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Development of the subsoephageal body cells and the pericardiac cells during embryogenesis with diapause in *Locusta migratoria* (Linnaeus 1758) (Orthoptera: Acrididae)

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ABSTRACT

During *Locusta migratoria* embryogenesis, the yolk is progressively degraded and the resulting metabolites are released in the haemolymph. We researched the organs possibly involved in the uptake of haemolymphatic proteins. Among organs originated from mesoderm, the SOB (suboesophageal bodies) situated in the embryonic head are remarkable by a very early acquisition of differentiated cytological characters, while most other cells of the embryo are undifferentiated. The SOB quite disappear before hatching. Just before katatrepsis stage, the other organs derived from mesoderm begin to differentiate, including the PC (pericardial cells) which take over from the SOB. These cells, situated in thorax and abdomen, are developed during the dorsal close of embryo. The development and the ultrastructural changes of the SOB cells and of the PC were studied during an embryogenesis with diapause. The morphology of embryos which enter diapause is comparable with that of a continuous development at the beginning of katatrepsis. However, the cells of SOB and PC cells suffer from remarkable changes not only physiologically but cytologically. At the beginning of diapause, the proteosynthetic activity practically disappears in the SOB cells and the lysis areas appear. Nevertheless, the exchanges between these cells and the haemolymph still remain important. For the period of cold, which is necessary to the resumption of development, the aspect of the SOB cells changes and in particular the areas of lysis become less wide. When the embryo reopens its development, the SOB cells show a proteosynthetic activity and the areas of lysis disappear. The changes of the SOB cells and of the PC cells are regularized during the resumption of the development: the SOB cells which had again taken a normal activity start to regress from the stage VII on, while the PC cells take over.

1. Introduction

The diapause in many species of insects was explored (Denlinger, 2002). According to species, the diapause is imaginal, nymphal, larval or embryonic. Most often, it is defined as a stop of development and falls of the metabolism (Lees, 1955; Mac Leod and Beck, 1963; Gelman and Hayes, 1982). Researches on the embryonic diapause were carried out more particularly on Lepidoptera, especially *Bombyx mori* (Gadenne et al., 1986; Kai and Nishi, 1976; Miya, 1985) and on an Orthopteran, *Melanoplus differentialis* (Agrell, 1951; Lees, 1955; Agrell and Lundquist, 1973).

The embryology of grasshoppers is well known in particular from the work of Roonwal (1937). The data relative to *Locusta migratoria* L. were supplemented by more specific analyses (Le Berre, 1957; Sanger and McCann, 1968; Maltête, 1962; Chapman and Whitham, 1968; Petavy, 1985; Ihsan, 1988; Ihsan et al., 1996; Harrat, 1999; Harrat et al., 1999, 2006). Briefly, the differentiation of morphological characters occurs during the stages I–IV; during blastokinesis, the embryo moves toward the centre of the egg, the head oriented to the posterior pole (anatrepsis, at the end of stage IV), and then it progressively turns upside down (katatrepsis, at stage V); the dorsal closure is complete at stage VII; from the stage VIII on, there is an increase of pigmentation and size until hatching.

In *L. migratoria*, the existence of polyvoltine strains without diapause and univoltine strains with diapause, associated with the great dimensions of eggs, seems favourable to a assess some aspects of diapause physiology during embryogenesis. In

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polyvoltine strains, the development is continuous until hatching, in contrast to univoltine strains, where the development stops at the end of stage IV, before blastokinesis. The termination of diapause needs low temperatures during several weeks, and the resumption of development takes place at temperatures exceeding 27 °C. Most works dealt primarily with the characteristics of the metabolism during the period of diapause, neglecting the pre-diapause period and the resumption of the development. Only Le Berre (1957) gave a comparative study of all the embryonic development of *L. migratoria* strains with or without diapause. Ihsan (1988) and Ihsan et al. (1996) analyzed the physiological and ultrastructural pre-diapause period, to compare the embryonic developments of polyvoltine and univoltine strains in the period preceding the katatrepsis of *L. migratoria*.

During *L. migratoria* embryogenesis, the yolk is progressively degraded and the resulting metabolites are released in the haemolymph. Three organs originated from mesoderm are possibly involved in the uptake of haemolymphatic proteins, the fat body, the SOB (suboesophageal bodies) and the PC (pericardial cells). The SOB, situated in the embryonic head, are remarkable by a very early acquisition of differentiated cytological characters, while most other cells of the embryo are undifferentiated. The SOB quite disappear before hatching. Just before katatrepsis, the other organs derived from mesoderm begin to differentiate, including the fat body and the PC, which take over from the SOB. These PC, situated in thorax and abdomen, are developed during the dorsal closure of embryo. The roles of both structures (SOB and PC) in the proteinic metabolism in the embryo in strains without diapause have been well investigated (Ihsan, 1988; Ihsan et al., 1996; Harrat, 1999; Harrat et al., 1999, 2006).

Our aim was to provide ultrastructural data on the developmental changes of these tissues from the pre-katatrepsis to the hatching, a period during which the diapause and the resumption of the development take place in univoltine strains. In this case of development, the embryos do not exhibit any morphological changes during the diapause, but the cells of the SOB and the PC cells suffer from remarkable changes physiologically and cytologically. Le Berre (1957) and Kessel (1961a, 1961b, 1962) specified that there is a specific metabolism to the diapause, as the cells of the SOB change into vacuolar with much bulkier granules. By histological investigation in the embryology of another Acrididae, *Aulocara elliotti*, Neumann-Visscher (1976) emitted the hypothesis that the SOB cells would secrete a carrier protein of the juvenile hormone (JH) and/or transform a soluble form of ecdysteroid probably secreted by the ventral head glands (equivalent to prothoracic glands). Following a more recent study, we know that the cells of the SOB undergo particular changes during the pre-diapause (Ihsan, 1988). As little is known on the cytological aspects of cells of the SOB and the PC during the diapause, we undertook a study on the ultrastructural changes of these two structures during the diapause.

2. Materials and methods

2.1. Animals

This work was conducted on eggs and embryos of a strain of *L. migratoria* ssp. *cinerascens*, the mixed "Espiguette" strain, collected in the South of France, close to the headlight of Espiguette, at 43°28N 4°E. The females laid eggs in jars with moist sand. Egg-pods were taken in the hour following the end of oviposition. They were put in boxes containing air saturated with water vapour and incubated at 27° ± 0.5 °C. For the development without diapause, the egg-pods were incubated at 27° ± 0.5 °C until the hatching. For the development with diapause, the egg-pods with eggs entering diapause were kept at 27° ± 0.5 °C for 12–45 days and then at 22° ± 0.5 °C for two to three months. Then they were put at 9° ± 0.5 °C for a period going from one to three months. After a 3 days stage at ambient temperature, the egg-pods were incubated at 27° ± 0.5 °C until hatching. To a better understanding of temperature effects, different combinations of conditions were submitted to the embryos. Embryonic stages were recorded according to terminology of Chapman and Whitham (1968), modified by Petavy (1985).

2.2. Light microscopy

The embryos were fixed in alcoholic Bouin. After rinsing with 70% ethanol, they were gradually dehydrated and embedded in paraffin wax, processed into serial sections of 5 μm thickness, and stained with haemalun-eosin. We have observed the serial sections of 6 specimens for each embryonic stage. Then, 1–1.5 μm sections of material fixed and embedded for electron microscopy were stained with toluidine blue and mounted in Depex® (Gurr.).

2.3. Electron microscopy

The embryos were fixed with 4% glutaraldehyde in a 0.2 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature. The fixing of the older embryos was less easy due to the dorsal closing and the secreted cuticle, and the embryos were separated into the head, thorax and abdomen prior to fixation. Endocytosis of proteins was studied by incubating isolated heads of embryos with 50 mg/ml ferritin in insect Ringer's solution. To ensure the fixation up to the structures studied in our work, the head was divided in two parts at the level of the cells of the suboesophageal body, and the embryos were cut at the thorax and abdomen levels, with elimination on the ventral side, for the study of the PC cells. The embryo fragments were post-fixed in 1% osmium tetroxide for 1 h at 4 °C. After a gradual dehydration, the material was embedded in Epon 812 (Luft, 1961). The 1.5 μm semi-thin sections were stained with toluidine blue and mounted in Eukitt. The ultra-thin sections cut with a diamond knife equipped to a Reichert ultra-microtome were contrasted with uranyl acetate and lead citrate (Reynolds, 1963). More than 20 sections by embryonic stage were selected and observed. They were examined with a Philips 300 electron microscope operating at 100 KV.

3. Results

3.1. Developmental changes of the SOB cells during diapause period

The occurrence of diapause, during which the embryo undergoes "internal modifications", depends on incubation temperature (Le Berre, 1957). We thus analyzed the developmental changes of the cells of the SOB of embryos incubated at 27 °C or 22 °C and of embryos placed successively at 27 °C and 22 °C. The effect of cold stays (9 °C) for a long period was assessed for embryos previously incubated to two different conditions: incubation at 22 °C or incubation at 27 °C and then at 22 °C.

3.2. Changes in embryos incubated at 27 °C

Among eggs incubated at 27 °C, some entered diapause, to stop the development at the stages IVb-Va (Fig. 1A). In the embryos, however, the proteosynthesis still remained very active in the cells of the suboesophageal body: the numerous cisternae of rough endoplasmic reticulum are regularly arranged in parallel (Fig. 1C).