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Quantitative changes in protein, glycogen and fat content in the eggs of the locusts, *Locusta migratoria migratorioides* and *Schistocerca gregaria* (Orthoptera), during embryogenesis

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Abstract. Changes in the content of protein, glycogen, neutral lipids and sterols were investigated in locust eggs from oviposition until larval hatching. The content of all of these nutritional reserves was higher in the eggs of *S. gregaria* because of their larger size, although relative changes in utilization or synthesis of these materials during embryogenesis showed a more or less parallel course. The amount of protein increased progressively during embryogenesis, while glycogen and neutral lipids were successively metabolized or utilized for development of the embryo. There appeared significant relative differences in the way of utilizing energetic reserves during embryogenesis between the two species. This was especially manifested by a larger relative decrease in the content of neutral lipids (mainly triglyceride) in the eggs of *S. gregaria*. Conversely, the eggs of *L. migratoria* showed a larger relative utilization of glycogen reserves. The content of steroids was higher in the eggs of *S. gregaria* during the initial 6 days of embryonic development. Later on, during advanced stages of pharate larval development, the steroids were rapidly utilized and decreased in both species. The described changes in utilization of the main energetic resources were correlated with periods of tissue growth and differentiation and with the cuticulogenesis of the growing embryo.

INTRODUCTION

Insect eggs represent a self-sustaining system which provides the raw materials for building the larval body and the energy reserves to cover all of the energy demands during embryogenesis (Sander et al., 1985). The development of the embryo is dependent upon the appropriate physiological and environmental conditions. The most important environmental condition for development of the embryo is favourable temperature and humidity (Hamilton, 1950).

It is known that lipid and carbohydrate reserves decrease as embryogenesis progresses. The carbohydrate metabolism in the eggs of the grasshopper *Aulocara ellioti* has been described by Quickenden (1970). The changes in lipid content and function have been surveyed in various insect species by Gilbert (1967). The aim of this study is to compare the parallel changes in protein, glycogen and lipid content in the eggs of *L. migratoria* and *S. gregaria* during embryogenesis.

MATERIAL AND METHODS

The eggs of migratory locusts, *Locusta migratoria migratorioides* (R. et L.) and *Schistocerca gregaria* (Forsk.) were used in the assay. These eggs were collected every day from our stock culture to have the samples of a known time of incubation. The eggs were incubated in wet sand at a temperature 32–35°C. They eclosed between day 11–13, eggs were sampled up to day 10.

The sampling methods

Each egg was homogenized in 70% methanol using an all-glass pestle homogenizer. The homogenate was transferred into a glass centrifuging tube and centrifuged at 4000 r.p.m./10 min. The supernatant was poured into another tube and an equal volume of petroleum ether was added to the supernatant and to the sediment. Then the samples were shaken vigorously, the sediment was centrifuged again, and the combined petroleum ether extracts were poured into thin-wall tubes and left to evaporate in digestory under room temperature. The dried residues were used for determination of neutral lipids using vanillin reagent according to Zoelner and Kirsch (1962). The results are given in equivalents of oleic acid.

Then the sediment was extracted again with methanol, centrifuged, and the combined methanol extracts were left to evaporate in a fumehoud at the room temperature. The dry remnants were dissolved in glacial acetic acid and used for determination of sterols according to method of Zlatkis et al. (1953). The results are expressed in cholesterol equivalents.

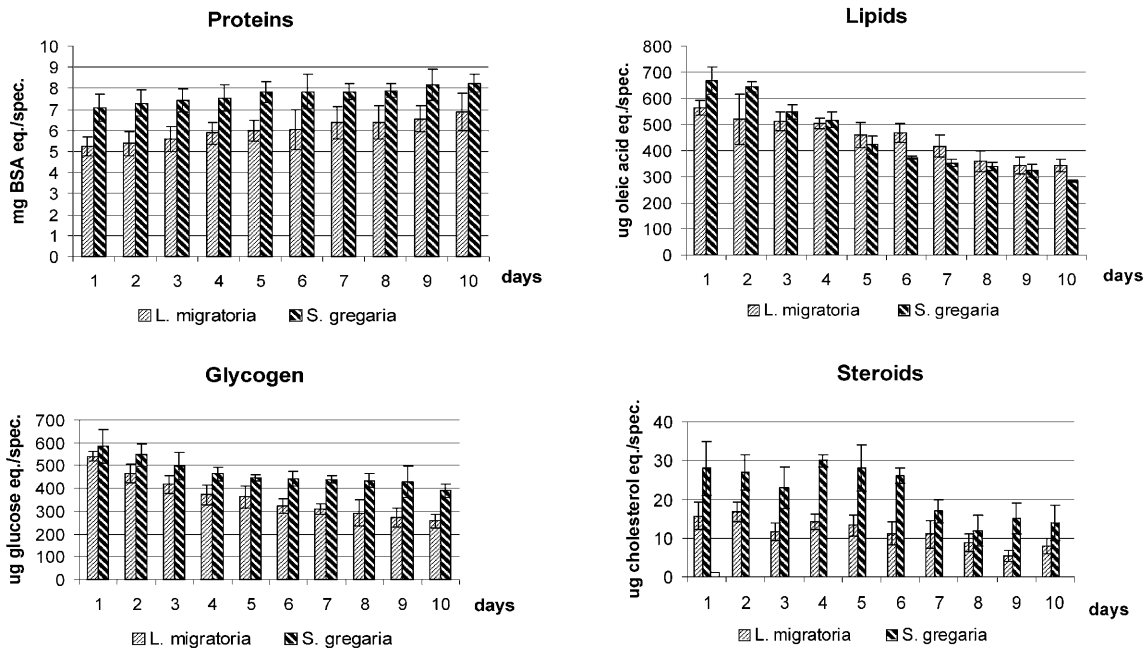
The sediment was used for determination of proteins and glycogen: The sediment was hydrolysed in 1N NaOH overnight at room temperature. Half of the hydrolyzate was used for direct determination of protein content using biuret reagent according to Goa (1953). The results were then calculated into bovine serum albumin equivalents. The other half was used for determination of glycogen using the method of Good et al. (1933) for isolation of glycogen and phenol - sulphuric acid reagent according to Dubois et al. (1956) for colorimetric determination. The results were calculated in glucose equivalents.

The data were statistically evaluated. The cummulative data represent the average and standard deviations of analyses in 15 eggs.

RESULTS

Changes in protein content

The quantity of proteins in the eggs of *S. gregaria* revealed no significant changes during embryogenesis. A slow increase of protein content was seen in the eggs of *L. migratoria* (Fig.1). Thus the rate of change of protein content could reflect the fact that the eggs of *S. gregaria* are bigger and heavier than that of *L. migratoria*.



Figs 1–4. 1 – changes of protein content (in mg of bovine serum-albumin equivalents) in the eggs of *Locusta migratoria* and *Schistocerca gregaria* during embryogenesis; 2 – changes of lipid content (in μg of oleic acid equivalents) in the eggs of *L. migratoria* and *S. gregaria* during embryogenesis; 3 – changes of glycogen content (in μg of glucose equivalents) in the eggs of *S. gregaria* and *L. migratoria* during embryogenesis; 4 – changes of steroid content (in μg of cholesterol equivalents) in the eggs of *L. migratoria* and *S. gregaria* during embryogenesis. (Means, $\bar{x} \pm$ s.d.; n =15).

Changes in glycogen content

The amount of glycogen decreased during embryogenesis, particularly in the eggs of *L. migratoria* (by 52% when compared with the glycogen content in the freshly laid eggs). The decrease of glycogen content in the eggs of *S. gregaria* is not so obvious (only about 33%) during the entire embryogenesis (Fig. 3).

Changes in lipid content

The content of lipids in the eggs of both locust species steadily decreased during embryogenesis, particularly in the eggs of *S. gregaria* (Fig. 2). When the changes in lipid content (in triglyceride) at the beginning and at the end of embryogenesis were compared, it was found to decrease by 39% in the eggs of *L. migratoria*, and by 58% in *S. gregaria*. It is evident that triglyceride is the main source of energy for all processes during embryogenesis.

Changes in steroid content

The steroid content in the eggs of two locust species differs greatly (Fig. 4). The eggs of *S. gregaria* have a two-fold higher content of steroids than those of *L. migratoria* during the six days of incubation. After the day seven the content of steroids in the eggs of *S. gregaria* decreased rapidly. The content of cholesterol in the eggs of *L. migratoria* also decreased, but not so rapidly as in *S. gregaria*. The decrease of steroid content in the eggs of both species at day 3 is probably connected with katatrepsis. The decrease at the end of embryogenesis is probably due to the synthesis of larval cuticle.

DISCUSSION

Locusts have probably one of the largest eggs of all insects. The development of the locust embryo lasted about 11–13 days in wet sand at a temperature 30–32°C. Because of the relatively slow development they are the ideal model for study of insect embryogenesis. This has been demonstrated in the studies of Roonwal (1936, 1937) performed on the African migratory locust, *Locusta migratoria migratorioides*. He confirmed the older data of Slifer (1932) who described the daily growth changes in the developing embryo of the grasshopper, *Melanoplus differentialis*. Later the study of Hamilton (1950) described the dependence of the rate of development on temperature and humidity in the eggs of *L. m. migratorioides* and *Schistocerca gregaria*.

The above mentioned authors followed the daily change in embryo growth. Shulov & Pener (1959) declared this method of study of embryogenesis inappropriate. They described in their detailed study dealing with the developing embryo of *L.migratoria* at least 23 developmental stages which lasted about 16 days at 27°C. However, the development of *Locusta* eggs in our stock culture was shorter, due to the higher temperature, as mentioned above. Along with the description of developmental stages they also estimated the daily changes in weight and water content in the locust eggs.

The eggs of *S. gregaria* differ from those of *L. migratoria* in several respects: they are bigger and heavier. The protein content in *L. migratoria* eggs during embryogenesis varied between 74 and 84% of that in *S. gregaria* (Fig. 1).

Differences between the eggs of *S. gregaria* and *L. migratoria* were also found in the rate of utilization the glycogen reserves. While the decrease in glycogen content in *S. gregaria* eggs was not so remarkable, in *L. migratoria* it was significantly greater (Fig. 3). It seems that glycogen in the eggs of *L. migratoria* serves to a larger extent as a source of energy. This fact was indirectly confirmed by estimation of the changes in lipid content during embryogenesis in both *S. gregaria* and *L. migratoria* eggs (Fig. 2). The data shows that lipids are utilized more extensively in the eggs of *S. gregaria* than in the eggs of *L. migratoria*.

The content of glycogen was found to be relatively low (see Fig. 3). It is in agreement with the data of Quickenden (1970) obtained by analysis of eggs of the grasshopper, *Aulocara*

ellioti. Most of glycogen was spent at the end of embryogenesis. The above mentioned author also estimated increased level of free sugars as embryogenesis progressed. The dominant free sugar was trehalose, which documents mobilization of the glycogen reserves.

Additional confirmation of our data are the results of Boell (1935) and recently Sláma (2000) who screened the respiration of the eggs of *S. gregaria*. Sláma discovered that the respiratory quotient of the eggs is 0.7. It is evident that the main source of energy for all metabolic processes during embryogenesis are lipids, particularly triglycerides. Our data on lipid utilization also corresponds with the results of Allais et al. (1964) obtained with the eggs of *L. migratoria* and Lipsitz and McFarlane (1970) with the eggs of house cricket, *Acheta domesticus*. Allais et al. (1964) found that the content of triglycerids decreased in the eggs of *L. migratoria* during embryogenesis by 52%, which corresponds with our data.

The content of sterols as was estimated varied between 2.2–3.4% of the total fat content. It is again in good agreement with the data of Allais et al. (1964) on the eggs of *L. migratoria* and with the data of Svoboda et al. (1966) with the eggs of *Aulocara ellioti*. They found that the dominant steroid is cholesterol (about 95% of all steroid content). According to the submitted results, the changes in steroid content probably reflected the processes of cuticulogenesis at the stage of katatrepsis and at the end of embryogenesis when the larval cuticle was synthesized (Fig. 4).

The size of eggs and nutrient content are influenced by various factors. McIntyre & Gooding (2000) found that egg size, egg content, hatch rate and biochemical characteristics in the eggs of *Musca domestica* correlated with the age and size of *M. domestica* female. Proteins, carbohydrates and lipids are transported from fat body to the eggs during oogenesis in the form of glycolipoproteins (vitellogenins) as was documented earlier (Chinzei et al., 1981; for survey see also Kunkel & Nordin, 1985).

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