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Macroscopic and microscopic study of the embryonic development of the desert locust *Schistocerca gregaria* (Forskal, 1775) (Orthoptera: Acrididae) in laboratory

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Abstract

Schistocerca gregaria (Forskal, 1775) is a serious pest of crops. The knowledge of its biology can contribute to the integrated pest management against locusts. In laboratory conditions (32 ± 2.9 °C and 70 ± 5.3% RH), the duration of the embryonic development of *Schistocerca gregaria* is 12.63 ± 0.55 days. The macroscopic study showed that the bright yellow egg at laying, changes to orange 2-3 hours after laying; then to Brown 1-2 days later and eventually becomes clear at the end of development. These color changes are accompanied by weight gain of 8.3-20 mg at the end of development. Observation by transparency of rudiments of organs was made from the 5th day. These transformations correspond to the stages of segmentation, gastrulation and organogenesis. These three stages interfere and overlap.Histological investigations reveal partial and superficial segmentation, characterized by an early Karyiokinesis followed by cytokinesis. After one hour and thirty minutes the periblastula is formed. Gastrulation is initiated immediately, with movements of cell proliferation, deployment, embolism and delamination leading to the formation of the embryonic layers within 24 hours. Clusters of cells from which will derive rudiments of organs are observed on sections of eggs of two days. Morphogenesis begins with cephalization with the formation of the optic lobes, then the thorax and abdomen. The whole morphogenesis lasts about ten days. Knowledge of the duration and timing of embryonic development will allow to provide the best times and the means of intervention to contribute to the integrated pest management (IPM) against locusts.

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Introduction

Desert locust *Schistocerca gregaria* (Forskal, 1775) is a serious polyphagous pest of crops. This species in its gregarious phase can cause up to 100% of crop loss (Simpson *et al*, 1999; FAO, 2012). The threat of an insect is due to its biotic potential resulting from the reproductive potential and the potential for survival. In favorable conditions, a female *Schistocerca gregaria* lays 4 times per year with an average of 50-70 eggs per clutch and the duration of the embryonic development is 12 days on average (Ould El Hadj *et al.,* 2004). All these factors allow it to increase its number quickly and reach the gregarious phase which is the most dangerous phase.

Despite repeated chemical treatments and with high doses, desert locust remains a real problem. It therefore requires a thorough study of the biology of these pests to find appropriate and sustainable solutions. The reproductive biology and especially the embryology of *S. gregaria* is very little known. The studies carried out focus on closely related species. They are not new and they are incomplete or inadequate especially in *Locusta migratoria* by Lecoq and Mestre (1988) and Thompson and Siegler (1993) in *Schistocerca nitens*, the embryogenesis was studied but not histologically (Bentley *et al*., 1979) and in *Schistocerca gregaria* by Duraton and Lecoq (1990) where the development was studied only at the macroscopic level.

This study will serve as a preliminary work to test factors (temperature, chemicals) that could affect or alter this temporal sequence of events during embryogenesis. This work which is part of preliminary studies of new prospects of the fight aims to deepen the knowledge of the embryonic development of *Schistocerca gregaria*.

This will be a first step to describe the various macroscopic changes of eggs following the chronology of the embryonic development in *S. gregaria*; in a second step, to make a histological study of the embryonic development by specifying the chronology.

Materials and methods

Breeding device of Schistocerca gregaria

Each cage contained 50 young and sexually immature locusts (25 males and 25 females) fed with young maize plants (*Zea mays* L. Poacae) of the Ferké 79 variety. The cages are cube-shaped, 50 cm square. Three of the four sides are made with metallic grid of 2 mm. The top and bottom sides as well as the fourth side face are in 5 cm thick plywood. This latter is perforated with a square opening of 20 cm square for the manipulation of insects inside the cages. Each cage is equipped with a 100-watt bulb lit with 12 hours of photoperiod, to illuminate the cage and to maintain the high temperature (29-35 ° C) and the relative humidity of 65-80 %. Plexiglass tanks (17,5 x11 , 5 x7 cm) filled with fine and moistened sand used as laying boxes were placed inside the cages to allow females to lay their eggs.

Duration of the embryonic development of S. gregaria

A hundred clutches were observed and numbered. The date of each spawning and that of the hatching of eggs were recorded. The duration of embryonic development corresponds to the time between the date of spawning and that of the emergence of the first larvae from this spawning. The duration of each of these hundred clutches was then recorded and an average was calculated. The estimated duration of each phase (segmentation, gastrulation and organogenesis) was performed by recording the time of incubation of eggs, their color changes, the weight of eggs, the amount and distribution of yolk as well as the appearance of the rudiments of organs observed by transparency.

Sampling of eggs for macroscopic study

Thirty mature unfertilized oocytes were collected from ovaries of mature females after dissection to be compared to eggs laid. Immediately after laying 30 eggs were collected using a flexible clip every 5 minutes for one hour. Then, every 30 minutes for 24 hours and then daily for 15 days. These eggs were

soaked in a mixture of "water / bleach 8 °" in equal proportions for 1 to 5 minutes depending on the age of the egg, to make them clear and transparent (Lecoq and Mestre, 1988). Then they were observed and photographed under a binocular microscope of brand BMK 31162.

Histology of eggs during embryonic development

Batches of 30 eggs collected in the same conditions as described above were fixed by immersion in an aqueous solution of Bouin for 7 days. Sections of 7 µ m were performed using Reichert Jung microtome. They were then collodionned and stained either with Hematoxylin-eosin or with Heidenhain Azan (Martoja and Martoja-Pierson, 1967). Observations and photographs were made using photomicroscope Zeisss of type "Axioskop" 40.

Results

Average duration of the embryonic development of S. gregaria

For the same clutch containing 40 to 50 eggs forming an ootheca, the first emergences are observed on the 11th day and the last on the 14th day after spawning but the largest number (57.62%) is observed on the 13th day. This corresponds to an average duration of embryonic development of 12.63 ± 0.55 days. The estimated average duration of the different stages of development are 1 hour 30 ± 30 min for segmentation, 21 ± 2 hours for gastrulation and 10 ± 1 days for morphogenesis (Table 1).

Table 1. External macroscopic changes of the eggs of *S. gregaria* during its embryonic development.

Stage	Incubation period	Eggs color	Eggs weight (mg)	Quantity of yolk
I	From spawning to first minute	Brig yellow shiny	8.3 ± 0.10	very abundant
II	2-3 hours	Yellow	8.3 ± 0.10	Abundant
III	1 day	Brown	10.0 ± 0.20	Abundant
IV	2-4 days	Brown	10.0 ± 0.20	Early resorption of yolk
V	5-7 days	Brown-clear	16.0 ± 0.40	This yolk at the centre of the germ
VI	8-9 days	clear	20.0 ± 0.40	Poor
VII	10-12 days	Whitish	20.0 ± 0.40	Very poor

External observations of eggs during embryonic development

Macroscopic observations of eggs allowed to distribute them in seven stages (Table 1) representing the three stages of embryogenesis namely segmentation (stages I and II), gastrulation (stages II and III) and organogenesis (stages III-VII).

Microscopy of eggs during embryonic development

Observations focused on the structure of the wall of the egg, yolk and the nucleus. The mature unfertilized oocytes (type I) yellow in color is protected by an epithelium lined with a yolk membrane adhering tacitly to the yolk; the chorion is not yet formed. The yolk appears in the form of a plate with a nucleus of granular appearance eccentric in the anterior pole of the egg. The abundance of yolk in the center would correspond to the type of centrolecithe egg. After fertilization, the eggs are light yellow and bright. Their micrograph demonstrates that the oocyte is protected by two cell layers (the chorion and yolk membrane) that adheres to the yolk. They are synthesized by the follicular cells that degenerate at the end of the development of the oocyte.

Thirty-five minutes after spawning, karyokinesis is advanced and many nucleis are observed. The yolk has many vacuoles, whose coalescence gives the appearance of an entangled network that serves as migration routes to nucleis. These nuclei will be distributed in the cytoplasm of the cell to the periphery and form a syncytium and later cytokinesis will follow**.** One hour 30 minutes after spawning, the egg of orange color, presents a continuous wall completely detached from the yolk. This is the stage of cellular blastoderm consisting of a single layer of cells surrounding the yolk. Segmentation is completed; the germ obtained is a periblastula having in its posterior pole polar plasma that is at the origin of germ cells (Fig. 1A, 2A). Between 2 and 3 hours after spawning the egg becomes more orange.

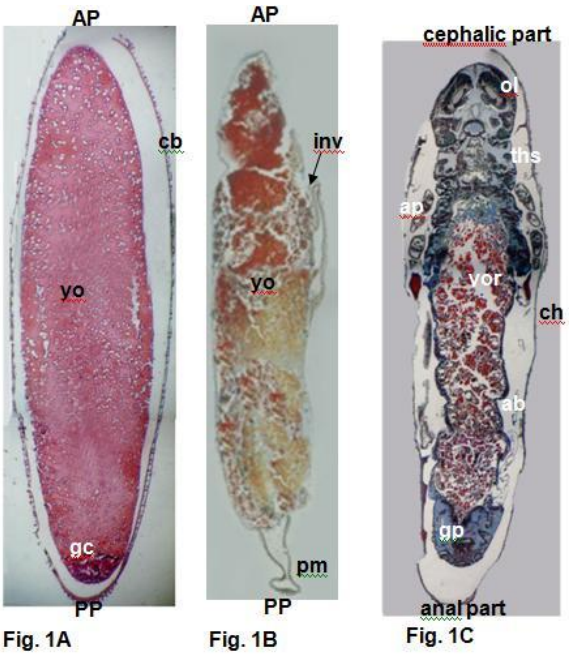

Fig. 1. Microscopy of eggs during the embryonic development of *Schistocerca gregaria*.

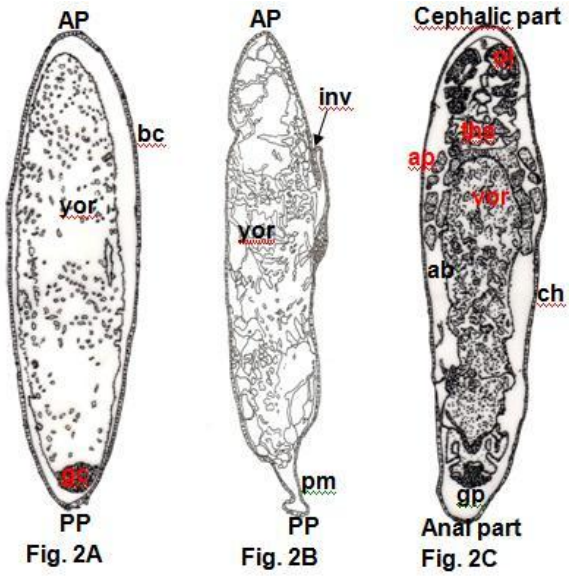

Fig. 2. Schematic representation of the histological observations of eggs during the embryonic development of *Schistocerca gregaria*.

Fig. 1A-2A: Sagittal section of the periblastula of *Schistocerca gregaria* presenting at its posterior pole a polar plasma

Staining: Hemalun-eosin (G x 40)

cb: cell blastoderm, yk: yolk, gc: Germ Cells, AP: Animal pole,

PP: posterior pole

Fig. 1B-2B: Sagittal section of a germ of *S. gregaria* during gastrulation
with a ventral invagination (G x 40)
Staining: Hemalun-eosin
Vi : ventral invagination; yk: yolk, em: extension of the membrane.

Fig. 1C-2C: Frontal section of an egg of *S. gregaria* 9 days after spawning:
Morphogenesis of *Schistocerca gregaria* (G x 40)

Staining: Heidenhain AZAN
ch: chorion, yr: yolk resorption; g: rudiment of genitalia, ol: optic
lobes, ths: thoracic segments, abs: abdominal segments,
al: Articles of legs.

Schistocerca gregaria

Gastrulation is initiated by a longitudinal cell proliferation in the anterior pole that will cause the formation of the germinal band. Then appears an intussusception in the ventral part of the germ that will cause the mesoderm. During this phase, we observe no cell division, but only reorganizations, transformations, extensions leading to a mono-cell layer. It will become very long, and roll up in the rear part of the blastoderm and this causes folds at the ends of the germ (figures 1B, 2B). The germinal band, after extension, will regress and get back on the ventral side. However, the mesoderm has spread under the ectoderm and is delaminated gradually into two stratums that can be_assimilated to germ layers. The micrograph of brown eggs of one to two days reveals that organogenesis has begun. The amnion and serosa resume their regularity and do not participate in organogenesis. They cover the yolk that is absorbed gradually by vitellophage cells. Between the 4th and 5th day after spawning, morphogenesis continues, cell clusters correspond to rudiments of organs. The first indications of the segmentation of the body are marked by constrictions defining the forebrain, the thoracic and abdominal segments. At the level of the head, appear side cell clusters that are assimilated to the differentiation of optical regions identified by transparency on the whole germs, as the eyes. The thoracic region is marked by rudiments of legs. The abdominal region has no visible appendix. From the sixth to the seventh day after spawning, histology reveals the formation of a body cavity. The germ gradually increases in size. The edges of the germinal band grow in the latero –dorsal direction to completely surround the yolk in resorption between the 8th and the 9th day. On day 9, morphogenesis is precise with the appearance of a very distinct larval form (figures 1C, 2C). At the level of the head there are the two optic lobes and cerebroid lymphs arranged symmetrically above the mouth parts. The thorax is segmented into different territories representing protothorax, the mesothorax and metathorax, cell clusters determine the articles of legs. The abdominal region presents 8-9 integumentary constrictions corresponding to the limits of future abdominal segments; the last is characterized by clusters of cells corresponding to the gonopods. On day 11, the muscular system is observed. Constrictions of the abdomen are more pronounced and yolk very low. The process will continue and complete the morphogenesis of the larva that will emerge between the 12th and 13th day. This microscopic description confirms the macroscopic observations.

Discussion

The average duration of the embryonic development of *Schistocerca gregaria* recorded in our breeding conditions is 12 days on average. This value is respectively 15 days (Duraton and Lecoq, 1990) and 12 days at 30 ° (Ould El Hadj *et al.,* 2004). Fertilization is internal as we have seen, so fertilized eggs are produced at the time of spawning as reported by Beaumont and Cassier (1991). During this development, color changes of yellow eggs at spawning which turn brown in the ground have been explained by the fact that as far as the cells are formed, the yolk is absorbed and egg darkens (Duraton and Lecoq, 1990). This resorption of yolk during embryonic development has been described by Bentley *et al.* (1979) in *Schistocerca nitens*, as well;

according to Ho *et al.* (1997) the homogeneous distribution of yolk at the laying of the egg becomes heterogeneous during development. As for the weight of eggs, our results showed that it increased from 8 to 20 mg before hatching against 5 mg to 19 mg (Hunter-Jones, 1964). In *Locusta migratoria* the weight increases from 6.3 mg to 14 mg from the laying to the hatching of the egg (Raccaud-Schoeller, 1980). This weight gain is due to the fact that eggs absorb about their own weight of water in the first five days of laying (Duraton and Lecoq, 1990). According to histological observations, the amount of yolk and its cytoplasmic distribution essentially determine the duration and progress of the mechanisms of embryogenesis (Borror *et al.*, 1981. Franquinet and Foucrier, 2003). In insects in general, eggs are centrolecithe as we have observed in *S. gregaria*. In addition, our investigations revealed that the segmentation is characterized by nuclear division or early karyokinesis followed by the migration of nucleis to the periphery. The formation of these energids has also been described by Ho *et al.* (1997) and Dearden and Akam (2001). They will completely surround the yolk and finally cytokinesis will take place. In addition, primordial cells, precursors of future germ cells were observed in the posterior pole of the egg at the end of segmentation. In Drosophila, the differentiation of these cells and their migration took place after the formation of syncytial blastoderm. The segmentation is meroblastic partial and superficial because only the peripheral part of the egg poor in yolk is the exclusive site of successive mitoses (Franquinet and Foucrier, 2003). These mitoses are at the rate of one mitosis every 10 minutes in Drosophila and the whole process takes 3 hours (Campos-Ortega and Hartensein, 1997). This rate appears to be consistent with that of *S. gregaria* in the sense that the segmentation begins in the genital tract of the female, and after an hour and a half after spawning, the segmentation is completed. The beginning of gastrulation is marked by cellular multiplication of a portion of the blastoderm followed by movements of deployment, of embolism and delamination. Among many insects at the end of segmentation, along a strip located ventrally, cells multiply and the blastoderm thickens to transform into a cylindrical epithelium called germinal band (Lamotte and L'héritier, 1969). Its two ends invaginate; the folds of the serosa will cover them and thus define the amniotic cavity. The germinal band will then be covered with a double membrane the outer part of which is the serosa and the inner part the amnion. The germinal band is also double. Its outer layer is the ectoderm marked with a median groove, the neural groove, the inner layer is the result of delamination (Johannsen and Butt, 1950). In *S. gregaria*, these two protective covers and the process of delamination were also observed. During these movements, the beginnings of organogenesis are manifested by cephalization followed progressively by the differentiation of the two other regions of the body of the insect. This process has been described in Drosophila (Franquinet and Foucrier, 2003) and in *Locusta migratoria* (Harrat and Petit, 2009) and in *Schistocerca nitens* by Bentley *et al.* (1979). The formation of the muscular system that we observed on day 11 has been described by Ball *et al.* (1985) on day 10 in *Schistocerca nitens*.

In Drosophila, the whole embryogenesis takes less than 24 hours; segmentation is done in 3 hours, gastrulation in 2 hours 30 minutes and organogenesis in 18 hours 30 hours (Franquinet and Foucrier, 2003). Compared to *S. gregaria* whose embryogenesis takes 12 days, the duration of segmentation has also taken about 3 hours, but gastrulation and organogenesis respectively lasted 2 days and 10 days. During their work, Bentley *et al.* (1979) and Shepherd and Laurent (2004) expressed the duration of the various phases as a percentage of the total duration of embryogenesis. These proportions are similar to those recorded in *S. gregaria*. Indeed, according to this author, at 5% of the development time, the energids are formed, this means that the segmentation is advanced and that karyokinesis is completed. At 15% of the time, he observed the cephalization of the embryo, which means that the organogenesis has already begun. These proportions correspond respectively to 10 hours and 1.8 day in the case of *S. gregaria* we

studied. Besides at 20% of the time of embryogenesis of *S. nitens*, the embryo develops and occupies two thirds of the length of the egg. In the case of *S. gregaria*, the 20% of the time correspond to 2.4 days and the embryo occupies only a third of the length of the egg; it occupies two thirds only on the seventh day. Moreover, eye pigmentation was observed earlier in this study, on the 5th day, which corresponds to 42% of the time of embryogenesis against 50% of the time recorded by Bentley *et al*. (1979) in *Schistocerca nitens* and Shepherd and Laurent (2004) in *S. gregaria*. Knowledge of the duration and timing of embryonic development will allow to provide the best times and the means of intervention to contribute to the integrated pest management (IPM) against locusts.

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