Eur. J. Entomol. **91**: 297–305, 1994
ISSN 1210–5759

Developmental changes during metamorphosis in *Tenebrio molitor* (Coleoptera: Tenebrionidae) studied by calorimetric thermography

AARE KUUSIK[1], URMAS TARTES[2], MARGUS HARAK[1], KÜLLI HIIESAAR[1] and LUULE METSPALU[1]

[1]Institute of Plant Protection, Estonian Agricultural University, Riia 12, EE2400 Tartu, Estonia

[2]Institute of Zoology and Botany, Estonian Academy of Sciences, Vanemuise 21, EE2400 Tartu, Estonia

Development, metamorphosis, basal metabolic level, heat production, thermography, muscular activity, apolysis, ecdysis, midgut purging, *Tenebrio molitor*

Abstract. Basal metabolism, evaluated as heat production during the period of metamorphosis in *Tenebrio molitor*, was precisely measured with the use of differential calorimeters. After larval-pupal apolysis characteristic and consecutive metabolic phases were observed: declining, plateau, ascending and pre-ecdysal steep-falling phases. At the moment of breaking the old integument, a sharp peak of the cooling effect, caused by exuvial fluid, was recorded on the thermogram; thus, the exact time of ecdysis and the length of the interecdysal period were determined. By using thermography, also regular periods of muscular activity in metamorphosis stages were monitored and midgut purging after feeding period was timed.

INTRODUCTION

Developmental events during metamorphosis can be observed by external morphological characters and by the course of the metabolic rate. If the changes of the metabolic rate are to be examined uninterrupted, in detail, in an insect during its metamorphosis, then most respirometric methods are quite unsuitable. Most respirometric equipment must be adjusted from time to time and experimental animals would inevitably be disturbed. Few fully automated and highly sensitive systems for long term measuring insect respiration have been described. In certain examples these are based on infrared gas analysis (IRGA) (e.g. Kestler, 1985; Sell et al.,1985; Lighton, 1988) or scanning microrespirographic method (Sláma, 1984; Sláma & Coquillaud, 1992; Sláma & Denlinger, 1992).

Sufficient data exist which indicate that metabolic changes are best reflected in changes of heat production. Curves relating oxygen consumption and heat production to metamorphosis in *Galleria mellonella* (Lepidoptera: Pyralidae) are parallel, both being U-shaped with a sharp decline just prior to adult emergence (Bell, 1940) and the same was demonstrated for *Pieris brassicae* (Lepidoptera: Pieridae) (Fourche et al., 1977) and *Tenebrio molitor* (Kuusik et al., 1985).

The influence of special experimental conditions on the normally occurring respiration behaviour must, at least, be checked as perturbation control (Kestler, 1985). During metamorphosis regular, stereotyped, body movements were observed which may also exert an influence on the metabolic level (Sláma, 1984; Kuusik et al., 1993). Differential calorimeters were often used as actographs when studying diurnal cycles of moving activity in individual animals, e.g., *Tenebrio molitor*, *Blattella germanica* (Dictyoptera: Blattellidae) (Loehr et al., 1976), *Apis mellifera* (Hymenoptera: Apidae) (Fahrenholz et al., 1989) and

Bombus lapidarius (Hymenoptera: Apidae) (Schultze-Motel, 1991, 1992). Special equipments also has been used for monitoring gut purging in insects (Lounibos, 1976).

There is lack of detailed studies concerning changes of metabolic level and muscular activity after alimentary period, pharate development and pupal moult. The purpose of this study was to examine developmental changes in *Tenebrio molitor* using calorimetric thermography.

MATERIAL AND METHODS

Insects

Larvae of yellow mealworm, *Tenebrio molitor* L. were reared in Petri dishes on flour with the addition of 1% dried yeast, at 70% R.H. The population was kept at 25°C under 12 h light each day (see Tyshchenko & Amadu Sheik Ba, 1987). The staging of the last instar larvae was determined according to the method of pigment retraction from lateral ocelli (ocellar retraction) (Stellwaag-Kittler, 1954). The larval instar was divided into three periods according to Connat (1983): the alimentary period (A-period), the pharate stage (pharate pupa or prepupa) (B-period), the transition period (T-period), i.e. a short period (ca 1 day) between A- and B-periods. At the end of the T-period apolysis occurred.

Calorimetric measurements

Six differential thermocouple (copper-constantan) calorimeters with recorders were used at the same time. Two adequate cylindrical boxes made of copper folium (0.2 mm), the insect chamber and the reference chamber, were connected with a constantan wire (∅ 0.1 mm). The insect box was sufficiently spacious in order to enable the pupa to make body movements inside it. We used chambers volume of 0.8–1.0 ml. The calorimetric boxes were placed into a penoplastic isolation surrounded by a metal screen. Temperature differences between the insect and reference chambers were measured, the calorimeter being operated according to the principle of difference thermo-analysis (DTA) (Hemminger & Höhne, 1979). Empirical calibration of the calorimeter was performed by means of a heating spiral of known resistance (15–20 Ω) inside the insect box. Such a simple differential thermocouple calorimeter was sufficiently sensitive (0.01 μV/μW) for recording not only heat production level during metamorphosis but also for recording heating effects during periods of muscular activity and the intermittent emission of CO_2 from the tracheae of an insect (weighing at least 30 mg). In addition the calorimeter recorded cooling effects resulting from ecdysis. Therefore we consider this method as calorimetric thermography.

Calorimetric measurement began after the last instar larvae finished feeding and shortly before the initiation of ocellar retraction (T-period). During measurements the calorimeter was kept in a thermostat (30 ± 0.1°C) inside a 1 l thermos flask.

Respirometric measurements

Respiratory measurements were performed by means of a continuously recording differential electrolytic microrespirometer combined with a calorimeter. The oxygen generating unit consisted of a 3-ml flask containing saturated aqueous copper sulphate solution as an electrolyte. Special electrodes for switching the oxygen generating unit on and off were not used in this respirometer. Oxygen generation was continuous, while the current level was changed according to changes in the insect's oxygen uptake rate (Kuusik et al., 1991, 1992).

The above-mentioned respirometer registered the down-spike resulting from a CO_2 burst and also from the increase in the body volume. The up-spike was recorded by a sudden and cyclic uptake of O_2 or by an abrupt decrease in body volume. The respirometer was sufficiently sensitive to register externally unnoticeable abrupt changes in the body volume. In this way the respirometer also performed as an actograph and this did not disturb respirometric measurements. The recordings of the aforementioned body movements were regarded as respirometric actograms.

IRGA measurements

In the recordings of the infrared gas analyzer (Infralyt 4, Dessau, Juncolor) the baseline was calibrated only in such cases when the duration of the CO_2 burst cycle lasted longer than 15 sec, and when each CO_2 burst did not overlap with its neighbouring bursts. In other cases, IRGA recordings were considered only as qualitative.

RESULTS

The different subsequent metabolic phases were observed according to the changes of the metabolic level.

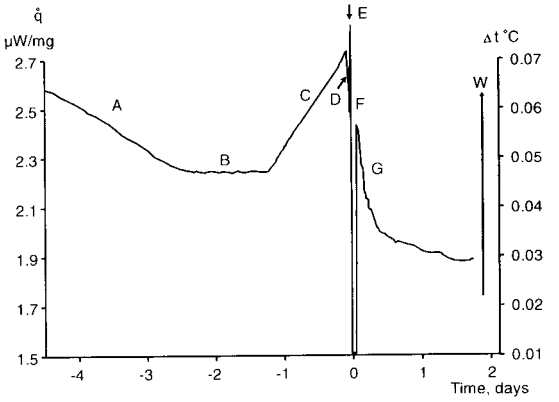

Fig. 1. Metabolic phases evaluated by heat production of basal metabolism in *Tenebrio molitor* from initiation of ocellar retraction until day 2 of pupal development: A – declining phase; B – plateau phase; C – ascending phase; D – preecdysal falling phase; E – moment of breaking of old exosceleton; F – postecdysal metabolic rise; G – beginning of U-shaped metabolic curve in pupa; Δt°C – temperature difference between insect chamber and control; W – direction of warming effect. The curve is constructed by a calorimetric thermogram (1 point per hour).

The first phase (A-phase) was characterized by a decline of the metabolic level (Fig. 1). During this declining phase irregular periods of muscular activity (bending and stretching rhythmic movements) occurred, which were reflected on calorimetric thermograms as sharp peaks of different height (Fig. 2). The metabolic baseline fell gradually, despite the relatively high activity of the prepupa. At the time when the metabolic level reached the lower plateau (B-phase), muscular activity periods became regular. One or two activity periods usually occurred during one hour. Single activity periods (bouts) lasted 2–3 minutes and the intermediate resting (interbout) periods were never interrupted by an accidental wave of strong muscular contractions. This metabolic plateau phase lasted 24–36 hours and was followed by the ascending phase (C-phase). It is remarkable that the transition from the plateau (B) phase to the ascending phase (C) did not take place gradually, but rather there was a distinct obtuse angle between these two phases (Fig. 1). Thus, the time of metabolic increase was easily recognizable from thermogram. The C-phase lasted 22–30 hours, at the end of this phase, regular periods of activity were abolished gradually while irregular periods of muscular contractions were now observed on thermograms (Fig. 3). Synchronous

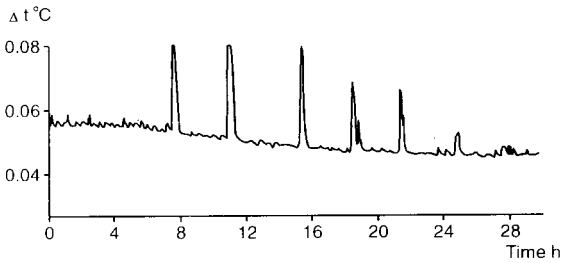

Fig. 2. Periods of active body movements (high peaks) in larvae, after cessation of feeding (showing the trend to locomotion). Between high peaks are seen lower peaks due to bouts of rhythmic bendings. Calorimetric thermogram.

measurements of heat production and respiratory rate confirmed a similar rise of respiration, as in the case of thermogenesis during the C-phase.

Two or three hours before larval-pupal ecdysis, the metabolic level fell steeply, while body movements ceased completely; however, this decrease of metabolic level was not associated obviously with lack of movement of the animal. The preecdysal fall of heat production and, consequently, basal metabolism, regarded conventionally as the metabolic D-phase, was an inevitable step at the end of the pharate pupal stage. This falling phase usually lasted for 20–30 minutes (Fig. 3).

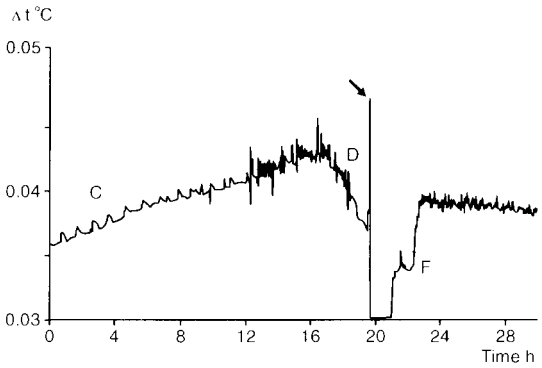

Fig. 3. Heat production measured as temperature differences before and after normal larval-pupal ecdysis. The arrow indicates the moment of breaking the old integument: C – ascending metabolic phase (C-phase); D – preecdysal fall of the metabolic level (D-phase); F – postecdysal metabolic rise before the U-shaped curve. Regular low peaks, in the right-hand part of the thermogram, indicate distinct periods of bending movements.

After this short resting period (D-phase) vigorous peristaltic body movements began suddenly, recorded as an upward peak of heating on the thermogram (Fig. 3). This peak of strong body motions commonly lasted 3–5 minutes. At the same time, the old exoskeleton broke and a downward peak appeared, due to the cooling effect resulting from the evaporation of moulting fluid, which is released from splits which formed in the old cuticle.

After the breaking of the old exoskeleton, at larval-pupal ecdysis, calorimetric measurements were blocked for some time until the liberating moulting fluid had evaporated completely. Usually, this "zero time" lasted mostly 1–2 hours and developmental changes during this period were evaluated with simultaneous respirometric measurements (Fig. 4).

Just at the moment of breaking the old integument, a great amount of CO_2 was liberated and this ecdysal CO_2 emission was also documented by IGRA recording (and by respirometry) (Figs 6 and 4).

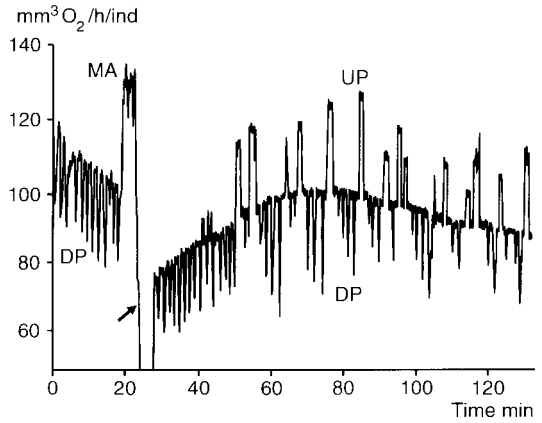

Fig. 4. Record of the automatic respirometer-actograph shortly before and after the larval – pupal moult of *T. molitor*. Upward peaks (UP) are denoting the bending bouts of pupa. Downward peaks (DP) indicate intermittent CO_2 emission. MA – muscular peristaltic activity before breaking the old exoskeleton. The arrow indicates the moment of splitting the exoskeleton and the release of relatively large amounts of CO_2.

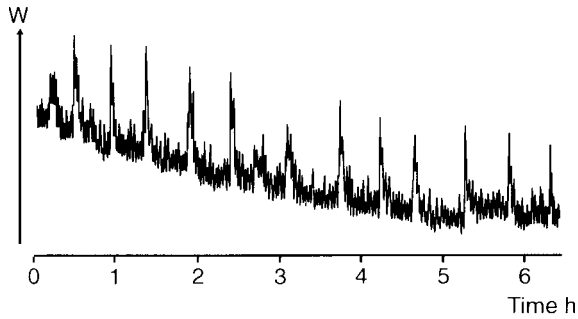

Fig. 5 (left). A section of calorimetric thermogram showing regular periods of rhythmical bending bouts (high peaks) and cyclical external gas exchange (lower peaks) between them during pupal development.

Immediately after the larval-pupal moult, the respiratory rate rose steeply, as seen from the respirogram, and this tendency lasted 1–2 hours. During such a postecdysal metabolic rise, the newly moulted pupa showed only slight activity. After this short metabolic period of rise, the pupa exhibited typical bending movements, but bouts of these movements were at irregular intervals. In the middle of pupal development (40–60%), when the bottom of the U-shaped curve was reached, muscular activity periods acquired a clear pattern, while every bout of bending movements caused one distinct peak on the thermogram. During the interbout periods lower frequent peaks of intermittent gas exchange cycles were visible (Fig. 5).

Normally, pupal development lasted 127 (± 13 SE) hours at 30°C. The decrease and the consequent increase of the metabolic U-shaped curve occurred rather gradually and showed no lowest plateau. The lowest point was noted on day 2 but the ascending part of the metabolic curve was always somewhat longer than the descending one.

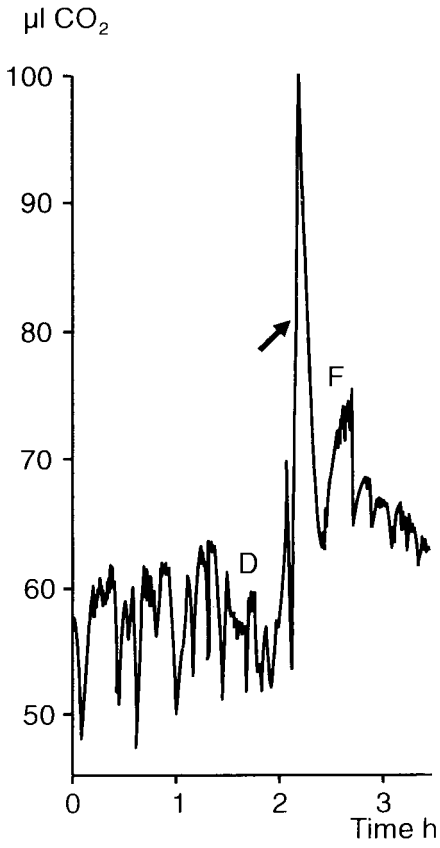

Fig. 6. IGRA record shortly before and after larval-pupal ecdysis. The peaks indicate periods (bouts) of abdominal rhythms motions. The arrow indicates the larger peak of CO_2 burst at the beginning of moult. D – preecdysal metabolic fall (D-phase); F – postecdysal metabolic rise.

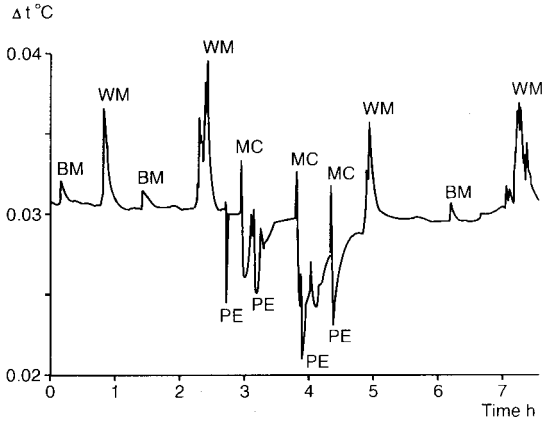

Fig. 7. Calorimetric thermogram from the *T. molitor* larva after initiation of retraction. WM – wriggling movements; BM – bending movements; MC – muscular contractions at defecation; PE – cooling peaks due to purged excrements.

During the last day before adult emergence a clear pattern of periods of bending disappeared and the character of irregular movements of the adult was then recognizable. Pupal development terminated in a similar short decline of the metabolic level described already as the D-phase prior to the larval-pupal moult.

Normally, the repeated cooling peaks (downward peaks) appeared also during the metabolic A-phase shortly before or after larval-pupal apolysis (Fig. 7). These peaks resulted from excretions by the larva during to the completion of evacuation of midgut contents (purging). Each of these cooling peaks was preceded by a sharp upward peak due to muscular contractions.

DISCUSSION

After the feeding period (A-period) the larvae showed a characteristic behaviour: in darkness they were present on the food surface, whereas in bright light they dug themselves into their food source. Thus, during 1–2 days after the end of the A-period, the last instar larvae were motile, but this decreased gradually, until they were not able to plunge into food, even in bright light. At the time of this transition period (T-period) the retraction of pigment from lateral ocelli (ocellar retraction) had begun. The onset of ocellar retraction was regarded as the time of apolysis.

The T-period is seen as a very important step in larval-pupal programme changes in *Tenebrio molitor*; a small ecdysteroid peak was recorded at this time (Connat et al., 1984). In *Galleria mellonella*, *Manduca sexta* and some other Lepidoptera, the transition period is known as the wandering period, when the switch-over from the larval to pupal developmental programme is committed by a small ecdysteroid peak in the absence of the juvenile hormone (Delbecque et al., 1978; Connat et al., 1984; Delachambre et al., 1984).

During calorimetric measurements the last instar larvae showed characteristic movements in the insect chamber during the T-period: since the locomotion was limited there, the animals twisted or wriggled aperiodically. On calorimetric thermograms high irregular peaks (Fig. 2) reflected the tendency of the larvae to locomotion.

It was seen from calorimetric recording that, starting from the end of the T-period (apolysis), heat production decreased gradually. The declining line on thermograms showed no exponential curve, typical of the U-shaped metabolic curve of the pupal stage;

rather it appeared as a straight line, regarded by us as the metabolic A-phase. The gradual decrease of basal metabolism was not due to reduction of activity, because the peaks indicating accidental twisting or wriggling were noted clearly on the baseline of thermograms (Fig. 2).

It is well known that a fall in respiratory intensity in insects may appear during the prepupal period, even before the moment of pupation, when the larva has stopped feeding (Agrell, 1964). It is probable that this descending phase reflects morphological changes which occurred before pupal preecdysal cuticle secretion.

It was possible to make temporal correlation between metabolic changes (estimated from our thermography) and events in epidermal cell cycles during the metamorphosis of *Tenebrio molitor* (Delachambre et al., 1984). It became clear that the metabolic B-phase in our experiments coincides roughly with the M-phase (pupal mitosis) preceding pupal preecdysal cuticle secretion. It was also clear that the metabolic ascending phase (C-phase) coincided with pupal preecdysal cuticle secretion, while pupal lateral abdominal spines became, step by step, more visible through the larval cuticle. Commonly, the initiation of ocellar retraction (apolysis) is regarded as the beginning of the pharate pupal stage. We consider only the C and D-phases as the period of real pharate pupal development.

Before larval-pupal ecdysis an abrupt fall of the metabolic level occurred (D-phase), while the animals exhibited only few and feeble muscular contractions. Respirometric measurements made simultaneously with those from the calorimeter confirmed the decrease of metabolic rate during the D-phase (Fig 4.).

It is possible that the decline of the metabolic level prior to ecdysis is related to events in the tracheal system, which occur just before the moult (see Miller, 1964; Wigglesworth, 1931, 1981). Nevertheless, respirograms reveal clear peaks of intermittent CO_2 emission up to the moment of the onset of shedding of the old integument (Fig. 4).

It can be suggested that the abrupt release of relatively great amounts (8–12 µl) of CO_2 at the beginning of moult is possible due to the accumulation of CO_2 and bicarbonates in the haemolymph and tissues during the D-phase, when gas exchange is suppressed.

The fast release of CO_2 by bursts is well known in Lepidoptera, in studies upon passive suction ventilation, and it has been supposed that most CO_2 was derived rapidly from tissue bicarbonate (see Buck & Keister, 1958; Miller, 1974).

Changes in the muscular activity pattern in the last instar larvae and pupae are also worthy of discussion. Regular low peaks on thermograms (Fig. 3) are not related to the twisting or wriggling of larvae. Each peak results from an increase in heat production occurring during the period of bending movements (bout). Bending is defined as regular and rhythmic forward movements of the abdomen, which are imperceptible by casual inspection (Kuusik et al., 1993). Often, it has been suggested that, in the larval stage, an insect shows no respiratory movements and it was assumed that diffusion takes place through the spiracles. In theory, the regular and scarcely perceptible movements in the larvae of *Tenebrio molitor* serve the purpose of ventilating the tracheal system; but the real functions of these movements have yet to be investigated. As it is seen from calorimetric thermograms, regular bouts of bending at times, were interrupted by stronger movements, indicated on thermograms as higher peaks (Fig. 2). Often, these irregular periods of strong movements occurred at the beginning of the A-phase and again at the end of the C-phase. Normally, during the plateau phase, only regular bouts of weak bending appeared.

In the case of normal pupal development the typical U-shaped metabolic curve always begins only after a postecdysal increase in the metabolic rate. Normally, a rapid increase of respiration lasted 10–20 minutes (Fig. 4) and, clearly, was related to filling the new tracheal system with gas. It is known that at the time of moulting the liquid in pupal tracheae must be replaced by gases (pneumatization) (see Miller, 1964; Wigglesworth, 1931, 1981).

In a "normal" pupa no wriggling or twisting was observed during the interecdysal period. The periods of bending movements were irregular during 10–20 hours after the larval-pupal moult and during 3–4 hours before adult emergence. At this time the rhythmical body movement was vigorous and bending often became stretching (backward rhythmic movements of the abdomen). Bending peaks on calorimetric thermograms acquired the clearest pattern on day 2 when basal metabolism was lowered. At this time, a bout of rhythmic body motions lasted 2–3 minutes, an interbout interval nearly one hour (Fig. 5).

It can be suggested that upward heating peaks on Fig. 7 denote hindgut contractions. Thus, the midgut evacuation did not occur on one occasion only, before larval-pupal apolysis, but happened repeatedly even during T-period. As far as it could be ascertained each individual viscous excrement, during defecation, caused one downward peak on thermogram.

Therefore calorimetric thermography may be recommended as a simple method for the continuous and long-term registration of changes in the level of basal metabolism and muscular activity in an undisturbed insect during metamorphosis.

ACKNOWLEDGEMENT. This research was supported by a grant from the Estonian Science Foundation, No. 189.

REFERENCES

AGRELL J. 1964: Physiological and biochemical changes during insect development. In Rockstein M. (ed.): *The Physiology of Insecta. Vol. 1.* Academic Press, New York, London, pp. 91–148.

BELL J. 1940: The heat production and oxygen consumption of pupae of Galleria mellonella at different constant temperatures. *Physiol. Zool.* **13**: 73–81.

BUCK J.B. & KEISTER M. 1958: Cyclic CO_2 release in diapausing pupae – II. Tracheae anatomy, volume and $_pCO_2$; blood volume, interburst CO_2 release rate. *J. Insect Physiol.* **1**: 327–340.

EDWARDS G.A. 1953: Respiratory mechanisms. In Roeder K.D. (ed.): *Insect Physiology*. New York, London, pp. 55–95.

CONNAT J.L. 1983: Juvenile hormone esterase activity during the last larval and pupal stages of Tenebrio molitor. *J. Insect Physiol.* **29**: 515–521.

CONNAT J.L., DELBECQUE J.-P. & DELACHAMBRE J. 1984: The onset of metamorphosis in Tenebrio molitor L.; effects of a juvenile hormone analogue and of 20-hydroxyecdysone. *J. Insect Physiol.* **30**: 413–419.

DELACHAMBRE J., BESSON M.T., QUENNEDEY A. & DELBECQUE J.-P. 1984: Relationship between hormones and epidermal cell cycles during the metamorphosis of Tenebrio molitor. In Hoffmann J. & Porchet M. (eds): *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones*. Springer Verlag, Berlin, Heidelberg, pp. 245–254.

DELBECQUE J. P., DELACHAMBRE J., HIRN M. & REGGI M.D. 1978: Abdominal production of ecdysterone and pupal-adult development in Tenebrio molitor (Insecta, Coleoptera). *Gen. Comp. Endocrin.* **35**: 436–444.

FAHRENHOLZ L., LAMPRECHT I. & SCHRICKER B. 1989: Microcalorimetric investigations of the energy metabolism of honeybee workers Apis mellifera carnica. 8-th Conf. Dev. Calorimetry. *Thermochim. Acta* **151**: 13–21.

FOURCHE J., GUILLET C., CALVEZ B. & BOSQUET G. 1977: Le metabolisme énergétique des nymphes de Pieris brassicae (Lepidoptères) au cours de la metamorphose et de la diapause. Essay d'établissement d'un bilan. *Ann. Zool. Ecol. Anim.* **9**: 51–61.

HEMMINGER W. & HÖHNE W. 1979: *Grundlagen der Kalorimetrie.* Verlag Chemie,Weinheim, New York, 256 pp.

KESTLER P. 1985: Respiration and respiratory water loss. In Hoffmann K.H. (ed.): *Environmental Physiology and Biochemistry of Insects.* Springer, Heidelberg, pp. 137–183.

KUUSIK A., SEIN E. & PIHU E. 1985: Synchronous measurement of respiration and heat production in insects. In: *Methods and Results of Investigations of Physiological State in Insects.* Institute of Zoology and Botany, Tartu, pp. 24–31 (in Russian).

KUUSIK A., HIIESAAR K., METSPALU L., & TARTES U. 1991: Gas exchange rhythms of Galleria mellonella L. (Lepidoptera, Pyralidae). *Proc. Estonian Acad. Sci. (Biol.)* **40**: 145–156.

KUUSIK A., METSPALU L., HIIESAAR K., TARTES U. 1992: Further investigations on gas exchange cycles in pupae of Galleria mellonella: records of body length changes, spiracular movements and CO_2 release. *Proc. Estonian Acad. Sci. (Biol.)* **41**: 14–24.

KUUSIK A., METSPALU L., HIIESAAR K., KOGERMAN A. & TARTES U. 1993: Changes in muscular and respiratory activity patterns in yellow mealworm (Tenebrio molitor) and greater wax moth (Galleria mellonella) pupae caused by some plant extracts, juvenile hormone analogues and pyrethroid. *Proc. Estonian Acad. Sci. (Biol.)* **42**: 94–107.

LIGHTON J.R.B. 1988: Discontinuous CO_2 emission in a small insect, the formicine ant Camponotus vicinus. *J. Exp. Biol.* **134**: 363–376.

LOEHR K.-D., SAYYADI P. & LAMPRECHT I. 1976: Heat production and respiration during development of two insect species. In Zotin A.I. (ed.): *Thermodynamics of Biological Processes.* Nauka, Moscow, pp. 136–141 (in Russian).

LOUNIBOS L.P. 1976: Initiation and maintenance of cocoon spinning behavior by saturniid silkworms. *Physiol. Entomol.* **1**: 195–206.

MILLER P.L. 1964: Respiration – aerial gas transport. In Rockstein M. (ed.): *The Physiology of Insecta III, 10.* Academic Press, New York, London, pp. 558–615.

MILLER P.L. 1974: Respiration – aerial gas transport. In Rockstein M. (ed.): *The Physiology of Insecta VI, 2.* Academic Press, New York, San Francisco, London, pp. 345–402.

SCHULTZE-MOTEL P. 1991: Heat loss and thermoregulation in a nest of the bumblebee Bombus lapidarius (Hymenoptera, Apidae). *Thermochim. Acta* **193**: 57–66.

SCHULTZE-MOTEL P. 1992: *Energiestoffwechsel und Soziale Thermoregulation der Steinhummel Bombyx lapidarius L. (Hymenoptera, Apidae).* Doktor-Dissertation Freie Universität Berlin, 111 pp.

SELL C.R., WEISS H.R., MOFFITT H.R. & BURDITT A.K. 1985: An automated technique for monitoring carbon dioxide respired by insects. *Physiol. Entomol.* **10**: 317–322.

SLAMA K. 1984: Microrespirometry in small tissues and organs. In Bradley T.J. & Miller T.A. (eds): *Measurement of Ion Transport and Metabolic Rate in Insects.* Springer Verlag, New York, Berlin, Heidelberg, Tokyo, pp. 111–129.

SLAMA K. & COQUILLAUD M.-S. 1992: Homeostatic control of respiratory metabolism in beetles. *J. Insect Physiol.* **38**: 783–791.

SLAMA K. & DENLINGER D.L. 1992: Infradian cycles of oxygen consumption in diapausing pupae of the flesh fly, Sarcophaga crassipalpis, monitored by a scanning microrespirographic method. *Arch. Insect Biochem. Physiol.* **20**: 135–143.

STELLWAAG-KITTLER F. 1954: Zur Physiologie der Käferhäutung. Untersuchungen am Mehlkäfer Tenebrio molitor L. *Biol. Zbl.* **73**: 12–49.

TYSHCHENKO V.P. & AMADU SHEIK BA 1987: Photoperiodic reaction of the mealworm beetle (Coleoptera, Tenebrionidae). *Zool. Zhurn.* **66**(1): 51–59 (in Russian).

WIGGLESWORTH V.B. 1931: The respiration of insects. *Biol. Rev. Cambr. Phil. Soc.* **6**: 182–220.

WIGGLESWORTH V.B. 1981: The natural history of insect tracheoles. *Physiol. Entomol.* **6**: 121–128.

Received October 26, 1993; accepted February 5, 1994