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HEAT DISSIPATION OF FLYING WAX MOTHS (GALLERIA MELLONELLA) MEASURED BY MEANS OF DIRECT CALORIMETRY

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Abstract

Heat production rates and flight speed of adult wax moths (Galleria mellonella) were investigated by means of direct calorimetry at T_A=20 and 30°C. Specific heat production rates were not significantly different between males and females at T_A=20°C (p_{TH}=747±123.7 mW g^{-1}, n=5 for males and p_{TH}=791±169 mW g^{-1}, n=5 for females) even with females having a higher body mass (M_B=83.8±21.6 mg, n=9 for males and M_B=146.4±25.7 mg, n=11 for females) and wing load. In females, heat production rates were dependent on temperature with higher heat production rates at T_A=20°C (p_{TH}=791±169 mW g^{-1}, n=5) than at T_A=30°C (p_{TH}=441±74 mW g^{-1}, n=6). Flight speed was also clearly correlated with T_A. Both males and females flew more slowly at T_A=20 than at 30°C.*

Keywords: direct calorimetry, Galleria mellonella, insect flight, metabolic rate

Introduction

The evolution of larval forms, which often differ morphologically and ecologically from adults to a great degree, can be viewed as a key innovation of nature leading to the enormous success of holometabolous insects such as coleopterans (beetles), dipterans (flies and mosquitos), hymenopterans (bees, wasps and ants) and lepidopterans (butterflies). In most cases, habitat and food resources are completely different between larvae and adults. Therefore, from an energetical point of view holometabolous insects have very interesting life histories since larvae, pupae and adults use free energy for very different purposes. Larvae take up most energy in order to grow and to accumulate food reserves for metamorphosis. Pupae do not feed anymore and rely on food reserves stored in their fat body, and adults need most energy for reproduction (retrievement of sexual partners and production of offspring).

Despite the economical and ecological importance of holometabolous insects, only a few comparative attempts have been made to investigate metabolic rates on all life stages [1], and even less investigators used direct calorimetry as method [2, 3].

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* Abbreviations: M_B–body mass, M_{TH}–thorax mass, P–heat production rate, p–specific heat production rate, p_M=P divided by M_B, p_{TH}=P divided by M_{TH}, v_F=flight speed, T_A=ambient temperature.

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The wax moth Galleria mellonella (Lepidoptera, Pyralidae) is a well-established model organism in insect physiology. The natural habitat of the larvae are honeybee colonies, where they feed on wax, pollen, honey and other organic material. Mass invasions of larvae can occur in weak bee colonies. Pupation takes place in silken cocoons inside the wax combs. Interestingly, the emerging adults have only degenerated mouthparts and do not take up any food or water during the rest of their lifespan. Therefore, they seem to be ideal objects for evaluating the cost of reproduction in adult insects. Detailed direct calorimetric investigations in wax moth larvae, pupae and resting adults have been published elsewhere [4, 5]. In this study, we complete the energetic picture of Galleria mellonella by measuring the metabolic rates during exercise, i.e. flight, by means of direct calorimetry.

Material and methods

Animals

Adult wax moths (Galleria mellonella) were obtained from a laboratory culture. Wax moths were kept at T_A=30°C in plastic containers (20×20×8 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. After pupation, individuals were separated and kept in petri dishes in order to protocol the time of emergence. As it turned out that young adults could be motivated more easily to fly in our calorimeter, all experiments were made with adults within 3 days after emergence.

Calorimeter

A detailed description of the flight calorimeter has been published elsewhere [6, 7]. The calorimeter prototype is placed in an isolated air bath of an LKB Flow-calorimeter (Typ 10700, LKB, Bromma, Sweden) with a temperature range from 18 to 42°C. The calorimeter chamber consists of an aluminium cylinder (diameter 180 mm, height 110 mm) connected to an outer aluminium cube via 10 Peltier elements (type TEC1-12705, Conrad electronics, Hirschau). The calorimeter is insulated from the air bath by a layer of styropor^{TM} (20 mm).

A two-armed, low-friction carousel of 62 mm radius for tethered insect flight is incorporated in the measuring chamber. The flight speed is measured by means of an inductive proximity switch activated at each revolution of the carousel arms. In some pre-experiments, it turned out that friction heat generated by the carousel is negligibly low (<1 mW).

The calorimeter interior is illuminated by two lightguides (Ø=5 mm) connected to a cold light source (Intralux 150 h, Volpi, Schlieren, Switzerland). The heat input to the calorimeter caused by illumination amounted to 2.5 mW, which was less than 3% of the total heat production rate of the flying moths. As adult wax moths are nocturnal insects the generated illumination intensity of 300 lx was high enough to allow flight activity. For calibration of the calorimeter, an electrical resistor was placed at the central axis of the carousel. Calibration rendered a sensivity of 63.6 mV W^{-1}.

Heat production rates were determined at T_A=20 and 30°C by integration (Digikon, Kontron, Munich) of the power-time curves.

In our earlier observations on flight metabolism of hornets [7] we determined wing beat frequencies by means of an incorporated microphone connected to a DAT-recorder and computer software for sound analysis. But wing beat frequencies of wax moths revealed to be very low (<20 Hz) and were thus outside the technical limits of our recording and computer software equipment.

Experimental procedures

Prior to the experiment, adults were kept for 20 min at 6°C in order to immobilize them. A small plastic tube (length=20 mm, diameter=2 mm) was glued onto the thorax. This tube was inserted into a thicker tube (length=15 mm, diameter=4 mm) attached to the carousel arm.

After determination of the baseline, the calorimeter was opened and the insect fixed to the carousel. This procedure took about 30 s and caused a heat artifact in the calorimeter signal which lasted for about 10 min after the start of the experiment. Therefore, heat production rates were only evaluated after this period. The duration of each experiment was about 20–30 min, and the experimental run was stopped when the insects ceased to fly.

Before and after the flight, the moths were weighed to the nearest 0.1 mg by means of a mechanical balance (type 414, Sauter/Ebingen, Germany). After each experiment, the moths were killed and sex and thorax mass determined after preparation. For evaluation of wing area, fore- and hindwings were dissected from the moths and their surfaces measured by a digital planimeter (Digikon, Kontron, Munich).

Results

It is known from personal experience, discussions with colleagues and literature data that only 30 to 50% of insects can be stimulated to perform tethered flight. In our investigations on wax moths, 20 experimental runs from a total of 64 could be evaluated for determination of heat production rates of flying wax moths (a success rate of 31%). Females could be motivated more easily to fly (11 successful experiments) than males (9 successful experiments). Figure 1 shows a typical calorimetric curve produced by a flying wax moth. After establishment of the baseline, the calorimeter chamber was opened and the moth fixed at the carousel arm. The moth still had a piece of polystyrene between its legs. The calorimeter signal changed to the negative range due to heat artifacts caused by handling the insect and by cool air from the colder calorimeter cabinet. After a short while (2–3 min) for initial thermal equilibration, the chamber was briefly opened to remove the polystyrene. The moth immediately started to fly, to be seen as carousel arm rotation by the pulses of the proximity sensor (dotted line in Fig. 1). After another 2–3 min, the calorimeter signal turned to the positive range and reached a maximum value. From this point on, the heat production rate of the insect could be evaluated until the experiment was terminated.

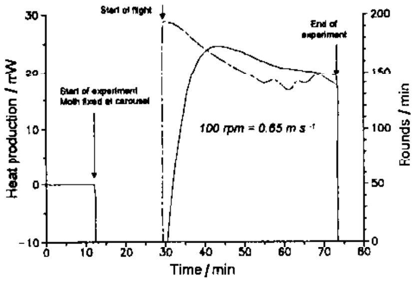

Fig. 1 Typical calorimetric curve of a flying wax moth. Time point 0 does not denote the beginning of the experiment; — heat production rate, – – – flight speed

At T_A=20°C, no difference could be observed in the heat production rates (P) of males and females. However, the specific heat production rates (p_M), defined as heat production rate P divided by total body mass (M_B) are significantly different at T_A=20°C (p_B=277.4±43.9 mW g^{-1} for males and p_B=181.4±42.8 mW g^{-1} for females), because males have a significantly lower body mass (M_B) (83.8±21.6 mg, n=9) than females (146.4±25.7 mg, n=11). But as most heat is generated during flight by the thoracic flight musculature, it seems more useful to divide heat production rate by thorax mass (M_{TH}) rather than by total body mass (M_B). Males and females have about the same thorax mass (29.1±5.0 mg, n=9 for males compared to 34.1±5.9 mg, n=11 for females). Table 1 summarizes the morphological features of male and female wax moths. As shown in Fig. 2, the p_{TH}, obtained by division of P by M_{TH}, are not significantly different for males and females at T_A=20°C. This is interesting, because females have a higher M_B/M_{TH}-ratio and a higher value for wing load than males.

In females, P and p_{TH} are correlated to T_A with higher heat production rates at T_A=20°C than at 30°C. Because of the low number of successful experiments which were performed with males at 30°C, no comparison of P or p_{TH} should be made.

The upper section of Fig. 2 shows the correlation between flight speed (v_F) and T_A. Both males and females fly significantly more slowly at T_A=20°C (0.37±0.10 m s^{-1}

Table 1 Morphological and physical characteristics of adult male and female wax moths. Males n=9, females n=11, SD=standard deviation

	Unit	Males		Females	
		Mean	SD	Mean	SD
Body mass	mg	83.8	21.6	146.4	25.7
Thorax mass	mg	29.1	5.0	34.1	5.9
Ratio of thorax mass to body mass	%	35.4	3.8	23.3	1.4
Wing area	mm^2	224	26	262	24
Wing load	N m^{-2}	0.381	0.068	0.557	0.077

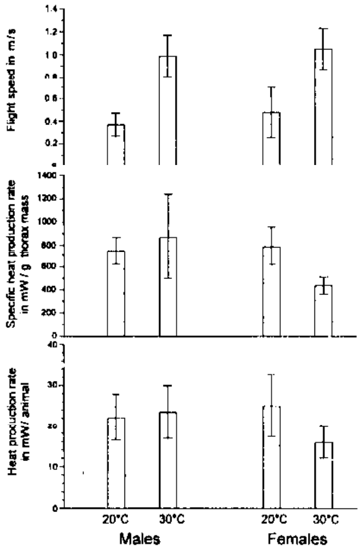

Fig. 2 Mean heat production rates and flight speeds of wax moths in relation to sex and ambient temperature. Bars indicate standard deviations. Number of experiments: males 20°C n=5, males 30°C n=4; females 20°C n=5, females 30°C n=6

for males and 0.48±0.22 m s^{-1} for females) than at T_A–30°C (0.99±0.18 m s^{-1} for males and 1.05±0.18 m s^{-1} for females).

Discussion

With this paper we present for the first time values for metabolic rates of flying wax moths or butterflies (lepidopterans) in general. The highest values of p_M in our experiments were recorded for flying wax moth males at 30°C, which amounted to 276.6±125.7 mW g^{-1} with average flight speeds of v_F=0.99±0.18 m s^{-1}. This lies in the range of flying honeybees producing between 278.2 and 292.6 mW g^{-1} during flight in a roundabout at v_F=1.1 m s^{-1} [8]. Regarding the different allometries between M_B and M_{TH} of males and females, it seems useful to distinguish between p_M and p_{TH}, as thoracic musculature produces the main amount of heat during insect flight. The above mentioned value for males at T_A=30°C can be converted to 872±370 mW g^{-1} thorax mass and again be compared with values for honeybees (1120 mW g^{-1} thorax mass at T_A=30°C; [9]). Resting metabolism of adult wax moths amounts to p_M=5.6±2.3 mW g^{-1} total body mass at T_A=30°C with no significant difference between both sexes [5]. The maximum metabolic rates of wax moths, which are achieved during flight, are therefore about 18 (females) to 49 (males) times higher than the resting metabolic rates even when regarding only p_M, which is lower than p_{TH}. The highest non-flight metabolic rates of wax moths recorded so far were produced by 5th-instar larvae showing values for p_M up to 160 mW g^{-1} [4].

Despite their anatomical differences, male and female wax moths show no differences in their values for P or p_{TH}. This is astonishing as females carry a more heavy load during flight, mainly because their abdomen is filled with well-developed ovaries. But one may also speculate that this result is due to the fact that the moths are fixed at the carousel arm and part of their mass is carried by the support. Further experiments are necessary to clarify this situation.

Wing load (which is defined as total body mass MB divided by the wing area) is higher in females than in males, even with females having slightly larger wings than males.

The heat production rate of flying female wax moths is temperature dependent with higher rates at low temperatures. This is in accordance with observations of other authors for honeybees [9] and own studies with our flight calorimeter performed on hornets [7].

Flight speed is also clearly correlated to ambient temperature. At 20°C, wax moths fly significantly slower compared to 30°C. Hornets show a reverse behaviour with higher speeds at low T_A [7]. The reason for this difference may lie in different thermoregulatory capabilities of these two insects. Hornets are more capable to produce endogenous heat in order to stabilize their body temperature [10]. Wax moths in contrast may have difficulties in producing a sufficient amount of heat for compensation at low ambient temperatures. Therefore, energy spent for temperature stabilization is lost for flight activities and wax moths thus fly slower at low T_A Clearly, this point awaits further investigations. In general, not much regard has been taken to flight speed in thermoregulatory studies of flying insects, and even less is known about flight speed under natural circumstances although there is some evidence that this parameter plays a so far underestimated role in thermoregulation.

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