of moisture have varying effects on the viability of eggs of these species. Salt (1949, 1952), in more detailed studies with Melanoplus bivittatus Say, has shown that external contact water is necessary for egg development. His studies show that water is absorbed very slowly during the first few days of egg development, but comparatively large amounts are absorbed during the eighth and ninth days. He also points out that no moisture absorption takes place during diapause but moisture must be absorbed before post-diapause development can proceed. Chin (1958), in his studies on the oriental migratory locust, Locusta migratoria manilensis Meyen, has shown that water absorption in this species is effected by membrane permeability changes and that absorbed water is necessary for normal development of the eggs. Matthee (1951) studied water absorption in the eggs of Locustana pardalina Walk. and found that it is absorbed in a definite manner and that absorption is a controlled energy-consuming process.

The role of temperature in embryonic development is similar to that in any biological system. That is, low development rates occur at low temperatures with an increase in rate to a maximum at optimum temperatures followed by a decrease in rate as temperatures above the optimum are reached. Parker (1930) found that if the eggs of

M.m.mexicanus were maintained at a constant temperature throughout their entire development, they progressed most rapidly at 27°C. He further observed that if the eggs were chilled for a period of time, the optimum developmental temperature was 37°. Matthee (1951) showed that the eggs of L.pardalina developed progressively faster when they were exposed to successively higher temperatures throughout the range of 8.5°C to 35°C.

Developmental rate is closely associated with respiratory metabolism. They both increase and decrease in response to temperature in approximately the same order. The rate of change with temperature, however, may differ with different species. For example, the eggs of Rhodnius prolixus Linn. double their respiration rate and developmental rate between 21°C and 25°C (Tuft, 1949).

The effect of temperature on respiration usually deviates from the normal at high temperatures near the death point and at temperatures approaching 0°C. It has been suggested by Edwards (1953) that these atypical responses are due to irreversible enzyme inactivation or to gradual domination by physical systems. He further theorizes that lipids may be liberated at high temperatures thus changing their ability to perform their normal function. Mitochondria, which contain large amounts of lipids, appear to be one of the first structures inactivated by high temperatures. Protein denaturation has also been suggested as a cause of heat injury, but heat coagulation of proteins involves vacuolization and vacuolization is not always visible in the heat-injured cell (Edwards, 1953). It has also been observed that respiration may continue at a rate higher than normal after an insect has been heat injured which is further indication that enzyme proteins are not all denatured (Birch, 1947). The work of Jefferson (1945) suggests that at high temperatures a combination of lipid displacement and enzymatic imbalance occurs.

Although preliminary investigations have been made on the enzyme systems of developing eggs, the data are not complete enough to demonstrate the specific interactions between these systems. Fitzgerald (1949) has placed the enzymes of grasshopper eggs into categories using the origin of the enzyme as the basis of classification. In the first group he has placed those enzymes he believes are of maternal origin since they become depleted during the life of the egg and are apparently not replaced. Methylbutrase and tributyrinase are two he placed in this group. In the second group he placed such enzymes as cytochrome oxidase and cholinesterase which are found only in the embryo. The last group which includes alkaline phosphatase, tyrosinase and catalase are found only in the extraembryonic fluids and apparently have their origin in this tissue. This unequal distribution of enzymes is not too surprising if the egg is regarded as a catabolic system within the yolk and an anabolic system within the embryo.

Catalase (Bodine et al., 1954) has been found to increase rapidly in both the yolk and embryonic tissue of pre-diapause eggs of *Melanoplus differentialis* Thos. During diapause a constant level of activity is maintained. From diapause termination until hatching catalase activity again increases rapidly. Succinic dehydrogenase, on the other hand, exhibits its greatest activity during the diapause period (Bodine et al., 1952a). It should be pointed out that these tests were made on embryo homogenates. It has been shown by Bucklin (1954) and also in this study, that when diapause embryos are separated from the rest of the egg metabolic activity increases. The succinic dehydrogenase may be present in considerable amounts in diapause eggs but may not be able to function. Consequently, the amount, and not the activity within the intact embryo may be the factor measured by Bodine et al. Enzymatic activity was shown by Bodine et al. (1952b) to be in the mitochrondia fraction of the cells and was shown to be depressed by sodium malonate, sodium iodacetate, and sodium oxalacetate. These workers further showed that nucleic acids had no effect on the activity of succinic dehydrogenase. Indophenol oxidase activity shows the same pattern of activity during ontogenesis as does catalase (Bodine and Boell, 1936).

Very little is known concerning fat metabolism in the insect egg. It can be assumed to play an important part in development since most of the stored energy in the newly laid egg is in this form. In *M. differentialis* Slifer (1930) has found that about 20% of the total weight of the newly laid egg is in the form of free and combined fatty acids. This value fell to less than 10% just prior to hatching.

Carbohydrates, in the form of phosphorylated hexoses, are utilized by embryos during development (Bodine and West, 1953). They also showed that magnesium ions enhance the utilization of these materials. Bodine (1953) states that aerobic glycolysis seems to be controlled by enzymes located only in the cytoplasm of the embryonic cells while oxidative phosphorylation occurs in the yolk or at the surface of these cells.

Phosphorus distribution changes have been studied by Lu and Bodine (1953). They showed that lipid phosphorus decreases during prediapause and remains low for the remainder of the embryonic developmental period. They also showed that acid-soluble phosphorus remains high during embryonic development but decreases just before hatching takes place. They further showed that the amount of phosphorus in RNA and DNA increases in proportion to the growth of the embryo.

Protein metabolism in insect eggs has been investigated by several workers. Shulov et al. (1957), Kuk-Meiri et al. (1954) and Lichtenstein et al. (1949) have shown proteolytic enzymes to be present in the eggs of several acridids. Shaw (1955) has isolated and detected seventeen different amino acids and closely related nitrogen compounds from grasshopper eggs. The sulfur containing amino acids have been investigated by Fu (1957) and Bodine and Norman (1953). In general it was found that methionine remains at a high level until after diapause at which time it is converted to cystine and cysteine.

The endocrine systems which are present in acridid eggs have not been described extensively. Ventral head glands are present in the embryo and have been shown by Jones (1956) to control pigment formation during the last stages of embryonic growth. This is brought about through the intermediation of the parapodia. Other endocrine systems are undoubtedly necessary to carry out the complicated processes associated with development. In addition, the diapause phenomenon has been shown to be under hormonal control in species where diapause occurs (Williams, 1947).

Endocrine activity with a resultant imbalance of hormones has been associated with high population densities. It has been observed that changes occur in morphology, physiology, and behaviour of locusts when they are subjected to crowding. These changes can be observed in naturally occurring populations and have been induced experimentally in the laboratory. Changes in morphology and behaviour are readily observed, but those associated with the physiology of nutrition and reproduction are more subtle. Differences in fat content, blood alkalinity and lactic acid content were shown to occur in different phases of locusts by Matthee (1945). Butler and Innes (1936) found that the metabolic rate of migratory locusts was higher than it was in locusts in the solitary phase. This was confirmed by Kruger and Bishai (1957) who found that there were differences also in the respiratory quotient between individuals in the two phases.

It has been shown that phase effects can be transmitted from one generation to the next. Albrecht and Blackith (1957) state that "the influence of crowding or isolation (is) shown to extend through at least three generations". Albrecht and Verdier (1956) also state that the reproductive potential of newly hatched hoppers is dependent on the density conditions of the grandparents. This is true since the number of ovarioles present in the hatchlings is dependent on size, which in turn, is dependent on parental phase.

In general, the accepted mechanism by which grasshoppers are changed from one phase to another is through 'mutual sensory stimulation'. Some workers feel that weather conditions may limit favorable habitat and thus concentrate grasshoppers in certain areas where this stimulation takes place. (Uvarov, 1932, Zolotarevsky, 1933). Under these crowded conditions increased activity takes place and some authors feel that it is this activity that results in the physiological change which is manifested by a change in phase. (Faure, 1932, Husain and Mathur, 1936). Chauvin (1941) however, feels that the change is brought about not by increased activity, but by a reflex response to sensory impressions of fellow locusts through nervous mechanisms, endocrine mechanisms, or both.

PROCEDURE AND RESULTS

Egg Development

The eggs of *A. elliotti* are laid in pods in the soil during July and August. The number of eggs per pod varies from three to ten, the average being eight. The eggs are approximately 4.5 millimeters long and 1.5 millimeters in diameter. The outside of the egg is covered with a thin non-cellular proteinacious layer, the chorion. Beneath this is the cuticle which is composed of two wax impregnated layers. The wax serves to waterproof the egg except in the region of the hydropyle where the wax is absent.

Under normal conditions the eggs begin to develop as soon as they are laid. At the time of oviposition the egg content is a more or less homogenous mass of yolk and fluid with germ and accessory cells within the mass. The embryo is first seen as a round thin layer of tissue lying on the surface of the yolk at the hydropyle end of the egg. Development of the embryo continues for about forty-five days. This is followed by a period of cessation in growth, termed diapause. During this stage the respiration rate drops and very little metabolic activity is evident. Diapause is obligatory in this species since only three or four eggs out of several hundred tested were able to complete morphological development without having been subjected to diapause-breaking conditions. The embryo is approximately two millimeters long at the diapause stage and structures such as the legs, mouthparts, antennae, and abdominal segments are discernible at this time. Observations show that there may be considerable variation in the developmental stage of the embryos when they enter diapause.

Unpublished data by VanHorn shows that the termination of diapause is brought about by prolonged exposure to low temperatures. At 3°C and 8°C the time required to terminate diapause is in the neighborhood of 160 days. Observations on eggs from field sources indicate that this time may be shortened by at least a month when optimum temperatures or other conditions prevail.

When diapause is terminated, the embryo rotates halfway around the egg on the surface of the yolk and comes to rest with the tail at the hydropyle end of the egg where the head had been previously. At this time respiration rate increases about fourfold and development resumes.

At the eighth to tenth day after rotation the embryo has grown to the point where all the yolk material has been engulfed. Muscular contractions are now common (although peristalsis is observed prior to this) and pigmentation, sclerotization, and internal organization are completed. Hatching usually occurs from fourteen to twenty days following the end of diapause.

<u>Collection and handling of eggs</u>

The eggs for this study were collected in the fall of 1959 and the winter of 1960 from three widely separated areas of Montana rangeland.

Area 1 is located in Blaine County east of Cleveland. This area is vegetated mainly by *Bouteloua gracilis*, *Stipa comata*, and *Agropyron smithii*. The numbers of grasshoppers observed on this area had been fairly high for several years. The population could be characterized as being vigorous, relative steady in density and remained so during the summer of 1960. Area 2 is located in Cascade County near Simms. Vegetation was mixed *B. gracilis*, and *A. smithii* with some *S. comata* interspersed. The population in this area was at a peak two years previous to the time the eggs were collected. Eggs were abundant, however, and easily found. The numbers of grasshoppers decreased rather sharply during the summer following the egg collections. Area 3 is in Meagher County north of White Sulfur Springs. The main grass species was *B. gracilis* with small amounts of *S. comata* and *A. smithii* interspersed. Populations of *A. elliotti* had been exceedingly heavy and widespread in this area for several years but the general infestation had diminished to a few localized spots during the summer preceding the egg collections. Eggs were difficult to find and the following year few grasshoppers were found on this area.

The eggs were collected by skimming the top few inches of soil and shaking it through a screen. They were then stored in a refrigerator at 3°C until they were used.

Egg Weights and Numbers

The egg pods were dissected, the eggs removed, counted and weighed. The balance used for weighing was designed to give accurate rapid results in the range of the eggs' weights. The working parts were made of quartz fibers. Figure 1 is a diagrammatic representation of this balance. It was constructed according to the principles employed by Neher (1942) and Kirk et al. (1947). Quartz fibers were used because they remain virtually unchanged in their physical characteristics through the range of the temperatures encountered. The working parts were mounted in a box to protect them from air currents and dust and only the tip of the balance beam and the object carrier protruded from the box. In practice, the dial was set to give a null point reading on the scale set into the front of the box. The reading was made by noting the position of the shadow of the balance beam on this scale. Light was furnished by an electric light source and a condenser lens. After the reading was noted the egg was placed on the object carrier and, due to the added weight, the beam was deflected downward. The dial was then rotated in a counter-clockwise direction until the torque on the quartz fiber returned the beam to its original position. The amount of torque necessary to return the beam to its original position was directly proportional to the weight of the egg. The amount of rotation of the dial which applied the torque to the beam was read on the stationary vernier scale.

The balance was calibrated with known weights and accuracy was in the range of .01 milligrams. The data and calculations for calibrating the balance are presented in Figure 2.

A. Rotating dial.
B. Stationary vernier scale.
C. Quartz torque rod.
D. Balance beam.
E. Counterweight.
F. Quartz tension bow.
G. Tightening screw.
H. Light source.
J. Condenser lens.
K. Shadow scale.
L. Object carrier.

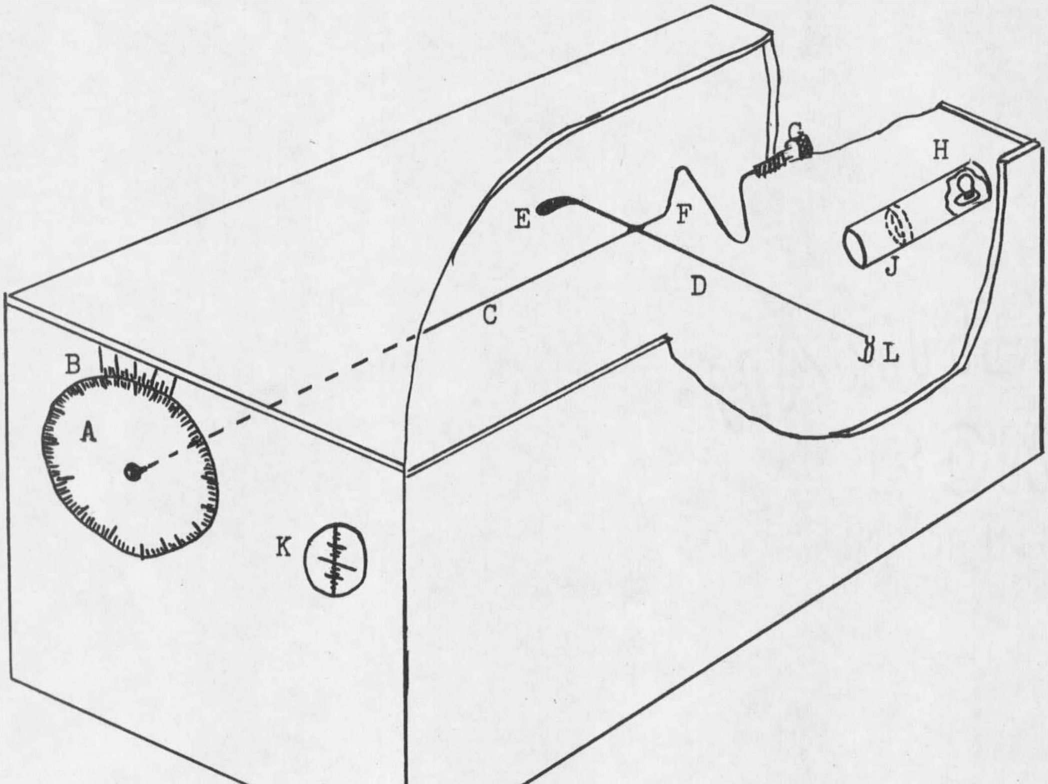

Figure 1. Balance constructed for weighing the eggs of <u>A. elliotti</u>.