Eur. J. Entomol. **93**: 23–35, 1996
ISSN 1210–5759

Different types of external gas exchange found in pupae of greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae)

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Gas exchange, Unidirectional Flow Microcycles, passive suction ventilation, abdominal rhythmic movements, tracheal ventilation, respirography, *Galleria mellonella*

Abstract. In outdoor and laboratory pupal populations of *Galleria mellonella* four types of external gas exchange were found: (i) a cyclic and sudden deep-air suction-intake, followed instantly by a rapid release of CO_2, occurring at every 8–14 s at 30°C and identified as UDFμC (Unidirectional Flow Microcycles); (ii) intermittent but slow emission of CO_2 without sudden air suction-intake; (iii) chaotic rhythm of gas exchange and (iv) continuous respiration.

Wandering larvae at rest and mobile prepupae in cocoons exhibited the same type of gas exchange and some individual breathing patterns which persisted during later pupal development.

Regular stereotyped abdominal movements acted as ventilating movements only in individuals which did not exhibit UDFμC in gas exchange. Externally imperceptible abdominal movements due to extracardiacal pulsations of haemolymphal pressure played an inessential role in active tracheal ventilation. The rhythms of abdominal movements persisted in pupae independently of the gaseous exchange type and individual breathing patterns.

Among pupae taken from beehives, individuals with UDFμC dominated, while in some laboratory generations only those with chaotic breathing rhythms were found. However, no distinct developmental factors were revealed that could exert influence on the individual formation of the gas exchange pattern.

INTRODUCTION

It is well known that certain developmental stages in a number of insects are characterized by continuous respiration in which no cyclic CO_2 release occurs ("acyclic" individuals). Many other species are known by their cyclic character of gas exchange often called discontinuous respiration or cyclic CO_2 release (Miller, 1974, 1981), where CO_2 is released either in the "constriction open flutter" (CFO) type in a diffusive open period (Schneiderman, 1960) or in the "constriction flutter ventilation" (CFV) type in an active ventilation period (Kestler, 1971). The occurrence of discontinuous gas exchange (DGE) has been summarized by Kestler (1985), Lighton (1988, 1994) and Sláma (1988, 1994).

Few data are available concerning the individual variation of the DGE type within a population. Sometimes a number of specimens lacking the DGE and showing continuous gas exchange pattern were found among "cycling" individuals that exhibited clear DGE cycles (i.e. Edwards & Miller, 1986). In a laboratory population of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) only 30–40% of pupae showed clear DGE whilst the rest were acyclic individuals or their gas exchange rhythms were irregular (chaotic) (Kuusik et al., 1991).

The cyclicity of DGE may be influenced by various factors. Injuring a diapausing pupa increases the metabolic rate and eliminates CO_2 bursts (Schneiderman, 1960). Intermittent CO_2 emission may be abolished in intoxicated insects (Buck et al., 1952; Kuusik et al., 1993; Kestler, 1991) obviously due to the permanent opening of spiracles.

At present several types of DGE cycles are described, while their discontinuous character often showed not only CO_2 emission but also O_2 uptake (see Kestler, 1985, 1991; Lighton, 1990, 1991; Miller, 1982; Sláma, 1984, 1988, 1991a, 1994). All DGE types may be characterized by the duration of CO_2 emission. The long duration of CO_2 release, lasting 10–15 min, is known in some large insect species, i.e. in diapausing silkworm pupae (Schneiderman & Williams, 1955) and in diapausing pupae of *Pieris brassicae* (Lepidoptera: Pieridae) the CO_2 emission lasted more than 5 min (Kuusik, 1977).

A very short duration (less than 10 s) of CO_2 emission due to the sudden displacement of CO_2 from tissue buffers, has been found in diapausing adults of *Bruchus affinis* (Coleoptera: Bruchidae) (Sláma, 1994; Sláma & Coquillaud, 1992). In these apnoic discontinuous ventilation cycles no CO_2 is released during the interburst period. It is similar to classical apnoic discontinuous ventilation (cf., Miller, 1974), where no gas exchange occurs between the ventilation periods. A short duration of CO_2 release (less than 30 s) has been found also in several other species (Tartes, 1990).

Passive suction ventilation (PSV) plays an essential role in DGE. It often occurs during the flutter (F) phase, when air is sucked in gradually through slightly open spiracles (Brockway & Schneiderman, 1967). In some insects PSV occurs as a sudden rapid air intake into the tracheae, while the spiracles are opened for a short time (less than 1 s). In pupae of *G. mellonella* the sudden air intake as PSV occurs every 30 s, with rhythmic changes in body length and were first recorded and described by Sláma (1984). Later, short DGE cycles were studied in *G. mellonella* during different stages of metamorphosis (Kuusik et al., 1991, 1992; Tartes, 1990).

During DGE cycles an active muscular suction ventilation (Hustert, 1975, cf. Kestler, 1985) and, in the CFV type, normal active ventilation (Kestler, 1971, 1978, 1985, 1991; Miller, 1974, 1981, 1982) may be involved. Extracardiacal pulsations of haemolymphal pressure were described by Sláma (1976) and later were found in several insects and ticks (see Farkaš, 1983; Sláma, 1984, 1986, 1991a,b, 1994). The hemolymphal-pressure pulsations may cause rhythmic, yet externally imperceptible abdominal movements, with an amplitude of only 2–5 μm, which can be recorded by special displacement transducers (see Kuusik et al., 1992; Lighton, 1994; Sláma, 1984, 1988). In order to ascertain if body movements act as ventilating movements, the spiracular coupling with ventilating rhythms need to be examined. A thermographic method was used for the simultaneous monitoring of air passage through several spiracles coupled with body movements (see Kuusik et al., 1992; Lighton et al., 1993; Lighton, 1994; Sláma, 1988).

In the present work we describe different types and patterns of external gas exchange that we observed in pupae of *Galleria mellonella*. Some data are given concerning early formation of the individual breathing mode, persisting during later pupal development. The shortest known regular cycles of DGE are examined. The role of stereotyped body movements in mechanical tracheal ventilation is studied in pupae of *G. mellonella*. A possible mechanism of the individual variation of the gas exchange pattern is discussed.

MATERIAL AND METHODS

Greater wax moths (*Galleria mellonella*) were reared at 30°C in constant darkness on a semi-artificial diet (Sehnal, 1966; King & Hartley, 1985). Pupae of known age (± 2 h) were used in the experiments.

"Diet" individuals are defined as animals reared on a semi-artificial diet consisting of 50% less of a natural honey and vitamine than a "normal" diet. Thus, the larvae were forced to eat a greater portion of the cereal ingredient.

A differential electrolytic micro-respirometer-actograph (DEMRA) was employed to record oxygen uptake level, gas exchange rhythms and body movements. DEMRA replaced consumed oxygen continuously by adjusting the current level according to pressure changes in the animal vessel (Kuusik et al., 1991; Tartes & Kuusik, 1994). Upward peaks on the recordings resulted from the sudden O_2 uptake and/or by the abrupt decrease in body volume. Downward peaks were due to CO_2 bursts and/or due to the increase in body volume. In this way O_2 consumed per hour and body rapid movements were registered on the same trace (Fig. 1–4).

In addition to the mentioned recorder, an integrator of current (X 606) complete with an impulse counter and a numeric recorder for averaging the results of 1 h were exploited. The current integrator was useful in case of a large amplitude of cyclic gas exchange.

A catharometer (thermal conductivity detector) of a gas-chromatograph was adapted for entomological studies (Kuusik et al., 1992). Catharometric recording proved the quickest method for separating cyclic from acyclic individuals. A heating-cooling semi-conductor microthermostat allowed the measurement of DGE cycles at constant temperatures of a wide range (from 8 to 35°C).

From the catharometric recordings the duration of single CO_2 outbursts was measured (Fig. 5).

Contact thermography (glass thermistors of 25 kΩ) was used to record, simultaneously, temperature changes from the dorsal line of two body segments. This equipment was sufficiently susceptible for recording DGE rhythms, abdominal pulses (AP) as well as heart beating (Fig. 6).

CO_2 concentration in the rearing bottles was analyzed with a gas chromatograph ("Biochrom").

RQ values were determined by means of a modified method of Mitchell (1973) and Tadmor et al. (1971), using the gas-chromatographic micromethod for respirometry. A gas-tight syringe (10 ml) was used as a respiratory chamber, connected to the dosage loop by a polyethylene tube (∅ 1 mm) (Kuusik et al., 1991).

Differential calorimeters with two adequate boxes (volume 0.5 ml) were exploited for the uninterrupted recording of DGE rhythms, periods of abdominal pulses and standard metabolism (as the heat production level) throughout all metamorphosis stages (Fig. 7). By using differential thermal analysis (DTA) (see Hemminger & Höhne, 1979) it was established if larval-pupal ecdysis may change the prepupal

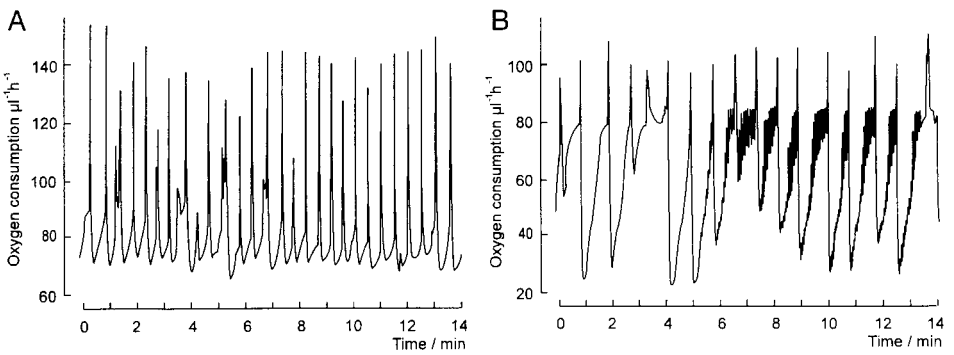

Fig. 1. Respirographic (DEMRA) recordings of UDFμC cycles. A – a 131 mg pupa of *G. mellonella* (♀) (RQ = 0.62). Sudden and exclusively deep air intake (upward peaks) into tracheae are followed, momentarily, by CO_2 bursts; at interburst periods bouts of abdominal twisting are seen. B – UDFμC cycles and abdominal pulses (AP) in a 115 mg pupa of *G. mellonella*, while AP did not eradicate peaks of sudden air-suction (sharp upward peaks) into tracheae.

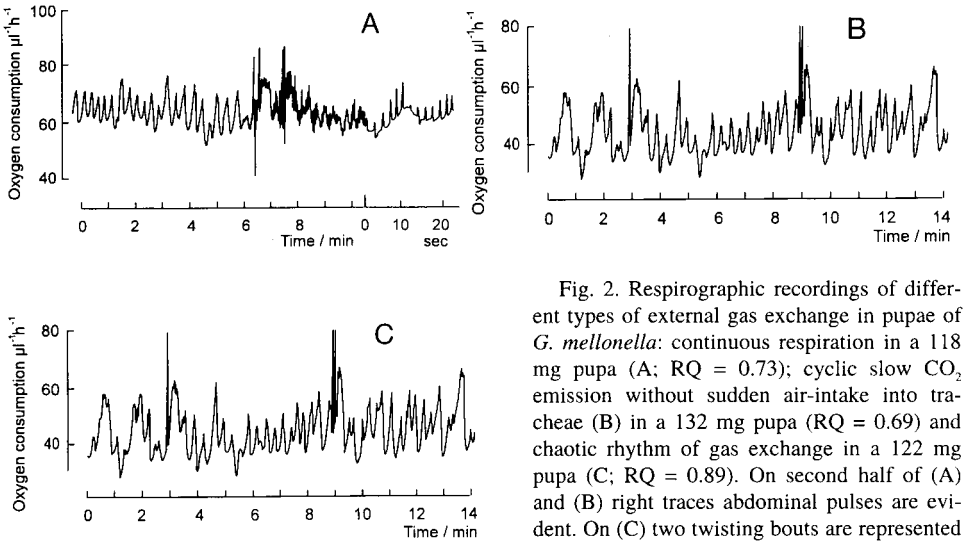

Fig. 2. Respirographic recordings of different types of external gas exchange in pupae of *G. mellonella*: continuous respiration in a 118 mg pupa (A; RQ = 0.73); cyclic slow CO_2 emission without sudden air-intake into tracheae (B) in a 132 mg pupa (RQ = 0.69) and chaotic rhythm of gas exchange in a 122 mg pupa (C; RQ = 0.89). On second half of (A) and (B) right traces abdominal pulses are evident. On (C) two twisting bouts are represented as higher peaks.

pattern of DGE. The exact time of larval-pupal ecdysis was indicated on the recording as a prominent downward peak due to liberating molting fluid (Kuusik et al., 1994).

Air passage through four abdominal spiracles was visualized by meniscus movement of a liquid drop in glass capillaries (inner diam. 0.2 mm) fixed to the spiracles of the same body side with low-melting-point wax. 1% water solution of KOH was used as liquid to decrease the surface tension. A flexible polyethylene tube was attached to the last abdominal spiracle (7A) (Fig. 8). The movements of the meniscus in four capillaries were observed under a stereomicroscope (capillary-microscope method, further "CM"). At sudden air intake a rapid inward shift of the meniscus from 0.15 to 0.2 mm occurred. Fluttering (vibrating in nanocycles) movements of the spiracles could be detected as a vibration of the meniscus.

In order to observe the contractions of somatic muscles in the microscope the soft intersegmental membrane was turned translucent by means of a spherical droplet (0.3 mm diam.) of glycerol.

Experiments with pupae were done on day 2 or day 3 after larval-pupal ecdysis (30 to 60% of pupal stage) mainly at 30°C and occasionally at lower temperatures. With wandering larvae experiments were done at 21°C.

RESULTS

Main types of gas exchange in *G. mellonella*

Mainly four types of respiration were observed in *G. mellonella* pupae. Naturally transitions exist between several types. Numerous individual modifications were also found within one gas exchange type. Here we describe only such gas exchange patterns that may be clearly regarded as a separate types.

Type A. DGE cycles with a sudden deep air suction intake into tracheae (lasting 0.2 to 0.5 s), followed instantly by rapid CO_2 release (microburst) were identified as continuous microcycles with passive suction ventilation (cf. Miller, 1974) and entire CO_2 release in microopenings. On DEMRA recordings every DGE cycle begins with a sharp high upward peak, due to sudden air suction-intake into tracheae, mainly, via fully opened abdominal spiracles 2A and 3A (Figs 1, 3). We did not find differences in spiracular movements

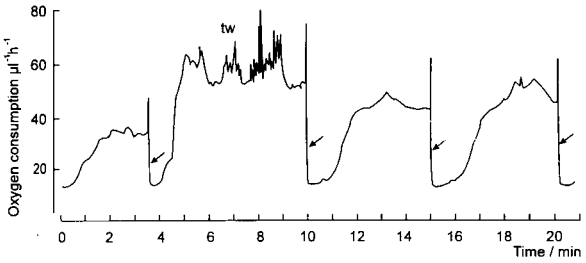

Fig. 3. Respirographic recordings of gas exchange cycles in a wandering larva (138 mg) at 21°C at rest (RQ = 0.93). Weak twisting (tw) movements are seen during an interpeak period. Upward sharp peaks indicate sudden air suction into tracheae. Arrows denote intermittent CO_2 emission.

between both body sides by CM and by thermography (see also Kuusik et al., 1992). No expiration movement occurred in spiracles 2A and 3A after sudden air suction-intake. The deeper the air suction, the higher the recorded sharp upward peak on DEMRA recordings. Almost instantly (after 0.2–0.3 s) after suction air inspiration, expiration burst was seen from spiracles 7A (Fig. 8) (see also Kuusik et al., 1992). By using catharometric measurements, it was documented that pure CO_2 was released cyclically from spiracles 7A. Thus, passive suction ventilation through the first abdominal spiracles was coupled with the release of all the CO_2, accumulated in the microconstriction period between the microopenings, through the last abdominal spiracles. As unidirectional flow, without active ventilation and by coordination between segmental pairs of spiracles, has never been described during microcycles, we propose the name unidirectional microcycles during continuous fluttering (UDFμC).

The duration of a UDFμC (defined as the time interval between two consecutive air intake peaks) varied individually from 8.2 to 14.6 s (10.4 ± 0.9 s; mean ± SD; N = 25 pupae) at 30°C. However, in single individuals, cycling was very stable and each pupa had its "standard" duration of UDFμC. In a 126 mg female pupa, 1,400 successive DGE cycles were measured precisely and the mean duration (10.6 ± 0.07 s), showing negligible deviation, was found.

Sometimes when the interpeak interval was shortened, this shift was compensated for by the following elongated interval; in this way the standard duration of the cycle was preserved.

With temperature reduced from 30 to 20°C the cycle was nearly twice as long. The CO_2 burst from the body lasted 3–5 s (4.2 ± 0.6 s; mean ± SE) at 30°C and 4–6 s (5.4 ± 0.9 s) at 20°C. Thus, the duration of a CO_2 burst depended less on temperature than the length of the UDFμC. Below 15°C the clear cyclicity of gas exchange was lost.

We did not detect any contractions of somatic skeletal muscles throughout the UDFμC, that might have been responsible for rapid CO_2 output from tracheae via spiracles. The

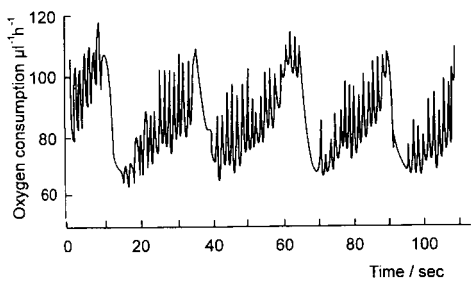

Fig. 4. Abdominal vigorous bendings during UDFμC cycles in a 126 mg pupa (RQ = 0.79) of *G. mellonella* recorded by DEMRA. Sudden air suction intakes are eradicated but cyclic CO_2 emission is preserved.

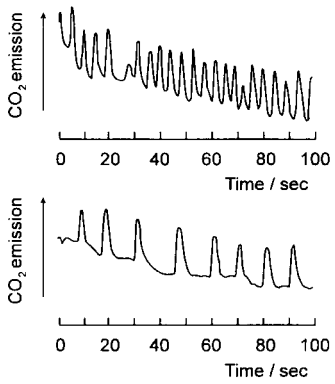

Fig. 5. Catharometric recordings of intermittent CO_2 emission during UDFμC cycles in a 128 mg pupa of *G. mellonella* at 28°C (upper trace) and at 15°C (lower trace). Relative units. Baseline is drifting.

periodicity of micro-contractions of abdominal intersegmental muscles or abdominal pulses (AP) did not coincide with CO_2 bursts. (Fig. 1).

During short DGE cycles of *G. mellonella* (lasting less than 20 s at 30°C), we did not observe any nanocycles that often occur in the flutter period (Kestler, 1985); however, at 21°C when the cycles lasted 40–60 s, nanocycles of vibrating movements appeared in wandering larvae.

Spiracular fluttering was seen as the vibrating of CM while at the same time slow air intake into tracheae via spiracles 2A and 3A occurred. Clear and longer lasting nanocycles due to shivering or vibrating movements (Kestler, 1985) were found in wandering larvae and in mobile prepupae in cocoons. In these developmental stages the duration of UDFμC was mostly 2–3 min at 30°C and 4–6 min at 21°C while nanocycles in a microconstriction period between microopening of PSV could be detected on DEMRA recordings as a more or less even metabolic level (Fig. 3).

Type B. Cyclic but relatively slow CO_2 release without sudden deep air suction intake was another gas exchange type in pupae of *G. mellonella*. On DEMRA recordings no upward peak of air suction was seen and the CO_2 outburst lasted 6–10 s at 30°C (Fig. 2B). In individuals possessing this pattern of DGE spiracles 2A and 3A showed no abrupt inspiration strokes, and slow rhythmic CM movements were seen that corresponded with peaks noted on DEMRA recordings (Fig. 2B). Any significant differences were not found in the spiracular movements of different abdominal segments. In contrast to UDFμC, CO_2 was released in this type of DGE via spiracles 2A, 3A, 7A and perhaps through other spiracles also. No unidirectional ventilation is suspected in this case.

Type C. Chaotic rhythms of respiration were mostly typical of individuals of high excitability. These animals exhibited irregular spontaneous but, mostly, weak contractions of skeletal muscles (Fig. 2C). The spiracular movements (2A, 3A, 5A and 7A were observed) showed an irregular character. The chaotic pattern of respiration had no direct relation to spontaneous muscular activity.

Type D. Continuous respiration as a special type of DGE showed neither regular nor irregular peaks on respirographic recordings (Fig. 2A). An uneven baseline of the recordings was typical of individuals possessing this type of respiration. The spiracles sometimes showed vibrating movements (nanocycles), without any clear inspiration or expiration strokes.

Formation of the individual mode and pattern of respiration

It is probable that the type and individual pattern of gas exchange is determined in larval life and, once acquired, the breathing mode persists during pupal development up to eclosion. Thus, clear UDFμC are formed in mobile larvae during the stage of light cocoon spinning (before larval-pupal apolysis). If, at the time of the prepupal stage, UDFμC are not formed then, as rule, they are not found during later pupal development either.

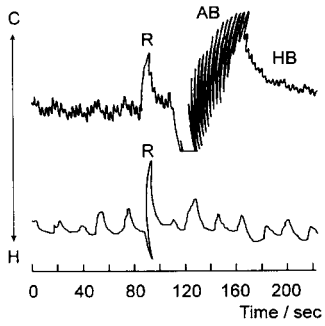

Fig. 6. Simultaneous contact thermography recordings of a UDFμC cycles from two abdominal segments of a 123 mg *G. mellonella* (♀) pupa at 25°C. In the upper trace the thermistor is dorsally contacted to the soft membrane between the 4th and 5th abdominal segments; sharp "comb-tooth" peaks are due to abdominal bending (AB), while a rotation stroke (R) is seen and short peaks denote heart-beat (HB). In the lower trace: the other thermistor is contacted to dorsal line of the 1st abdominal segment. C – cooling, H – heating.

However, UDFμC can be lost irreversibly in individuals used repeatedly in laboratory tests.

Individuals programmed to continuous respiration exhibited this individual breathing mode already at the time of larval-pupal apolysis. The irregular breathing pattern may have been acquired in larval life as well as during larval-pupal transformation.

Pupae (N = 100) from a "natural" population (taken from beehives) exhibited predominantly (73%) clear UDFμC while continuous respiration was also common (18%); a smaller part (9%) showed a chaotic DGE pattern. Independently of the individual breathing mode, pupal development proceeded normally, with the emergence of externally normal adults. However, the transpiration rate in pupae with chaotic DGE was higher than in individuals with UDFμC (A. Kuusik, in prep.).

Special laboratory experiments with undernourished larvae resulted in a larger proportion of individuals with a chaotic DGE pattern (63%) and a smaller proportion (23%) with cyclic slow CO_2 emission, while the rest showed continuous respiration (N = 200 pupae). No animals with UDFμC were found in these test series.

When the aeration in rearing bottles was insufficient and the CO_2 concentration in bottles was constantly 5–6%, UDFμC of respiration appeared seldom in pupal development (only 3% of individuals, N = 200).

Nevertheless, in two laboratory generations we found no pupae with clear DGE cycles despite normal feeding and aeration (N = 160). Thus, factors involved in the individual formation of the DGE pattern are yet to be investigated.

Influence of body movements on the respiratory pattern

We observed several modes of abdominal stereotyped rhythmic and periodically recurrent movements in *G. mellonella* pupae.

A. Abdominal pulse (AP) is defined as abdominal movements that are externally imperceptible to the naked eye and the tip of the abdomen bent rhythmically with an amplitude of 0.01–0.05 mm. As far as we could ascertain, the AP was not caused by active contractions of somatic muscles in movable abdominal segments but by pulses of hemolymph pressure reflected indirectly to the tip of abdomen as AP. In translucent pupae rhythmic microcontractions of intersegmental muscles were detected in immovable abdominal segments only.

From DEMRA recordings it is seen that AP do not abolish or even disrupt UDFμC, while AP are noted on respirograms as a "saw-toothed" pattern (Fig. 1B). In the period of AP no significant rise of the metabolic level occurred.

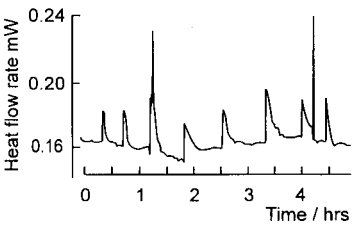

Fig. 7. Muscular activity periods (bouts) as peaks of heat production on a calorimetric recording in a *G. mellonella* pupa at 30°C (initial body mass 132 mg).

AP periods (bouts) are noted as distinct peaks on calorimetric recordings (Fig. 7). Thus, the DTA method proved most suitable for the uninterrupted registration of the periodicity of abdominal movements throughout all stages of metamorphosis (see also Tartes & Kuusik, 1994).

During UDFμC, spiracular movements (on 2A and 3A) were not coupled with APs. Thus abdominal pulsation obviously did not serve as active tracheal ventilation in UDFμC of *G. mellonella*.

B. The vigorous rhythmic bending of the abdomen (AB) was visible externally, while the tip of the abdomen bent with an amplitude of 0.4–1.2 mm. In AB movements somatic skeletal muscles of movable abdominal segments were involved and, thus, AB differed principally from AP movements. Abdominal bending did not abolish UDFμC either, but peaks of sudden air suction-intake were often shortened, indicating clearly that ABs occurred then at partially-opened spiracles (Fig. 4).

In pupae with continuous respiration and in individuals with a chaotic gas exchange pattern, AB often (but not always) acted as tracheal ventilation as estimated by the CM method.

In the first half of pupal development APs prevailed, during the second half mostly ABs occurred. It should be mentioned here that AP movements in a pupa at the pharate adult stage were transformed into AB at the end of every period of muscular activity.

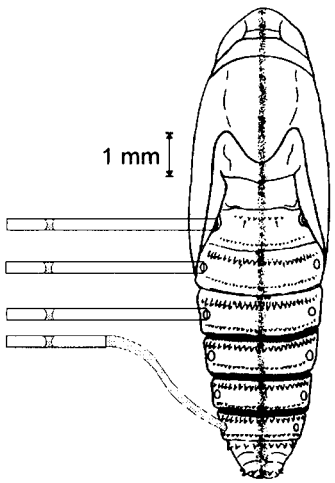

Fig. 8. A scheme for the simultaneous visualization of the passage through the left abdominal spiracles (2AL, 3AL, 4AL and 7AL) by movement of liquid meniscus in glass capillaries fixed upon spiracular plates.

C. Abdominal rotation is a common stereotypic twisting movement in lepidopteran pupae. In *G. mellonella* rotation occurs periodically, and one activity period (bout) consists, mostly, of 2–3 full turns (strokes) made with the abdomen. The first half of the rotation stroke commonly acted as an "expiration" movement due to a decrease in the body external volume, the second half as an "inspiration" movement due to an increase in the body volume. On DEMRA recordings a rotation stroke is, thus, noted as a sharp upward-downward peak (Fig. 2C).

We found three most common modes of spiracular coupling during twisting. In pupae with UDFμC, rotations occurred mostly, at fully closed spiracles, or with some of them leaking, and rotation strokes usually coincided with the O_2 interpeak period (microconstriction). Thus, the clear pattern of UDFμC was not disrupted by twisting movements (Figs 1A, 3, 6).

In the case of pupae with chaotic DGE rhythms, as well as in individuals with continuous respiration, rotations were, mostly, at fully opened spiracles (2A and 3A were observed), and rotating body movements

acted as tracheal ventilation. In the mentioned groups of pupae, 4–10 rotation strokes were commonly made during one bout (Fig. 2).

During the pharate adult stage, a specific mode of spiracular coupling with abdominal rotating occurred: rotations were made at fully closed spiracles, but immediately after the last stroke, spiracles were opened and rapid deep air suction intake followed (mainly via 2A and 3A). Rotation periodicity was not related to abdominal bending or pulsation periods.

Effects of sealed spiracles

If all abdominal spiracles were sealed, then, according to respirographic measurements, the oxygen consumption level dropped rapidly to zero and the body stretched and stiffed. In *G. mellonella* pupae, prothoracic spiracles have insignificant role in gas exchange and thus the head and thorax region must be mostly ventilated via abdominal spiracles.

If all spiracles, except both 2A, were sealed the metabolic level remained the same, while DGE rhythm was modified. In UDFμC, sudden air suction intake was then lost. The metabolic level was not affected even if only 2AR or 2AL remained nonsealed.

If only one spiracle, either 7AL or 7AR, was not sealed, cyclic DGE acquired a continuous character and the metabolic level decreased (60–70% of the earlier level). If both spiracles 7A, were left unsealed, the metabolic level fell first by one-third, but, after a few hours, the previous level was attained.

The sealing of spiracles from 4A to 6A exerted no considerable influence on the DGE pattern and level.

DISCUSSION

Primarily external gas exchange in *G. mellonella* is of interest due to the principally different DGE types represented in the pupal stage. It is evident that larval developmental conditions play a key role, determining in the later DGE type in pupae and, possibly, the individual pattern.

The general gas exchange type in an individual was formed at the time of larval-pupal apolysis and the same type (as well as some individual features) persisted throughout later pupal development. Thus, a prepupa exhibiting continuous gas exchange showed no cyclicity after larval-pupal ecdysis either.

Which is the normal gas exchange mode in *G. mellonella* pupae? All individuals developed into externally normal adults, regardless of their breathing mode. Continuous respiration appeared to be as normal as discontinuous respiration (UDFμC) in pupae. The only argument for regarding UDFμC as being the most "normal" is the predominance of this type in outdoor pupal populations.

Short regular cycles in *G. mellonella* are analogous with the microcycles of CO_2 occurring during flutter (F) in some large insects, e.g. *Hyalophora cecropia* (Lepidoptera: Saturniidae) (see Brockway & Schneiderman, 1967), as far as these microcycles, as a rule, display a regular pattern in segmental spiracular pairs. They differ from the microcycles of the flutter period as they do not lead to CO_2 retention, but are connected with a release of the entire quantity of accumulated CO_2 which enables continuous fluttering without the large CO_2 burst of the (C)FB cycles (CFO and CFV type). It may be due to active suction ventilation (cf., Kestler, 1985) first recorded by electrophysiological methods by Hustert (1975) in *Locusta migratoria* (Orthoptera) and termed regular "miniature inspirations".

In most cycling insects, there exists a distinct F phase (cf., Kestler, 1985; Lighton et al., 1993; Miller, 1974; Sláma, 1988) but no true F phase has been found in some grasshoppers (Quinlan & Hadley, 1993).

We did not detect a true F phase in *G. mellonella* pupae during fast UDFμC (5 to 6 per min). The spiracles were opened only for a short time (less than 1 s), allowing air intake via 2A and 3A and a relatively rapid (3 to 5 s) CO_2 egression via 7A. The spiracles must have been fully opened, in this case, in order to enable such rapid gaseous bulk flow into the tracheae and outward. If UDFμC lasted over 40 s (e.g. in wandering larvae at 21°C and in pharate pupae at 30°C) then a typical vibrating movements (nanocycles) could be detected in the microconstriction periods of the UDFμC's (Fig. 3).

Microopening occurring at every 10 s (on the principle of PSV) through the first abdominal spiracles and a rapid subsequent CO_2 release from tracheae outward through the last abdominal spiracles is sufficiently effective to serve not only the abdominal but also the head and thoracic regions in *G. mellonella* pupae, but in a hitherto unknown way in our UDFμC's. The volume flow of passive suction ventilation may be used to expel subsequently the CO_2 through the last abdominal spiracles. The coordination of the segmental ganglia in this new spiracular behaviour will be analyzed further in this new type of respiration. No mechanical ventilation, coupled with CO_2 bursts, was found in *G. mellonella* pupae, which again confirms the effectiveness of UDFμC in respiration.

Significant mechanic ventilation at CO_2 bursts was not found either in *Cataglyphis bicolor* (Lighton et al., 1993) or *Camponotus vicinus* (Lighton, 1988) (both Hymenoptera: Formicidae). Nevertheless, active muscular ventilation during CO_2 cyclic emission is a common event in adult insects, that has been examined, e.g., in the desert locust *Schistocerca gregaria* (Orthoptera) (Hamilton, 1964, 1972) as apnoic discontinuous ventilation. The (C)FV type has been described for the first time by Kestler (1971) in *Periplaneta americana*, *Blaberus discoidalis* (Blattodea), *Schistocerca gregaria* and *Carabus problematicus* (Coleoptera: Carabidae) (cf. Kestler, 1985, 1991; Miller, 1981, 1982). The (C)FV has been described in *Attacus atlas* (Lepidoptera: Saturniidae) adults by Wasserthal (1981), in *Blaberus craniifer* by Edwards & Miller (1986), and in *Camponotus detritus* by Lighton (1990). In contrast, Hadley & Quinlan (1993) assume an apnoic discontinuous ventilation without a flutter period.

Extracardiac pulse, reflected by rhythmic abdominal movements in *G. mellonella* pupae, did not depend on the cyclicity of gas exchange. The regular periodicity of AP and AB bouts were expressed in all pupae independently of their gas exchange type.

However, in some insects extracardiac pulse may be influenced by toxic and bioactive substances (see Farkaš, 1984; Sláma & Lysenko, 1981; Sláma & Miller, 1987; Sláma et al., 1993). In *G. mellonella* pupae the rhythmicity of AP was changed by the action of some toxic substances and DGE cycles were fully abolished (Kuusik et al., 1993).

The haemolymphal pulses are controlled by the autonomic nervous system (coelopulse) with the centre located in thoracic ganglia (Coquillaud et al., 1990; Sláma, 1986; Sláma & Miller, 1987; Sláma et al., 1979). In insects the coelopulse mechanism is engaged actively in the exchange of respiratory gases through the spiracles and it controls the circulation of haemolymph through the appendages and regulates water balance (Sláma, 1988, 1994).

Twisting stereotypic abdominal movements in *G. mellonella* are surprisingly similar to those described by Sláma (1991a) in pupae of several silk moths. In *G. mellonella* pupae abdominal rotations are the most vigorous mode of mechanical ventilation if made at opened spiracles. However, it is probable that this is not the only function of this type of

motion. In natural conditions, when the pupa is closed in the cocoon, the rotating part of its body is the midbody, since the tip of the abdomen is fixed to cocoon by old exuviae. It is suggested that the inside of the cocoon is ventilated by active movement of the body.

The abdominal movements (AP and AB) of pupae of *G. mellonella* with UDFμC did not act as ventilating movements, or their ventilating effect was nonessential, as had been shown by Brockway & Schneiderman (1967). Abdominal movements (including rotations) played a more important role in active ventilation in individuals whose gas exchange type was different from UDFμC.

All rhythmic abdominal movements, due to the contraction of skeletal muscles in *G. mellonella* pupae, may be functionally associated with haemolymphal circulation and heart-beat, and has been shown for *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae (Tartes & Kuusik, 1994). In *Galleria mellonella* during late pupal development, strict synchronization occurs between rhythmic abdominal movements and heart pulse, while each heart activity period is triggered by a bout of rotation (unpublished).

In the pupal stage, the employment of stereotypic body movements for different functions appears to be an inevitable way of saving energy. The ability of muscular ventilation in lepidopteran pupae is altogether restricted if compared with adult insects. Sudden passive air suction into the trachea coupled with the UDFμC appears to be a useful adaption in developing pupae that have a relatively high level of standard metabolism (in *G. mellonella* 400...600 μl $O_2.h^{-1}.g^{-1}$).

ACKNOWLEDGEMENTS. This research was made possible in part by Grant No. LGC 000 from the International Science Foundation and Grants from the Estonian Science Foundation, No. 189 and No. 858.

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Received November 15, 1994; accepted March 15, 1995