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Calorimetric measurements of energy contents and heat production rates during development of the wax moth *Galleria mellonella*

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Abstract

Changes of the energy content of different developmental stages in the wax moth *Galleria mellonella* were investigated by means of combustion calorimetry. The energy content (Q) is highest in L_7-larvae (2.94 kJ per individual), the last stage in *Galleria* ontogenesis which takes up food, and the specific energy content (q) is highest in adults (37.5 ± 2.4 kJ g^{-1}) with fat as energy reserve. *Galleria* takes up no further food after pupation and in adult life. The energy reserve for metamorphosis and adult life can be calculated to 1.65 kJ per moth.

Heat production rates of wax moths from L_7-instars until death of the adult were recorded continuously by means of long-time calorimetric experiments. Different develomental phases could be clearly distinguished in the calorimetric curves because larval instars, prepupae, pupal instars and adults each showed a specific pattern of heat production. Developmental events such as moultings could be observed with own characteristic patterns of heat production rates. Mean specific heat production rates ranged from 13.7 ± 3.3 mW g^{-1} during cocooning of the L_7-instar, to 5.1 ± 1.3 mW g^{-1} ($n = 6$) in prepupae. Adults showed a circadian pattern of heat production with resting metabolic rates of 5.6 ± 2.3 and 10.4 ± 2.1 mW g^{-1} ($n = 6$) during periods of activity.

Keywords: Calorimetry; *Galleria mellonella*; Metamorphosis; Energy content; Energy metabolism

1. Introduction

Heat production rates of animals strongly depend on the activity status of the individuals. The scope between resting metabolic rate and maximum metabolic rate can be about 10-fold (e.g. mammals) to 150-fold (some flying insects) [1]. Usually, only equal life stages (in most cases mature adults) are compared and no regard is made on differences of metabolic rates of the various developmental stages of the individuals. In animals with direct development, i.e. without larval life forms, the change of heat production rates is rather simple with highest specific rates in the beginning of ontogenesis and lowest rates in mature adults [2]. When larvae occur during development, e.g. in holometabolous insects, processes become more complex since larvae have habitats and lifestyles which often differ strongly from those of the adults. In this study, we compare heat production rates of different developmental stages of the wax moth *Galleria mellonella* in order to get information on the metabolic scope for specific heat production rates during the lifetime of

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this insect. *Galleria* is a well-established model organism for insect physiologists, and our group has published two papers on heat production rates of wax moths so far [3,4], so that together with the present paper, sufficient data will be available to calculate the metabolic scope not only for different activity states (e.g. resting and flying) but also for comparison of developmental stages (e.g. larva and adultus).

The energetic picture of *Galleria* would be incomplete without informations about the amount of energy which is accumulated in biomass during ontogenesis. For this end, we investigated all life stages of *Galleria* by means of combustion calorimetry in order to obtain values for changes in mass, water content and energy content of all larval stages, pupae and adults.

2. Experimental

2.1. Animals

Adult wax moths *(G. mellonella*) were obtained from a laboratory culture. Wax moths were kept in darkness at $T_A = 30°C$ in plastic containers (20 cm× 20 cm× 8 cm).

Larval food contained 22% maize flour, 11% wheat flour, 11% bruised wheat, 11% milk powder, 5.5% yeast, 17.5% bees wax, 11% honey and 11% glycerol.

Larval stages were determined by measurement of the head capsule width, which is the most accurate parameter for defining larval instars [5]. It was not distinguished between first and second larval instars as it turned out that they could not be evaluated safely due to their very small size. The sex of the wax moth was determined only in adults as it is impossible to differentiate gender in larvae and pupae [6].

For measurement of adult lifespan, males and females were kept under two different conditions after pupation: (a) isolated (in a petri dish) or (b) in a group which consisted of 17 females and 14 males (in a plastic container). The adults were inspected once a day for being alive.

2.2. Combustion calorimetry

Oxygen microbomb calorimetry was performed on samples of all larval instars, pupae and adults. All animals were killed in a deep freezer at −18°C, and weighed by means of a mechanical fine balance (Sauter Typ 414/13, Ebingen, Germany) to the nearest 0.1 mg. Samples were lyophilized in a vacuum freeze drier (Trivac Type D4A, Leybold Heraeus, Hanau, Germany) at a partial pressure of 10^4 Pa and a temperature of 4°C for 2–7 days until no changes in mass were detectable. The dried samples were homogenized with a mortar and pestle. As it turned out to be difficult to pellet the samples, portions of 15–25 mg were filled in gelatine capsules with a known specific energy content of 19.6 kJ g^{-1}. These capsules were burnt in a modified Phillipson Oxygen microbomb calorimeter (constructed by I. Lamprecht after [7]; Ignition device: Micro bomb calorimeter Serial No. 52D, Gentry and Wiegert Instruments Inc., Aiken S.C., USA) under high pressure oxygen atmosphere (31×10^5 Pa). The calorimeter was connected to a chart recorder (BD 41, Kipp and Zonen, Delft, The Netherlands) and an Atari computer. The combustion bomb was calibrated with benzoic acid as reference substance of known energy content.

2.3. Batch calorimetry

As heat production rates of eggs and larval instars have been determined in an earlier study [3], we measured only heat production rates of pupae and adults by means of an isoperibolic batch mirocalorimeter (Calvet, SETARAM, Lyon, France; volume 100 ml) connected to a chart recorder (L 2005, Linseis, Selb, Germany).

Heat production rates were determined continuously from the time of spinning of L_7-instars (cocooning) before pupation until death of the adults for a time span of up to 30 days. Shorter experiments were performed on pupae and isolated adults. Before and after each experiment, mass was determined with a mechanical fine balance (Sauter Typ 414/13, Ebingen, Germany) to the nearest 0.1 mg. Heat production rates were determined at a temperature of 30°C by integration (Digikon, Kontron, Munich) of the power–time curves.

3. Results

3.1. Body mass

Fresh and dry mass (m_F and m_D) of wax moths was determined for all larval stages exclusive stages L_1–L_3

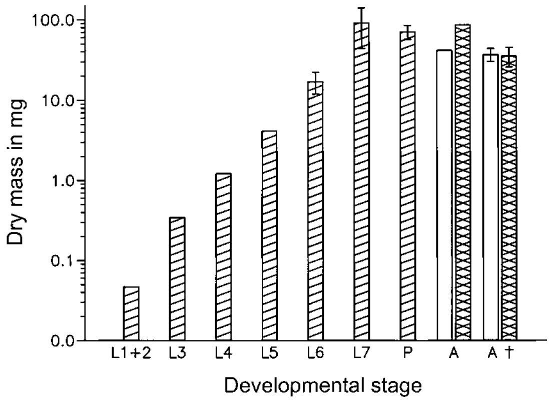

Fig. 1. Changes of dry mass during development of *G. mellonella.* L1+2,. . .,L7 = larval instars $L_1 + L_2,\ldots,L_7$; P = pupal instar; A = freshly emerged adults; A† = dead adults; simple crossed bars = no differentiation of sex; double crossed bars = females; open bars = males. Error bars indicate standard deviation.

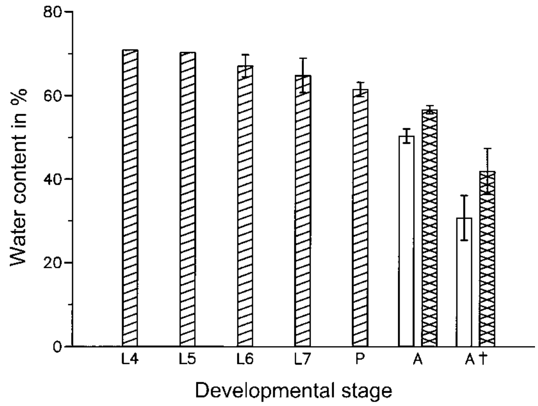

Fig. 2. Water content of *G. mellonella* determined as difference between fresh and dry mass given in percent of fresh mass. L4,. . .,L7 = larval instars $L_4,\ldots,L_7$; P = pupal instar; A = freshly emerged adults; A† = dead adults; simple crossed bars = no differentiation of sex; double crossed bars = females; open bars = males. Error bars indicate standard deviation.

in order to avoid artifacts as it turned out that very young larvae were extremely sensitive to mass loss due to desiccation. Sex was only differentiated in adults as it is undistinguishable in larvae and pupae.

As shown in Fig. 1, m_D increased exponentially during larval development and decreased during pupation and adult life. Females had a significantly higher m_F and m_D than males ($m_F = 189 \pm 28$ mg for females, $n = 41$, compared to 129 ± 28 mg for males, $n = 17$) at time of emergence. At time of death, mass of males and females was statistically undistinguishable. The relative water content m_W, which is calculated by substraction of dry mass from fresh mass given in percentage of fresh mass, decreases only slightly during larval development and more clearly during pupation and adult life (Fig. 2).

3.2. Lifespan of adults

The length of adult lifespan was remarkably dependend on whether the moths were kept isolated or in a group (Table 1). Males lived about eight times longer when kept in isolation. This difference is much less pronounced in females, which live only about 2.5 times longer in isolation. Duration of the pupal phase was not statistically different between the sexes.

3.3. Energy content

In the samples for the determination of energy contents of *G. mellonella*, individuals were pooled, as (i) m_D differed from about 3 mg in early larval stages up to 100 mg in old larvae and (ii) sample size was limited with 15 mg at minimum. Therefore the number of individuals per sample ranged from 2050 larvae for $L_1 + L_2$-stages to nine individuals per sample in freshly emerged females.

During larval development, the energy content Q increased exponentially from an initial value of 1.3 J in $L_1 + L_2$-larvae up to 2.94 kJ in L_7-larvae, whereas the specific energy content q remained relatively unchanged (Fig. 3). Values for q slightly increased

Table 1
Lifespan and duration of pupal phase of male and female wax moths (*G. mellonella*) in isolation or in a group

	Adult lifespan				Duration of pupal phase	
	Isolated (days)	*n*	Group (days)	*n*	No. of days	*n*
Males	23.9 ± 10.7	11	3.2 ± 1.7	14	11.4 ± 0.6	13
Females	8.9 ± 2.6	21	3.5 ± 1.8	17	12.2 ± 0.8	21

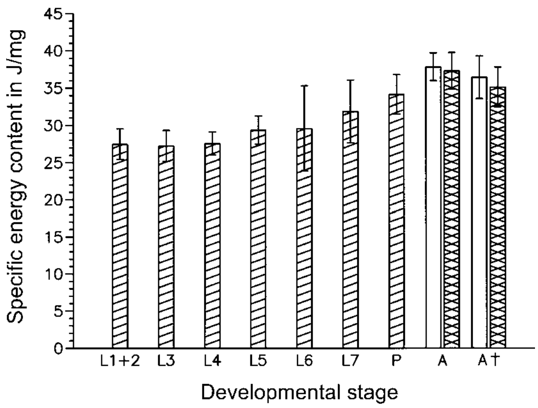

Fig. 3. Specific energy content of *G. mellonella* during development. L1+2,. . .,L7 = larval instars L_1 + L_2,. . .,L_7; P = pupal instar; A = freshly emerged adults; A† = dead adults; simple crossed bars = no differentiation of sex; double crossed bars = females; open bars = males. Error bars indicate standard deviation.

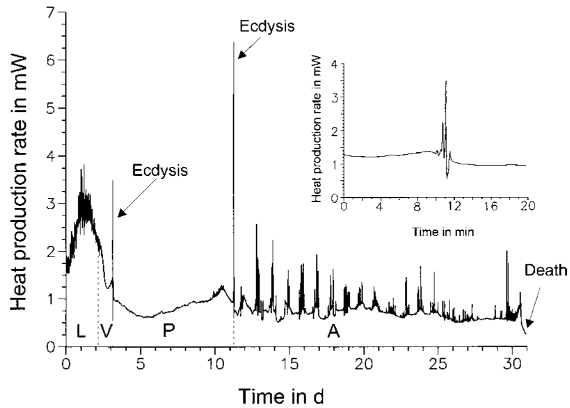

Fig. 4. Heat production rate of a female wax moth (*G. mellonella*) during development from L_7-instar to adult. Figure insertion shows details of pupation(= ecdysis (= moult) from prepupa to pupa; first ecdysis shown in main figure). L = L_7-instar, V = prepupa, P = pupal instar, A = adult.

during pupation and reached a maximum value of 37.5 $\pm$ 2.4 kJ g^{-1} in freshly emerged adults. From that time to death, q decreased as expected since adults do not take up food. Values for q did not differ significantly between the sexes.

3.4. Heat production rates

In order to follow the heat production of *Galleria* from pupation to the death of the adult, two long-time experiments of about one month each were made. In the beginning of our experiment, L_7-larvae were placed in the calorimeter vessels. During the experiments, the heat production rates of four ontogenetic phases of *Galleria* (L_7-larva (L), prepupa (V), pupa (P) and adult (A)) could be observed continuously without interruption (Fig. 4). Prior to pupation, the heat production rate showed strong fluctuations due to intense locomotor activities during cocooning of the larva. The maximum heat production rate during this phase amounted to 3.4 mW. After completing the cocoon, the L_7-larva undergoes a prepupa-stage, in which all activities are ceased. The heat production rate in this phase is slightly U-shaped, with a decrease down to 1.2 mW and an increase of heat production shortly before the larval–pupal moult. The moult into the pupal instar is clearly visible in the heat production curves as two strongly visible successive exothermic peaks followed by an endothermic peak (insert in Fig. 4). The heat production rates of the pupal instars show no fluctuations, as the pupa is motionless, and is lower compared to the heat production of the prepupa. Nevertheless, again a U-shaped pattern of heat production can be recognized in the time course. The heat production decreases down to 0.7 mW at days 2 and 3 of the pupal phase. Afterwards, it slowly increases again. Moulting (“ecdysis”) and emergence of the adult produced again the same pattern of heat production as visible in pupation (see above), but with a much stronger second exothermic peak rising from 1 up to 6.5 mW. The heat production rates of adult wax moths show a circadian rhythm.

More experiments were made on two of the events described above. First we investigated the phase of cocooning and pupation, and second, the circadian patterns of heat production rates of the adults. Cocooning and pupation were investigated in a total of six wax moth larvae. The duration of cocooning varied considerably between 25 and 45 h with no correlation between time spent for cocooning and heat production rate during this event. The mean specific heat production rate (p) during cocooning amounted to 13.7 $\pm$ 3.3 mW g^{-1} (n = 6). As mentioned above, prepupae produced less heat, and the mean value for p in prepupae was 5.1 $\pm$ 1.3 mW g^{-1} (n = 6).

Adult wax moths showed periodical activities (Fig. 5). The heat production rates of four adults of each sex were investigated. Six of them (three males

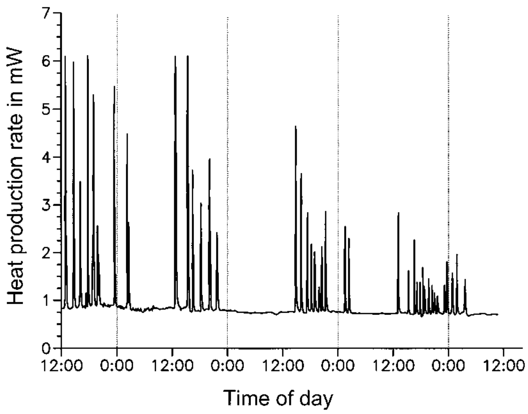

Fig. 5. Heat production rate of an adult female wax moth (*G. mellonella*) during 4 days. The moth was kept in continuous darkness.

and four females) exhibited periodicity in the heat production rates. Periods of activity bursts resulted in 8–15 successive exothermic peaks, which were followed by periods of quiescence with no fluctuations of the heat production rate. The duration of the activity phases varied between 450 and 830 min occured with a mean period of 24.3 $\pm$ 0.7 h ($n = 6$) and can thus be clearly denoted as circadian rhythms. The mean specific heat production rate (p) during the active period (i.e. the mean of p from beginning to end of the active phase, which includes all exothermic peaks as well as short intermittent periods of ceased activity) amounted to 10.4 $\pm$ 2.1, and to 5.6 $\pm$ 2.3 mW g^{-1} during the non-active period with no significant differences between the sexes.

4. Discussion

The specific energy content (q) of the ontogenetic stages of *Galleria* remains stable during larval development with values ranging from 27.3 kJ g^{-1} in L_3-instars up to 29.6 kJ g^{-1} in L_7-instars and grows slightly during metamorphosis of the pupa and in adults with values of 31.2 kJ g^{-1} for pupae and 37.8 kJ g^{-1} for adult males. The increase of q is accompanied by a slight decrease of the relative water content m_W. The changes of q and m_W are indicators for a substitution of proteins and carbohydrates by fat as internal food reserve. Fat has average values of q (about 39 kJ g^{-1}) which are similar to those of adult wax moths, whereas the lower q values of larval instars of around 27 to 29 kJ g^{-1} indicate that even in the last larval instars, protein (q = 17.5 kJ g^{-1}) and carbohydrates (q = 16.5 kJ g^{-1}) are part of the energy reserves of *Galleria.* Adults seem to rely solely on fat as food substrate. This finding is supported by a lower relative water content of pupal instars and adults compared to larval instars, because fat needs less water for storage in the body than proteins and carbohydrates do. This result fits well with previous reports about the chemical composition of holometabolous insects during development [8]. L_4- and L_5-instars of *Galleria* have remarkably high heat production rates of up to 160 mW g^{-1} [3]. This finds no reflection in the specific energy content of these instars, which remains unchanged.

The first larval instars have a total energy content (Q) of 1.3 J per individual, which culminates during ontogenesis up to 2.94 kJ in the last larval instar. The time for larval development is about 30 days [5], which renders a mean daily increase rate of 98 J/day for Q. From pupation to dead, we determined an average lifespan of 35 days for isolated males and 21 days for isolated females. The mean energy content of dead *Galleria* adults amounts to 1.29 kJ, which means that adults use 1.65 kJ during metamorphosis and adulthood. The mean daily decrease of Q is therefore 47 J/day for isolated males and 78 J/day for isolated females. When kept in groups, wax moth adults live much shorter, and males and females have a lifespan of 3.2 and 3.5 days, respectively. Individuals of *Galleria* are able to reproduce themselves in this time, and it is known from fruitflies and nematodes that sexual reproduction can reduce the lifespan of animals [9,10]. The amount of energy which is available for adults is used in a much shorter time with a mean daily energy loss of 515 J/day in males and 470 J/day in females, when living in groups.

The heat production rates of *Galleria* during metamorphosis show a U-shaped pattern, which is in good agreement with other reports on energy metabolism during insect metamorphosis [11]. Moulting of wax moths from larval instar to pupal instar and from pupa to adultus was visible in the calorimeter curves as two steep exothermic peaks followed by an endothermic peak. Both exothermic peaks are presumably the result of strong locomotor activities of the insects in order to break and shed the old cuticle (exuvia). The endothermic peak is most likely caused by evaporation of the exuvial fluid, which lies between the new and the old cuticle and is released during the moult.

Adults exhibited a pronounced circadian rhythm in their heat production rates. This phenomenon seems to be common in insects and has been previously reported elsewhere [12]. Simultaneous activity rhythms in *Galleria* males and females may synchronize sexual activities such as the release of sexual pheromones and mating. In non-active periods, wax moth adults have only a resting metabolism of about 6 mW g^{-1} at $T_A = 30°C$ (no difference between the sexes). This can be compared with maximum activity metabolism during flight which renders 230 mW g^{-1} in males and 101 mW g^{-1} in females at the same ambient temperature [4]. The metabolic scope (i.e. the difference between resting metabolism and maximum metabolism) is 95 mW g^{-1} for females (difference 17-fold) and 224 mW g^{-1} for males (difference 38-fold).

During ontogenesis, the specific heat production rates of different developmental stages vary considerably. It is almost impossible to measure true resting metabolism rates in wax moth larvae, as larval instars constantly move and feed. Nevertheless, heat production rates of active larvae have been measured calorimetrically [3]. The first and second larval instars have specific heat production rates of 55 mW g^{-1}. Highest heat production rates can be measured in L_4- and L_5-instars, with values up to 160 mW g^{-1}. The heat production rates decrease in the subsequent L_6- and L_7-instars. The latter have specific heat production rates of 18 mW g^{-1}, whereas prepupae, which were investigated calorimetrically in this study (prepupa: L_7-larva in a cocoon, preparing for pupation) have heat production rates of 5.1 mW g^{-1}, a value close to the resting metabolism of adults. The lowest heat production rates in the complete lifespan of *Galleria* can be found in pupal instars with values of about 4 mW g^{-1}. This is surprising at the first glance, because metamorphosis (in pupae) is thought to be an intense, energy-consuming process. Nevertheless, a strong selective pressure for energy conservation in pupal instars can be predicted, as all energy saved during metamorphosis can be used by the adult for reproductive activities. In this connection, comparative studies in energy consumption in pupal instars of holometabolous insects with different ecological strategies could be of interest. The circadian activity patterns of adult wax moths are also clearly a mean of energy conservation, which is necessary as adults cannot take up food.

In future, the differences in energy investment of male and female wax moths in reproduction should be investigated. Both sexes have only limited energy reserves, but follow different sexual strategies, which makes them good model objects for studies on the cost of reproduction and reproductive strategies in insects.

5. Nomenclature

m_F	fresh mass
m_D	dry mass
$m_W = (m_F - m_D)/m_F \times 100$	relative water content
P	heat production rate
$p = P/m_F$	specific heat production rate
Q	energy content
$q = Q/m_D$	specific energy content

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