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# Correlation between metabolic depression and ecdysteroid peak during embryogenesis of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae)

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**Key words.** Acrididae, *Schistocerca gregaria*, ecdysteroids, oxygen consumption, morphogenesis, metamorphosis, embryogenesis, respiratory metabolism, pharate stages

**Abstract.** Respiratory metabolism of developing eggs of *Schistocerca gregaria* has been individually monitored by means of scanning microrespirography. The freshly oviposited eggs consumed 7 nl of $O_2$ /min./egg (50 µl $O_2$/g/h) while the pharate 1st instar larvae shortly before hatching consumed 141 nl of $O_2$/min./egg (550 µl $O_2$/g/h), which shows 20-fold metabolic increase during the egg stage. The output of $CO_2$ was also regular, without discontinuous bursts throughout the whole embryonic development. The amounts of $CO_2$ produced were constantly close to R.Q. ratio of 0.7, suggesting that lipid was the main energetic source. The vermiform, pharate 1st instar larvae emerging from the eggs exhibited very high respiratory rates (up to 3,000 µl $O_2$/g/h). During initial phases of the egg stage, $O_2$ consumption steadily increased until day 6, which was associated with katatrepsis or blastokinesis stage of the embryo (61 nl of $O_2$/egg/min. = 240 µl $O_2$/g/h). Since blastokinesis, respiratory metabolism of the egg remained constant or decreased steadily until day 10, when it rose sharply again towards hatching. The temporary metabolic depression was closely correlated with endogenous peak in ecdysteroid concentration within the embryo. These results corroborate validity of the reciprocal, high ecdysteroid – low metabolism rule previously known from insect metamorphosis. They extend its application into the period of embryogenesis. Practical implications of certain physiological, morphological and evolutionary consequences of these findings are discussed with special emphasis on the connecting links between embryogenesis and metamorphosis.

## INTRODUCTION

Insect morphogenesis is divided between the two distinctive developmental periods, embryogenesis and metamorphosis, which are temporarily interrupted by larval somatic growth (Novák, 1966). The periods have a number of physiological and biochemical features in common. For example, both of them represent a non-feeding, predominantly immobile developmental stage adjusted to the proliferation and reorganization of tissue and cells. At the beginning of 20th century, Antonio Berlese (1913) developed a theory which later became known as the desembryonization theory. He concluded, quite reasonably, that external morphological structures of larvae were determined in different insect groups by the extent of the morphogenetic changes that occurred during embryogenesis.

With respect to hormones, embryogenesis and metamorphosis are distinguished by the absence of juvenile hormone (JH). Another remarkable endocrine feature of these two stages appears to be special peaks in endogenous concentration of ecdysteroid hormones (see Hoffmann & Lageux, 1985; Koolman, 1989 for a review). The peaks are almost exclusively located in the pharate, non-feeding developmental periods. They are virtually absent during the feeding periods of larval growth. At the beginning of ecdysone research, ecdysteroids were thought to function as a centrally produced hormone secreted from prothoracic glands for stimulation of insect ecdysis (cf. Sehnal et al., 1988). According to Sláma (1980, 1998) however, the pharmacokinetic action of ecdysteroids does not fulfill the function of a centrally produced growth hormone. The biological status of ecdysteroids has been defined as the peripheral feedback tissue factors which synchronize tissue growth with some important developmental events like ecdysis or oviposition (Sláma, 1980). The relationships between total body metabolism and morphogenesis have so far been investigated only during the period of metamorphosis. A long time ago it was recognized that the process of larval-pupal-adult transformation was always associated with a U-shaped metabolic response. The first observation of this was ascribed to August Krogh (cf. Fink, 1925). At present, we can find a number of earlier, as well as new, respirometric data that confirm the presence of these U-shaped metabolic trends. They have been found in all major endopterygote groups (see Wigglesworth, 1965 for a review) and also during the second-half period of the last larval instars in certain exopterygotes (Sláma, 1960; Sägesser, 1960). Physiological reasons for existence of the U-shaped metabolic changes are usually explained by the specific nature of the histolysis-histogenesis process.

In 1982, I discovered existence of a reciprocal, or inversely proportional correlation between the peaks in ecdysteroid concentration and the rate of total body metabolism. The peaks always coincided with the moments of minimum metabolic intensity in the pharate developmental stages of a number of unrelated insect species (Sláma, 1982). The initial part of these ecdysteroid-metabolic correlations resulted from interaction of three variables: (a) ascending ecdysteroid content; (b)

decreasing metabolic rate, and (c) rising amount of cell divisions and morphological reorganizations among tissue and organs. Stages with relatively weak histolysis-histogenesis reorganizations showed proportionally smaller ecdysteroid peaks and smaller metabolic depressions (Sláma, 1982). In certain species (*Dermestes vulpinus)*, the peaks of ecdysteroids (Delbecque & Sláma, 1980) and the metabolic depressions (Sláma & Hodková, 1975) were absolutely independent from the presence or absence of the prothoracic glands. In addition, the prothoracic glands of *Galleria* showed absolute minima in their intrinsic metabolic activity just at the time of the ecdysteroid peak in the whole prepupal body (Sláma & Malá, 1984). This lack of direct causal relationships between ecdysteroid peaks and the prothoracic glands was also found during the embryonic development of *Schistocerca* (see Sbrenna, 1991 for a review).

It is important to realize that injections of exogenous ecdysteroid do not cause immediate metabolism stimulating or inhibitory effects during metamorphosis (Sláma & Hodková, 1975; Sláma, 1982). However, nothing is known in this respect about embryogenesis. The eggs of certain exopterygote insects (*Locusta, Schistocerca)* contain a large amount of ecdysteroid conjugates of maternal origin in yolk and, in addition, they also show pronounced peaks in ecdysteroid concentration that are produced within the embryo itself (reviews by Steel & Vafopoulou, 1989; Sbrenna, 1991). In this work I have investigated changes in respiratory metabolism during the entire embryonic period in the desert locust, *Schistocerca gregaria.* The aim was to elucidate possible ecdysteroid-metabolic interactions in embryogenesis. The eggs of *Schistocerca* have been selected because biosynthesis and metabolism of ecdysteroids have been intensively investigated and are probably the best known (cf. Gande & Morgan, 1979; Gande et al., 1979; Scalia et al., 1987; Tawfik et al., 1999).

## MATERIAL AND METHODS

Adult desert locusts *Schistocerca gregaria* (Forskål) were reared at a constant temperature of 28–30°C under a 12L : 12D photoperiodic illumination. Egg pods laid into sterilized wet sand were collected daily. Individual pods were incubated separately in wet sand at 28–30°C. The eggs for respirometric measurements were kept in Petri dishes on moistened filter paper under aseptic conditions. They were incubated in darkness at 28–30°C together with the rest of undisturbed eggs from the same pod. These control eggs were measured only once at different developmental periods and the values were used for determination of regularity in development and water absorbtion.

$O_2$ consumption was recorded in individual eggs by a scanning micro-respirographic method (Sláma & Denlinger, 1992). This continuously recording technique enabled daily $O_2$ consumption to be measured in at least 16 separate eggs, with an accuracy better than one nanoliter of $O_2$ per min. The eggs were transferred untouched, lying on small discs of moist filter paper, into opened plastic disposable syringes, 2 ml total capacity. The closed syringes with the eggs and $CO_2$ absorbent (25 µl of 1% KOH) were clipped into the outlets of the respirograph; the measuring temperature was set to 28 ± 0.04°C. After 20 min of temperature equilibration, $O_2$ consumption of individual eggs was monitored on a linear recorder or PC for about 30 to 60 min. Respiratory quotients were obtained from two sets of measurements, i.e. with and without the $CO_2$ absorbent. The final $O_2$ consumption curves for each individual egg were constructed from the daily measurements. The obtained values were occasionally compared with those of the undisturbed control eggs which were measured only once. Values obtained from eggs that later showed some deviations from normal embryonic development were discarded.

Larvae in the control groups of eggs hatched after 14–15 days. The majority of experimental specimens also hatched during this period; however, some of them showed delayed hatching which could be due to the daily manipulation and transfer of eggs to and from the respirograph at a room temperature. In order to obtain consistent average values of $O_2$ consumption we did not use the data of specimens that hatched after 15 days. In addition, values obtained with the group that hatched on the 15th day were interpolated and joined to 0–14 day group. This was necessary because $O_2$ consumption of the mobile, pharate 1st instar hatchlings was much higher and variable (not shown in the Figures) in comparison with the advanced pharate 1st instar larvae enclosed within the egg shell (day 13). The recordings of $O_2$ consumption were made on individual eggs in 5 consecutive series (each consisting of 4 to 8 eggs), the total number of eggs measured was 36.

## RESULTS

### Regularity in embryonic $O_2$ consumption and $CO_2$ release

The freshly deposited eggs (0 to 20 h) show rather low, but definite and very regular oxygen consumption of 7 nl $O_2$/egg/min. They release simultaneously $CO_2$ at a constant rate of about 5 nl /min which indicates the values of the respiratory quotient (RQ) close to 0.7. Evidently, the hydrogen proton fuel that drives metabolic energy of the eggs has been mostly recruited from neutral fat or fatty acids. The RQ ratio of 0.7, which is also characteristic for metamorphosis, has been found throughout the entire period of embryogenesis in *Schistocerca.* In addition, it has been also found in the early postembryonic development of *Schistocerca*, before initiation of larval feeding.

In the advanced pharate 1st instar larvae still enclosed within the egg shell, $O_2$ consumption reaches 141 nl of $O_2$/egg/min. These results show 20-fold increase in metabolic rate during the 13-day period of the egg stage. The final embryonic stages are represented by pharate 1st instar larvae (vermiform larvae), which are mobile. They rupture the egg chorion case by means of two eversible epidermal pouches at the pronotal region ("egg bursters"). The liberated (vermiform) larvae of the pharate 1st instar are vigorously digging their way out of the sand. After reaching the surface, the pharate larvae promptly ecdyse into the regular, ventrognathe 1st instars that are able to feed. These larvae show extremely high $O_2$ consumption values of 0.5 to 1 µl $O_2$/min. On a unit of mass basis, these larvae consume 1,500–3,000 µl of $O_2$/g/h which represents a maximum metabolic rate in the life cycle of the desert locust.

Fig. 1 (A) shows a small segment of an authentic, cummulative, scanning respirographic record to demonstrate the constant $O_2$ consumption of an individual egg. The figure shows that a 2-day-old egg consumed continuously

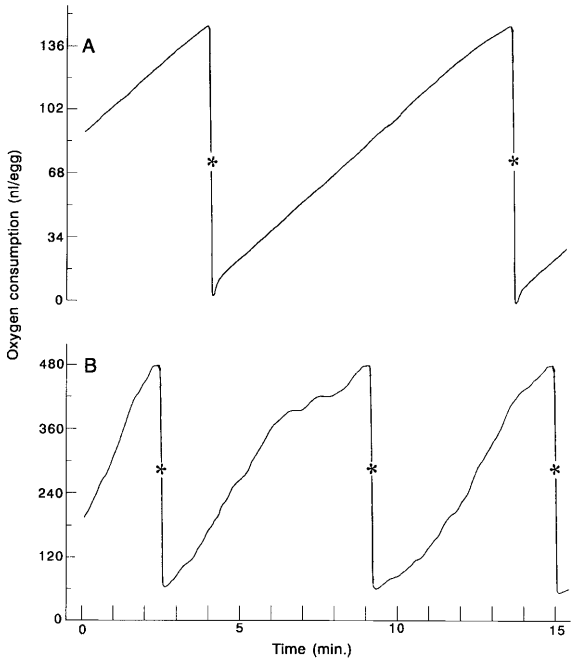


Fig. 1. Examples of respirographic records of $O_2$ consumption illustrating performance of the scanning microrespirographic technique. A – small fraction of a record showing steady or regular $O_2$ consumption in 2-day-old egg of *Schistocerca gregaria*; B – irregularities of $O_2$ consumption in the advanced pharate 1st instar larva one day before hatching. Asterisks indicate the moments of automated electronic zeroing; 28°C.

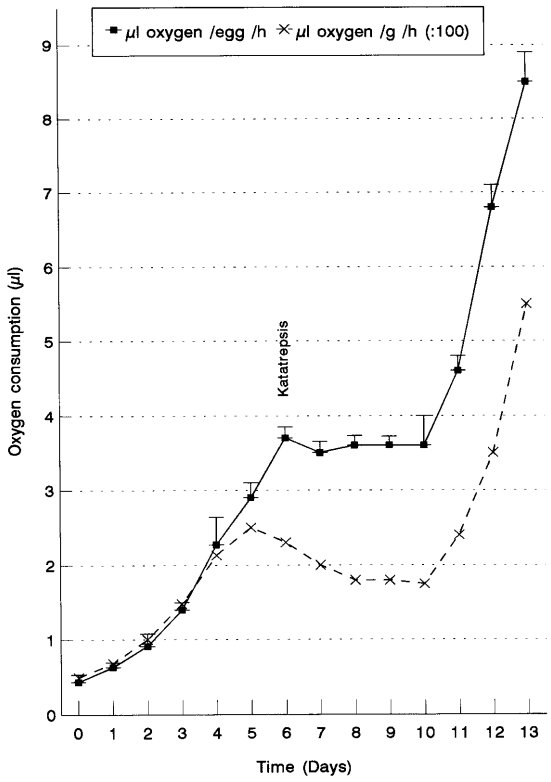


Fig. 2. Oxygen consumption during egg development in *Schistocerca gregaria* ($n$ = 8–16 eggs for days 0 to 5, $n$ = 16–21 eggs for days 6 to 13; vertical bars indicate S.E.M.).

15 to 16 nl of $O_2$ per minute. Other recordings with the eggs of different age revealed a similarly constant $O_2$ consumption, as in Fig. 1(A). Only the advanced, pharate 1st instar larvae ready to hatch showed certain irregularities in $O_2$ consumption. These larvae were able to move within the egg case. A sample of such an irregular respirographic record is given in Fig. 1 (B). It shows periods of elevated $O_2$ consumption (90–150 nl/min. = 270–450 µl $O_2$/g/h) alternating with the periods of lowered $O_2$ consumption rate (relative rest, or reciprocal discontinuous $CO_2$ release?).

### Changes in $O_2$ consumption during egg development

Fig. 2 shows the averages of $O_2$ consumption recordings from oviposition until larval hatching. One of the two curves shows average values in µl of $O_2$ consumption expressed on per egg per h basis. The second shows conventional units in µl of $O_2$ consumption per g per h. The results indicate a more or less continuous rise of $O_2$ consumption of the eggs from oviposition until day 6 of egg development. This moment is associated with the katatrepsis or blastokinesis stage. At this stage, the respiratory metabolism levels temporarily or it slightly decreases until day 10 which is associated with apolysis and secretion of cuticle of the 1st instar larva. From day 10, the rate of $O_2$ consumption sharply rises again in connection with the final biochemical maturation of larval tissues and with the preparation of the muscular and digestive systems of the 1st larval instar for active metabolic functions.

Comparisons of individual records revealed that the final average course of $O_2$ consumption, as shown in Fig. 2, has been true for all individual eggs. This fact can be documented also by relatively small values of S.E.M., see Fig. 2 The metabolic depression following the katatrepsis stage of the embryo and culminating at the time of cuticle secretion clearly shows that the U-shaped, or in this case perhaps better a J-shaped, metabolic curve really occurs in the second-half of embryogenesis. The depression starts after the katatrepsis stage and it is evidently related to histogenesis and differentiation of tissues of the future first larval instar.

### Relations between the metabolic depression and ecdysteroid peaks

The yolk of insect eggs contains relatively large amounts of conjugated ecdysteroids of maternal origin. With respect to developmental regulation, however, the most important fractions are the free ecdysteroids circulating in the body cavity of the embryos. Fig. 3 shows the comparison of the metabolic curve from Fig. 2 with the literature data concerning biosynthesis and metabolism of ecdysteroids in the eggs of *Schistocerca*. It is obvious that the curve for free ecdysteroid content in the developing embryo shows an inversely proportional, or a mirror image reflection of the metabolic rate. The peak of endogenous ecdysteroid concentration at day 10 coincides exactly with the lowest metabolic point, which is associated with intensive cell differentiation and with the secretion of larval cuticle. This may provide satisfactory

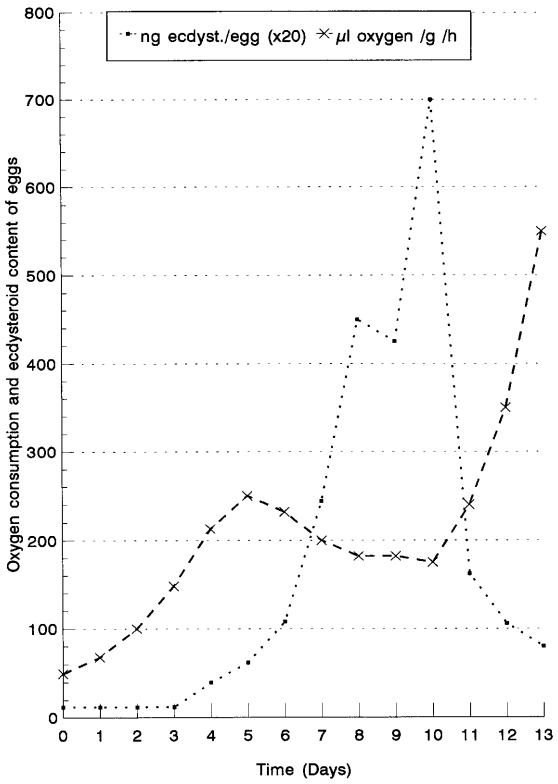


Fig. 3. Inverse relationships between $O_2$ consumption and formation of free ecdysteroid (ecdysone and 20-hydroxyecdysone) during embryogenesis of *Schistocerca gregaria*. The values of $O_2$ consumption in µl of $O_2$/g/h have been taken from Fig. 2; the formation of free ecdysteroid within the embryo of *Schistocerca* has been calculated from values given by Gande & Morgan, 1979; Scalia et al., 1987.

experimental evidence for validity of the reciprocal, high ecdysteroid – low metabolism metamorphosis rule in insect embryogenesis.

## DISCUSSION

### The U-shaped metabolic depressions in insect embryogenesis

Respiration of terrestrial insect eggs is always in conflict with water loss (Tuft, 1950). This problem is partly compensated by special structural adaptations of the chorionic egg shells which can supply oxygen to developing embryo with a maximum water retention (Wigglesworth & Beament, 1950; Hinton, 1962). Attempts to measure the respiration of silkworm eggs had been made in 19th century (Luciani & Piutti, 1888), but the best information concerning the respiration and respiratory enzyme activity of insect eggs was obtained much later by Bodine and his associates in 1930–1950. They investigated respiratory metabolism in the eggs of an American grasshopper, *Melanoplus differentialis*. There was an obligatory initial metabolic rise during the early embryonic development, but this was interrupted after blastokinesis by embryonic diapause with very low metabolic rates. After termination of diapause, the metabolic activity of the developing embryo was resumed (Bodine & Boell, 1934a, b; Bodine & Lu, 1950). This course of metabolic changes may also be common for diapausing eggs of Lepidoptera, e.g. *Bombyx mori* (Luciani & Piutti, 1888) and *Lymantria dispar* (Bell, 1989).

Due to the small size of insect eggs, their respiration was usually measured in large groups, which occasionally included some unfertilized or irregularly developing specimens. The respirographic method used in this study is superior in that it gives continuous readings of $O_2$ consumption for individual eggs. The metabolic depression between days 6 and 10 (Figs 2, 3) is statistically highly significant ($P < 0.001$).

Tangl (1903) assumed that the amount of energy required for embryonic development was greater in comparison with the energy required for metamorphosis. The main argument was that embryonic tissues and organs had to be constructed de novo, whereas adult tissues during metamorphosis were mostly rebuilt from the prefabricated, old larval structures. In 1925, Fink investigated changes in respiratory metabolism during embryonic developoment and metamorphosis in several non-diapausing insect eggs. He noticed the refractory metabolic periods in the middle of egg development and concluded that the curves of $O_2$ consumption had similar U-shaped curve during embryogenesis as in the pupal stage. A temporary metabolic depression in the middle of embryogenesis has been also observed in eggs of the Japanese beetle, *Popilia japonica* (Ludwig & Wugmeister, 1955) and in the eggs of the American cockroach, *Periplaneta americana* (Kubištová, 1955). These results are in good agreement with our respirometric data obtained from *Schistocerca* (see Fig. 2). When considering the relatively small proportion of the histolysing tissue during embryogenesis (cf. Tangl, 1903), the metabolic responses which we have found in the eggs of *Schistocerca* could be perhaps expressed more accurately by a J-shaped curve instead of the U-shaped metabolic response.

### Ecdysteroid-metabolic relationships during embryogenesis

The results shown in Fig. 3 provide clear experimental evidence that ecdysteroid peaks are associated with metabolic depressions not only during metamorphosis but also during embryogenesis. This shows that the rule of inverse metabolic-ecdysteroid relationships (Sláma, 1982) can be applied to insect morphogenetic process in general. The eggs of *Schistocerca* are a very convenient model for ecdysteroid-metabolic interactions because the biosynthesis and metabolism of ecdysteroids have been investigated in this material by a number of authors (Gande & Morgan, 1979; Gande et al., 1979; Scalia et al., 1987; Tawfik et al., 1999; see also reviews by Hoffmann & Lageux, 1985; Steel & Vafopoulou, 1989; Sbrenna, 1991).

Extensive description of changes in ecdysteroid metabolism in the eggs of *Schistocerca* was made by Scalia et al. (1987), and more recently also by Tawfik et al. (1999). The basic conclusions of these studies are as follows: (1) shortly after oviposition, there are conjugated ecdysteroids of maternal origin in the yolk; (2) the conju-

gated ecdysteroids successively diminish during embryogenesis; (3) when the yolk becomes engulfed after dorsal closure, free ecdysteroids (ecdysone, 2-deoxyecdysone and 20-hydroxyecdysone) are produced within the growing embryo, with a sharp peak around day 10 (see the dotted line in Fig. 3); (4) the peak of free ecdysteroids coincides with high mitotic activity, apolysis of the second embryonic cuticle and secretion of the cuticle of the 1st larval instar. It has been also observed that apolysis of the embryonic cuticle and the de novo synthesis of ecdysteroids during the peak are not dependent on the presence of the prothoracic glands (Sbrenna, 1991).

The conclusions of Scalia et al. (1987), reached on ecdysteroids during embryonic development, support previous views (Sláma, 1980, 1982) that ecdysteroids may serve the role of peripheral tissue growth factors. The association of ecdysteroid peaks with mitoses, cell proliferation and finally cuticle secretion (Scalia et al., 1987) provides a good physiological explanation for the metabolic depression, since growth and multiplication of cells are always in conflict with the metabolic efficiency of tissue and organs (Sláma, 1982). In other words, the newly born cells require an obligatory period of "biochemical maturation" before they reach full metabolic capacity. This may also help to explain the fact that a large ecdysteroid peak never occurs within a feeding larval period, a period when the metabolic machinery of the cells is working at maximum speed.

The reciprocal ecdysteroid-metabolic relationships found in the eggs of *Schistocerca* (see Fig. 3) are similar to the temporary depressions of $O_2$ consumption found in the eggs of other insect species, i.e. *Periplaneta americana* (Kubištová, 1955) (Blattaria); *Leptinotarsa decemlineata* (Fink, 1925), *Popillia japonica* (Fink, 1925; Ludwig & Wugmeister, 1955) (Coleoptera); *Lymantria dispar* (Bell, 1989), *Bombyx mori* (Gharib & De Reggi, 1983), *Manduca sexta* (Warren et al., 1986) (Lepidoptera), and; *Hylemyia cilicrura* (Fink, 1925) (Diptera).

From a purely developmental point of view, the effects of ecdysteroids may be superficially antagonistic to the effects of JH. For instance, occurrence of ecdysteroid peaks are incompatible with high JH titers during larval feeding, while JH is invariably absent when large ecdysteroid peaks occur during metamorphosis. According to pharmacokinetic criteria, JH shows all the characteristics of a centrally produced growth hormone. Its action is purely qualitative, all or nothing, at the level of individual cells, depending on whether the JH-responsive gene was activated or not (Sláma & Weyda,1997). By contrast, ecdysteroids exert a profoundly different, concentration-dependent or quantitative dose-response relationships (Sláma, 1998). We have now determined that the above described differences in the action of JH and ecdysteroids, which were derived mostly from metamorphosis, may be true for the period of embryogenesis as well. This is in agreement with the action of JH on embryogenesis, first found by Sláma & Williams (1966) in *Pyrrhocoris apterus* and then investigated in other species, including the eggs of *Schistocerca* (Novák, 1969; Injean et al., 1979; Truman & Riddiford, 1999). It has been determined that exogenous JH can affect the process of embryogenesis at relatively early stages of blastokinesis (Enslee & Riddiford, 1970), which is in good agreement with the onset of free ecdysteroid production within the embryo (Gande & Morgan, 1979; Scalia et al., 1987). Unfortunately, this hormonal story cannot be completed because the effect of exogenous ecdysteroid on egg development is still unknown due to technical difficulties involved in ecdysteroid injection into the egg.

**Hormones, embryonic metabolism and evolution**

The U-shaped metabolic depression (Fig. 2), which is associated with the peak in concentration of free ecdysteroid (Fig. 3) in the eggs of *Schistocerca,* takes place during the period of the pharate 1st larval instar. The next large U-shaped metabolic depression coupled with an ecdysteroid peak can be found in the pharate adult stage (late last larval instar) of most exopterygote insects (Sláma, 1960). This shows that there are two dominant metabolic-ecdysteroid interactions in the ontogeny of exopterygote insects. One of them is associated with the pharate 1st larval and the second with the pharate adult period. In between there are intercallated cycles of somatic larval growth under influence of JH. In the Endopterygota there are also two conspicuously large metabolic-ecdysteroid interactions, the first occurs during the pharate pupal period and the second during the pharate adult period (Sláma, 1982).

Exact ontogenetic positions of the above described ecdysteroid-metabolic interactions can be actually derived from the theory of Berlese (1913) which shows essentially that larvae of the Exopterygota hatch from the eggs at a more advanced ontogenetic stage in comparison with larvae of the endopterygotes (de-embryonization). In 1959, the morphological conclusions of Berlese were further extended and completed by a comprehensive endocrinological analysis by Novák (1959, 1966). He assumed that larval morphology of various pterygote insects was actually determined by the onset of the "status quo" action of JH at various steps of embryogenesis. Novák also outlined homologous embryonic and larval structures for representative insect orders. The conclusions of Novák (1959), concerning the role of JH in embryogenesis were tested and corroborated by exogenously applied JH activity on the eggs of *Schistocerca* (Novák, 1969).

Some hormonal conclusions of Novák (1959, 1966) have been recently "rediscovered" by Truman & Riddiford (1999), who propose a new hormonal hypothesis for the origin of insect metamorphosis. The corner stone of their hypothesis is intimately related to the pharate 1st instar larvae of *Schistocerca*, which have been also investigated in this study. The pharate 1st instar larvae of *Schistocerca* are special because they can move, forcibly break and leave the egg shell, bore their way out of the soil and finally ecdyse on the surface. The freshly ecdysed, true 1st instar larvae have a large, perpendicularly oriented head capsule that is illdesigned for digging,

but is good for feeding. The mobile, pharate 1st instar larvae as well as the mobile pharate adults are quite common stages in insects (Wigglesworth, 1965). In our experiments we have also measured $O_2$ consumption of these mobile pharate 1st instar larvae, which occasionally hatched from the eggs and immediately ecdysed during the recordings. The respiratory rates of these mobile larvae were 3 to 4-times larger (1,700–2,400 µl $O_2$/g/h; not shown in Figs 2 and 3) in comparison with larvae of the same age but still enclosed inside the egg (500 µl $O_2$/g/h; see Figs 2 and 3).

The new hypothesis of Truman & Riddiford (1999) claims that the advanced pharate 1st instar larva of the locust might constitute a special morphogenetic stage called a "pronymph". It has been further speculated that the "pronymph" could be equivalent to an evolutionary ancestor of endopterygote (holometabolous) larvae. They have in fact revitalized the outlived doctrine that exopterygote (hemi-metabolous) larvae are sufficiently different from the larvae of endopterygotes and thus should be called "nymphs" instead of larvae. Their suggestion to use a succession of terms pronymph-nymph-adult instead of the common larva-pupa-adult scheme looks original but not professional. Unfortunately, Truman & Riddiford (1999) seriously underscored or ignored previous papers dealing with hormonal hypotheses in the field of insect evolution.

The hormonal hypothesis of Truman & Riddiford (1999) is theoretically based on the old, outdated doctrine of Piepho (cf. Sláma & Weyda, 1997) concerning the action of JH. Moreover, it adheres strictly to the conservative belief that ecdysteroids are nothing more than a centrally produced hormone of the prothoracic gland. They did not realize that the term "pronymph" was long time ago conventionally reserved in entomology to designate the non-feeding prepupal instars of Hymenoptera (sawflies). In addition, the term "nymph" was once used for exopterygote larvae at some prehistoric age of insect physiology, being later reasonably abandoned by the pioneers of insect morphology, H.E. Hinton and V.B. Wigglesworth, some 40 years ago (for more details see the books by Wigglesworth, 1965; Novák, 1966; Sláma et al., 1974).

The locust egg shows a fascinating succession of embryonic developmental forms (Shulov & Pener, 1959). It is well known that there are actually two embryonic moults (Hoffmann & Lageux, 1985; Scalia et al., 1987). The pharate 1st instar larva ("pronymph" according to Truman & Riddiford, 1999) shows certain advanced morphological structures which never occur in endopterygote larvae. There are 3 pairs of large thoracic legs, partly developed compound eyes, developed optic lobes of the brain and everted imaginal discs of the wings. These structural features may be homologous to the advanced pharate pupae, but not to the larvae of endopterygotes.

According to Novák (1966, 1969), the early embryonic development of a locust shows several morphogenetic stages that may be homologized with a potential ancestor of an endopterygote larva. These are the concealed early embryonic stages occurring around the first embryonic moult (Novák, 1966). This stage has nothing in common with the advanced pharate 1st instar larva or "pronymph" of Truman & Riddiford (1999). A major obstacle with the "pronymph" would be to specify for which group of endopterygote (holometabolous) insects it might be ancestral; whether for the apod larvae of Diptera, polypod larvae of Lepidoptera or the oligopod larvae of Neuroptera or Coleoptera? Recent analysis of the phylogeny of endopterygote insects reveals incredible diversification of the particular ancestral forms (Kristensen, 1999).

Endocrinological arguments against existence of direct hormonal analogy between exopterygote larvae and endopterygote pupae were presented by Novák & Sláma (1959) more than 40 years ago. The true exopterygote stage corresponding to the pupa was localized into the second half-period of the last larval instar. This view was accepted by H.E. Hinton, who stated that "The pupa is phylogenetically derived from the final larval instar of the exopterygote ancestors of the endopterygotes" (Hinton, 1963). This shows that exopterygote larvae are true larvae, not "nymphs" until the advanced pharate adult stage. The neuroendocrine regulations of juvenile larval-larval cycles of somatic growth constitute the most important hormonal condition in the determination of larval stage. Therefore, a larva which undergoes several moulting cycles under the influence of JH is physiologically a larva, no matter if it is a maggot, caterpillar or a locust by external anatomy.

The respirographic results of our study, described in Fig. 3, are also in conflict with the conclusions of Truman & Riddiford (1999), who claim that the pharate 1st instar larva ("pronymph") of *Schistocerca* might resemble an ancestral form of the endopterygote larvae. The metabolic depression coupled with large ecdysteroid peak, which we have found in the pharate 1st instar larvae ("pronymphs"), are normally characteristic for the pharate pupal and pharate adult stages of all Endopterygota (Sláma, 1982). However, they never appear in the young larval instars. It has been already indicated that both Exopterygota and Endopterygota have two periods of extensive morphogenesis, which are always associated with: (a) large ecdysteroid peaks, (b) U-shaped metabolic depression, (c) far-reaching proliferation of tissue and (d) absence of JH activity. In exopterygotes, one is realised in embryogenesis and the other occurs in the pharate adult period at the end of larval life. In endopterygotes, both of them (pharate pupal and pharate adult period) are known to occur during metamorphosis.

The number of juvenile, feeding larval-larval instars under the influence of JH is largely variable and unimportant. Irrespective of whether we call them larvae or "nymphs", they all undergo perfectly coordinated cycles of feeding, somatic growth and moulting. These cycles are regulated by the common neuroendocrine physiological pathways in all groups of the pterygote insects. It thus appears quite unrealistic or incompetent to propose a new hormonal hypotheses on the basis of rather unimportant morphological differences between larvae and

"nymphs" (cf. Truman & Riddiford, 1999), while ignoring the imperative endocrinological premise that the JH of insects is acting only in larvae and adults, but never in the pupae ("nymphs"). This fact was evidently realized long ago by Sir Vincent Wigglesworth (1965) who reasonably refrained from using the term "nymph" for larvae of his favourite bug, *Rhodnius prolixus*. It is difficult to understand the motivation of Truman & Riddiford (1999) in their appeal for ignoring the established terminology of Exopterygota and Endopterygota and the reinstallation of the abandoned terminology "Hemimetabola", "Holometabola", "nymphs" and "pronymphs". Such confusion would inevitably take the field of insect physiology backwards 40-years. Practical use of the current terminology has been recently defined in the detailed phylogenetic analysis of the Endopterygota, the most successfull group of insects (Kristensen, 1999). It may be noted, finally, that the described metabolic-ecdysteroid interactions in embryogenesis (Fig. 3) and metamorphosis (Sláma, 1982) are in favour of the hormonal theory (Sláma, 1995, 1998), which shows that the true nature of the particular external anatomical structure does not depend only on a combination of hormones. It is determined primarily by the species-specific, hormonally-sensitive, start-stop codes that have been imprinted on the inherited tape of the morphogenetic process.

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