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Thermochimica Acta 251 (1995) 241–245

thermochimica acta

Calorimetric investigations on thermoregulation and growth of wax moth larvae *Galleria mellonella* ☆

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Received 22 August 1994; accepted 30 August 1994

Abstract

Laboratory cultures of larvae of the wax moth *Galleria mellonella* show drastically increased temperatures a few days after the start of cultures. To examine this phenomenon, we performed direct calorimetric measurements on isolated eggs and larvae of different larval stages. Fourth and fifth instar larvae have significantly increased mass-specific heat production rates (up to 160 mW g^{-1}). These high heat production rates are obviously the reason for the increased temperatures in wax moth cultures. In addition to these results, larvae were reared successfully in the measuring chambers of the calorimeters in long-time experiments, and power–time curves were obtained continuously for the whole period of larval development (up to 40 days).

Keywords: Calorimetry; *Galleria mellonella*; Larval development; Metabolism; Thermoregulation

1. Introduction

The larvae of the wax moth *Galleria mellonella* (Pyralidae, Lepidoptera) live in honeybee colonies where they feed on wax, honey, pollen and other organic material. Mass invasions of larvae can occur in weak bee colonies. Earlier observa-

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☆ Presented at the Ninth Conference of the International Society for Biological Calorimetry, Berlin-Schmerwitz, 27–31 May 1994.

SSDI 0040-6031(94)02094-9

tions have shown that accumulations of these larvae produce conspicuously high temperatures. In laboratory cultures of wax moth larvae the temperatures inside a culture box suddenly increased from 32°C at the start of the culture up to 40°C after 3–4 days at an ambient temperature of 28°C [1]. As the mentioned study [1] was concerned with the temperature of groups of wax moth larvae and not with isolated animals, it is unclear if the high temperatures in wax moth cultures result from increased heat production of individuals. As larvae live together inside silken tubes, increased temperatures could also be the effect of aggregation when the total surface/volume ratio, and thus the heat-loss rates, are reduced. In the present study direct calorimetric measurements were made of isolated larvae during different stages of development, and individual heat production rates were determined.

2. Materials and methods

Eggs and the different larval stages of *G. mellonella* were kept in a laboratory culture at 30°C. The food for the larvae contained 22% maize flour, 11% wheaten flour, 11% bruised wheat, 11% milk powder, 5.5% yeast, 17.5% bees wax, 11% honey and 11% glycerine.

The heat-production rates of larvae and eggs were determined at 28°C using two isoperibolic batch calorimeters (Calvet, SETARAM, Lyon). Each calorimeter had two measuring and two reference vessels of 15 ml volume. The sensitivities were 67.8, 62.5, 63.1 and 60.3 $\mu V\ mW^{-1}$, respectively. The larval weight and the width of the head capsule were measured before and after each experiment to determine the larval stage. During the experiments the larvae had free access to food. Before and after the experiments the zero line was recorded from the measuring vessel charged only with food.

Heat production was recorded over an experimental time of 5 h. The average heat-production rates were determined by electronic integration (Digikon, Kontron, Munich) of the $P(t)$ curves. In the case of long-time experiments heat production was monitored for up to 40 days. In these experiments the weight of the larvae as well as the larval stage were determined every second day and the food in the measuring chamber was renewed.

As the heat production rates of eggs and larvae in stages L1 and L2 were very low, we used up to 20 larvae or up to 670 eggs for a single experiment.

3. Results

The weight increased exponentially with increasing larval stage (Fig. 1). Correspondingly, an increase in the individual heat production rates with each new larval stage was observed except for the L6 and the L7 stages (Fig. 2). On a first glance, the individual heat production rates show no dramatic changes, but the analysis of the mass-specific heat production rates reveal a different picture (Fig. 3). The L4 and L5 stage larvae exhibit considerably higher mass-specific heat production rates,

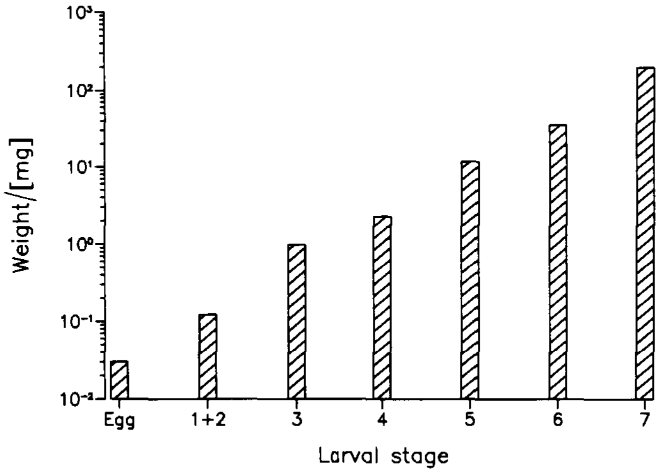

Fig. 1. Weight of eggs and larval stages of the wax moth *Galleria mellonella*.

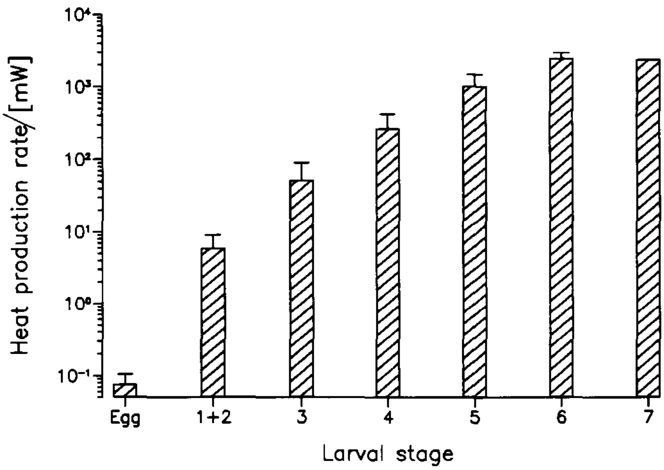

Fig. 2. Mean heat-production rates of eggs and larvae. Bars indicate standard deviation ($n = 30$).

in accordance with the temperature increase in the wax moth cultures during these stages.

The course of the individual heat production during larval development was observed in long-term experiments. Fig. 4 shows the change in heat dissipation of one isolated larva during the larval stages L4 to pupa with a 10-fold increase to the L7-stage around day 11. The temporal fluctuations in the power–time curve are due to locomotor activities of the larva and do not occur during the relatively immobile pupal stage. These patterns are most distinct during the 6th and 7th larval stages. Moreover, a pronounced decrease in heat production (and thus in metabolism) appears during each larval moulting (Fig. 4) with rates of only 7–20% of the

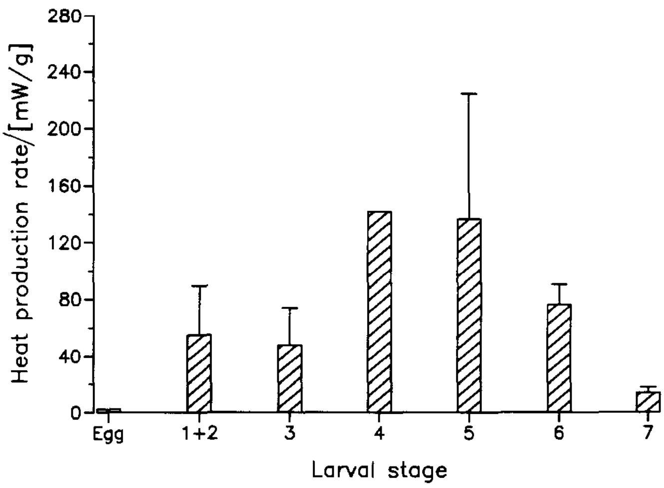

Fig. 3. Mean mass-specific heat-production rates of eggs and larvae. Bars indicate standard deviation ($n = 30$).

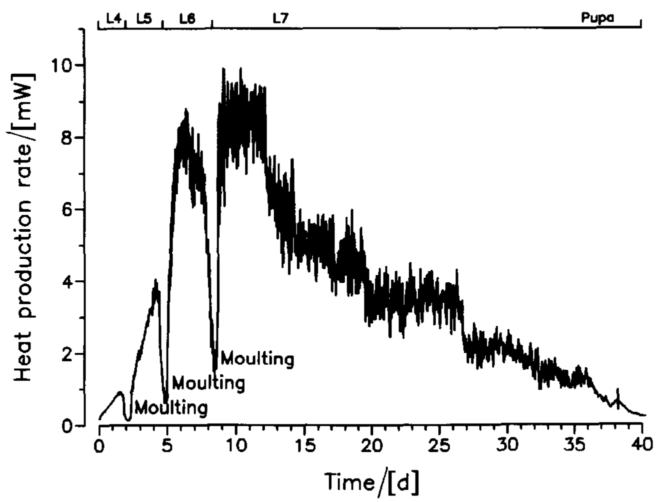

Fig. 4. Power–time curve of a wax moth larva during a long-time experiment. The larva was placed in the measuring chamber at the L4 stage and continued to develop until the pupa stage. Note the decrease in heat production during ecdysis (moulting).

preceding values. Half a day later the larva regains its former metabolic level and its heat-production rate increases to a new maximum. Four days after the last larval moulting (day 13) the heat-production rate decreases continuously until pupation. The lowest heat-production rates were observed at the pupal stage. It should be mentioned that, during the experiment reported in Fig. 4, the wax moth larva underwent its normal development, which means that it moved and fed.

4. Discussion

Lepidopteran larvae are known to exhibit decreasing individual development times by aggregation, as shown for *Vanessa io* and *V. urticae* [2]. Based on the present calorimetric experiments it is unlikely that this mode of behaviour is responsible for increased temperatures in wax moth cultures. Firstly, mass-specific heat production of individual isolated larvae was highest at the 4th and 5th larval stages. These stages had been found in our wax moth culture 3–4 days after the start of the culture, i.e. at about the same time that the temperature increased drastically [1]. Secondly, as shown in Fig. 1, the total biomass of the culture increases exponentially with increasing individual larval weight. This means that the increasing biomass density inside the culture box causes an elevated heat dissipation and, consequently, a higher temperature. In conclusion, we assume that the increase in the temperature of the culture was caused by the individually intensified metabolism and probably also by artifical circumstances during laboratory rearing of the larvae.

Similar development patterns of individual metabolism as presented here have been reported for larvae of the beetle *Tenebrio molitor* [3] and thus propably may be typical of the larvae of holometabolous insects. Nevertheless, the reason for and the adaptive significance of increased heat-production rates during the development of wax moth larvae are as yet unclear.

The individual heat-production rates of *G. mellonella* decrease during each larval ecdysis (moulting). The larvae cannot feed in this time, because the fore- and hindgut are moulted together with the rest of the cuticule, and locomotor activities are strongly reduced.

The long-term experiments show that the time course of the individual development of small animals can be determined by direct calorimetry, even over several weeks. This kind of experiment reveals the complete temporal patterns of heat dissipation during individual development and offer a unique possibility of examining the total amount of energy required for growth.

Acknowledgements

We thank the AG Parasitology from the Institute for Zoology at the Free University of Berlin for giving us wax moth larvae to initiate our laboratory culture, and Prof. Dr. I. Lamprecht for valuable and helpful comments on the manuscript.

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