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Energetics of honeybee development
Isoperibol and combustion calorimetric investigations

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Dedicated to Professor Edwin H. Battley/Stony Brook (N.Y.) on the occasion of his 80th anniversary and to decades of friendship and cooperation.

Abstract

Energetics, growth and development of workers and drones of the European honeybee *Apis mellifera carnica* were determined by classical isoperibol Calvet calorimetry and by combustion bomb calorimetry during one breeding season. Development was followed from the moment of egg laying till to the hatching of young adults. Mass, heat production rate and specific heat production rate served as experimental parameters registered daily. Two worker and two drone larvae remained after capping for 2 weeks in the calorimeter to continuously monitor their metabolism, their locomotor activity, moulting and hatching. Drone mass and metabolism were significantly higher than those of workers in all states of development.

The energy content during the development of workers and drones was evaluated by combustion calorimetry and by CHN analyses combined with the Dulong equation or derivatives of it.

Keywords: CHN analysis; Energetic development; Honeybees; Isoperibol and combustion calorimetry

1. Introduction

Honeybees belong to the holometabolous insects, a group that shows a distinct separation of larvae, pupae and adults during their development. Larvae can be taken as “feeding automatons” that have to accumulate mass and store energy for the following stages. Pupae perform the metamorphosis of the larval mass to the final adult structure without any energy take-up, while adults are mainly concerned about reproduction with mating, laying eggs or supporting and nursing the brood. To meet these demands during development, an initially extreme specific energy consumption rate can be observed in larvae that steeply drops to the pupae state, as more and more metabolically inactive depots are created. Due to the different caste tasks no linear relationship between age or mass and energy turnover can be expected so that holometabolous insects are fascinating objects for biological calorimetrists [1–3].

A number of calorimetric experiments have been performed on holometabolous insects concentrating on the mealworm *Tenebrio molitor* [1,2] and the greater wax moth *Galleria mellonella* [4,5]. Although the development of *Tenebrio* with about 90 days is 10 times longer than that of honeybees (10 days), general structures of this process can be easily compared.

Social insects like honeybees, bumblebees, wasps and hornets have been intensively investigated in the Berlin group with a steady emphasis on honeybees and their questions of life: energetics of a hole hive [6], summer and winter clusters [7], development [3], sleeping at night [8], infestation by bee pests [9,10] or communication through pheromones [11,12]. Moreover, energetic and morphologic comparison of the common European honeybee *Apis mellifera carnica*

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with the much smaller and more aggressive African honeybee *Apis mellifera lamarckii* rendered interesting details about their adaptation to different forms of environment [13].

In the present paper we concentrate on energetic aspects of honeybee development, energy content of the different bee states and the efficiency of several essential tasks in the life of a beehive like rearing brood or collecting honey, pollen and water.

2. Experimental

2.1. Honeybee samples

Honeybees of the subspecies *A. mellifera carnica* originated from hives of the working group for Bee Research of the Free University of Berlin a few meters away from the laboratories for the calorimetric measurements. Although it was intended to use genetically homogeneous brood from one queen, offspring from three queens entered the investigations due to api-technical problems. The results of the three broods showed no significant differences so that they could be pooled for the evaluation.

To know the age of the experimental animals by observation and not only by morphological differences, the bees were kept in a standard observation hive [8]. A laying queen was transferred to this hive with empty combs (no food or brood in the cells) for 24 h to lay eggs. Thus it was known with an accuracy of 1 day how old the chosen larvae or pupae were. Animals were always taken directly from their cells for experimental investigations. Larvae in their combs were cut out from the comb and quickly but with much care prepared from the cells and taken out as the bottom of the cells is covered with a nourishing fluid (“food sap”) whose evaporation would significantly overlay the biological heat production. The larvae were dried superficially with a soft paper towel, weighed (precision balance type 414/3, SAUTER, Ebingen, Germany) to the nearest 0.1 mg and transferred to the calorimetric vessels. Capped brood could be measured in its cells to reduce disturbance of the development.

Because of their very low thermal signals, in some developmental stages groups of individuals were used for one measurement: eggs had to be monitored in samples of 30–40, 1st-day drone larvae of three to four, 1st-day worker larvae of about 10th and 2nd-day worker larvae of five to six individuals. Capped brood (metamorphosing pupae) was kept in the cells till to emergence, experiments on free larvae were stopped after 60 min as their situation in the calorimetric vessel was far from being normal. In the hive they enjoy a large surplus of food sap and up to 140 visits by nursing bees during 1 day for worker and up to 1300 for queen larvae [14]. At the end of the experiment the animals were weighed again and killed by freezing.

2.2. Isoperibol calorimetry

Three isoperibol twin calorimeter of the Calvet type were used in the present investigation, two MS 70 instruments (SETARAM, Lyon, France) with 15 ml vessels and one with 100 ml, and an additional twin calorimeter (Triflux, THERMANALYSE, Lyon, France) with only 1.2 ml. The sensitivity varied between 44 and 63 $\mu V\,mW^{-1}$ for the SETARAM instruments and amounted to 81 $\mu V\,mW^{-1}$ for the Triflux. All were operated at 35 °C, the average temperature in the brood area of the beehive. Thermal signals as consequence of the metabolic as well as the locomotor activity of the animals were recorded as power–time curves (*p*–*t* curves) and evaluated by means of a digital planimeter (Digikon, KONTRON, Munich, Germany) for total heat output, maximal and minimal turnover rates and for movements of the animals.

2.3. Combustion bomb calorimeter

A customer built modification of the Phillipson microbomb calorimeter [15,16] was used for the energy content determination. The original Phillipson bombs with spherical bottoms and a weak contact to the heat flow sensor were changed to bombs of the same size (about 12 cm^3 active volume) but with flat bottoms in perfect contact with a ringed plate above a Peltier element as heat flow sensor. In this way, the sensitivity could be increased by a factor of about 10 allowing for smaller samples and higher stability. Combustion experiments were run at room temperature and a pressure of 2.5 MPa. Ignition was obtained by an ohmic discharge of a 5000 μF capacitor and a nickelin fuse wire of 0.1 mm diameter (combustion heat 3.0 $J\,cm^{-1}$).

Samples for combustion calorimetry were carefully dried to weight constancy by lyophilisation (type Trivac D4A, LEYBOLD HERAEUS, Hanau, Germany) at 4 °C and a pressure of <10 Pa. Fresh and dry weights were determined by a microbalance (type 414/3, SAUTER, Ebingen, Germany). The dried samples (10–20 mg) were homogenized in an agate mortar, pressed to pills or burnt in gelatine capsules that were kept till use above silica gel in an exsiccator. The combustion heat of gelatine was experimentally determined and amounted to 19.6 $kJ\,g^{-1}$. If traces of soot were observed after combustion in the bomb, the run was discarded because of incomplete combustion. A whole experiment took only about 20 min so that a larger number of specimens could be burnt during the day.

The bomb calorimeter was calibrated with commercial benzoic acid (RIEDEL DE HAEN; Seelze, Germany; as calibration standard of known 26,470 $J\,g^{-1}$ combustion heat) and rendered – depending on the different bombs used – sensitivities between 100 and 110 $J\,mV^{-1}$ without any pre-amplification. The thermal signal of the calorimeter was registered in the usual way and graphically analysed for the heat of combustion.

2.4. Energy content from elemental determination

In addition to the bomb calorimetric determination of energy contents the elementary composition of samples was determined by means of a Perkin-Elmer PE CHNS/O 2400 Series Elemental Analyzer. Benzylthiuronium chloride was used as a calibration standard. When the mass fractions for carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) are known, the energy content can be estimated applying the Dulong equation or one of its modifications as well as the Giese equation [16,17]. The elemental determination was performed by combustion at 925 °C in a pure oxygen atmosphere rendering CO_2, N_2 and H_2O fractions from which the distribution between C, N, H, and O can be calculated [17]. Besides these volatile compounds the ash content was evaluated after ashing at 450 °C for 12 h, rendering by subtraction the biomass in the total mass.

2.5. Statistics

Results are given as means plus standard deviations. The small sample numbers are due to the fact that animals could not be taken directly from the usual beehive with thousands of larvae and adult worker bees. To know the age of the samples the laying queen had to be separated from her hive for 1 day—a procedure that disturbs the development of the colony and cannot be repeated often. Therefore, progeny of three queens entered the investigation. As no differences in the results of the three sets were seen, they were pooled.

3. Results

3.1. Growth and development

Growth (taken as mass and not as length increase) of honeybee workers and drones follows a sigmoid curve before capping and an exponential drop in the capped cell. The maximum rate of mass gain is observed during the 3rd day after hatching and amounts to 2.2 per day (factor of 9.3 per day) for workers and to 1.66 per day (factor of 5.2 per day) for drones. Such rates are only possible due to the fact that the young larvae are permanently provided with food sap that contains about 18% proteins, 10–15% carbohydrates and 5.5% fat (in total around 7.7 kJ ml^{-1}) [18]. The growth rate of drones is lower since they have 1 day more for their uncapped development and since they get an additional small provision of food in their cells.

A dramatic mass increase takes place during ontogenesis from the egg to the 1st day in a capped cell, a 1300-fold increase for worker bees (Table 1) and a 2200-fold one for drones (Table 2). The scatter in the mass values is rather high due to the fact that the time of depositing of a specific egg was uncertain within 24 h (see above). Body mass decreased significantly after capping (by 38 and 36% for workers and drones, respectively) as the larvae were no longer fed by the nursing bees. Drones are more than double as heavy as workers due to a more intensive feeding during the first larval days.

Table 1
Mass of honeybee workers during their development from egg to adults at 35 °C

Day of development	Mass (mg)	Range of masses (mg)	Sample size
Egg	0.14 ± 0.01	0.13–0.15	151
L1	0.32 ± 0.15	0.18–0.55	58
L2	3.7 ± 3.1	1.2–10	103
L3	22 ± 9	11–35	26
L4	52 ± 9	40–70	12
L5	109 ± 19	90–130	5
L6 (capped)	185 ± 17	165–203	4
L7 (capped)	173 ± 24	150–196	4
Adults	115 ± 7	94–128	20

As there is a large scatter in the first larval days (L1–L3), the ranges of values are given additionally to the mean, the standard deviation and the number of independent samples. L1–L7: larvae from day 1 to day 7.

3.2. Heat production during development

The strong initial mass increase is also seen in Fig. 1a that shows the heat production rates of growing drones during their ontogenesis. Each point is the mean together with the standard deviation of independent calorimetric measurements of drones. The number of experiments for points in the larval, pupal and adult state is cited in the figure legend. The first four points in Fig. 1b demonstrate that the mass specific heat production rate is constant, i.e. the heat production rate is directly proportional to body mass. In the following days the body mass increases further while the heat output drops so that the changes in the mass specific curve are by far more impressive than the slope above it. It renders the U-shaped form that is well known for the ontogenesis of many holometabolous insects [5,19–21]. For both genders, the initial values are nearly the same (10 and 11 mW g^{-1} for workers (not shown) and drones, respectively) and also the minima (1.6 and 2.0 mW g^{-1}) with reductions to 16 and 18% of the maximal value.

Table 2
Mass of honeybee drones during their development from egg to adults at 35 °C

Day of development	Mass (mg)	Range of masses (mg)	Sample size
Egg	0.18 ± 0.01	0.16–0.19	91
L1	1.0 ± 0.2	0.7–1.2	22
L2	5.3 ± 4.1	2.0–15	36
L3	36 ± 12	20–60	23
L4	119 ± 21	90–175	20
L5	233 ± 28	190–290	18
L6	388 ± 25	340–410	10
L7 (capped)	402 ± 19	390–440	11
Adults	256 ± 13	240–280	11

As there is a large scatter in the first larval days (L1–L3), the ranges of values are given additionally to the mean, the standard deviation and the number of independent samples. L1–L7: larvae from day 1 to day 7.

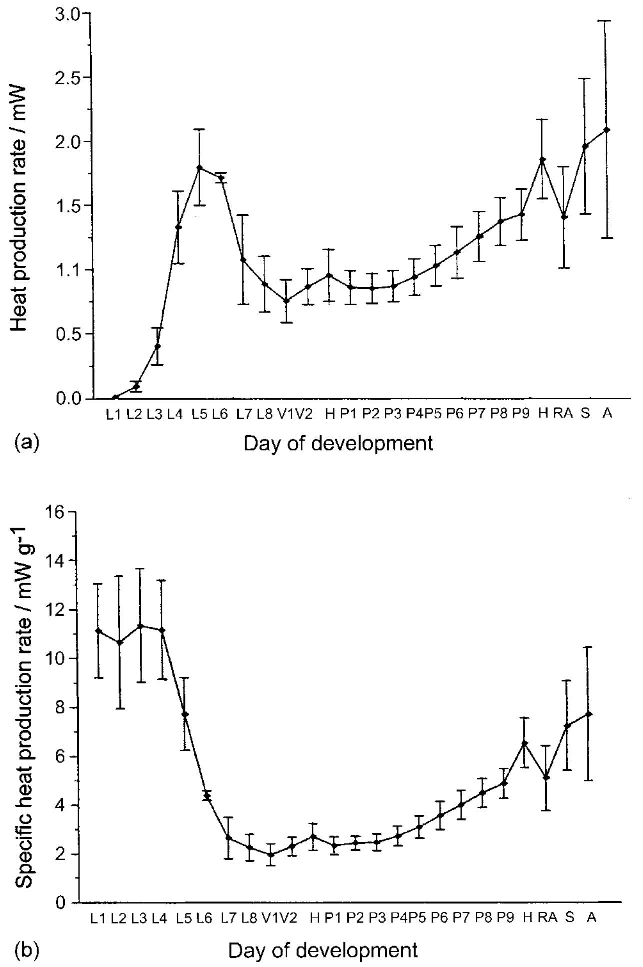

Fig. 1. Heat production rates (a) and mass specific heat production rates (b) during the development (given in days) of honeybee drones. Each data point originates from one animal that did not enter the experiment again. The larval stage continues from L1 to L8, that of pupae from P1 to P9. V1 and V2 correspond to the prepupal days, H to moulting, RA to the resting and A to the active adult, while S indicates hatching. Standard deviations concern 12.3 ± 6.6 (range 4–22) independent experiments for larvae, 8.3 ± 2.4 (range 4–10) experiments for all pupal stages, 7 for moulting, resting adults and hatchings and 4 for adults.

The minima are followed by a more or less steady increase of heat or mass specific heat output with some deviations around the pupal and imago moulting and the resting adult. Both moults, which are so clearly indicated in Fig. 2, are less impressive in Fig. 1 due to time smearing in the moment of hatching. Standard deviation is also high for “adults” as they have to be caught in the moment when opening and leaving their uncapped cells. As they escape in less than one hour, only four adults could be caught for the investigations.

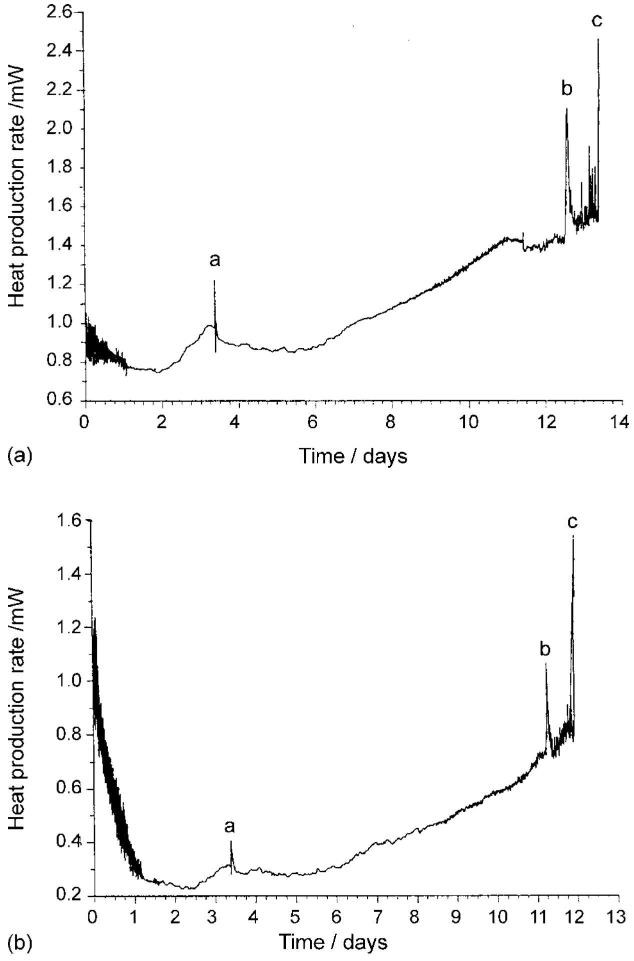

Fig. 2. Heat production rates of one honeybee drone (a) and one honeybee worker (b) during their development after capping. Time 0, the moment when the capped cell is introduced into the calorimeter, corresponds to the 2nd day of capping, i.e. day L7 for the drone and L6 for the worker. Pupa moulting (a), imago moulting (b) and hatching from the capped cell (c) are clearly indicated by the strong deviations in the *p*–*t* curves. Smaller and denser fluctuations at the beginning and the end of the graphs are due to locomotor activity.

Additionally to the few-hours point-wise measurements for each day during their development, two worker bees and two drones were continuously monitored after their cells were capped (Fig. 2). They remained for about 2 weeks in their individual cells in the calorimetric vessels of 100 ml, which were open to air so that no oxygen shortage could disturb the ontogenese. All four animals developed normally and hatched in a proper state. The two *p*–*t* curves show that the larvae of both genders exhibit locomotor activities in the first days and become quiescent afterwards till to the late pupal state when after the 9th day denser fluctuations in the graph become visible. The earlier fluctuations around the 5th day with longer periods are due to instabilities of the calorimetric zero line in these long-term experiments. (One has to keep in mind that the calorimetric registration had a length of about 300 cm and was compressed to the present 10 cm.) It is interesting to see that nearly no (energetic) preparation occurs prior to the larval moulting and that pupal moulting also appears more or less suddenly. After the second moult, locomotor activities are more impressive. Moulting documents itself by one or two strong exothermic peaks including one endothermic peak.

The recorded maximum amplitude of the *p*–*t* curve during this transition is damped because of the thermal inertia of the calorimetric signal. It can be expected to be two to three times higher in reality than seen in the curve. Such extremely high heat outputs are due to the necessary force to break open and shed the old cuticle, while the endothermic effect happens because of the evaporation of ecdysial fluid in the moment of moulting. Assuming a desmearing factor of about 3 for the calorimetric signal, the hatching amplitude of a drone would amount to more than 2.7 mW (10.5 mW g^{-1}; total turnover in that moment 4.3 mW or 16.8 mW g^{-1}), the worker amplitude to 2.1 mW (18.3 mW g^{-1}, total turnover 2.9 mW or 25.2 mW g^{-1}). These energy turnover rates seem high but are by far not comparable with those of flying honeybees (about 50 mW) [22–25].

Both graphs in Fig. 2 show *p*–*t* curves in the classical form of continuous registration for a drone (Fig. 2a) and a worker (Fig. 2b) and Fig. 1a that of a daily point-wise measurement for an ensemble of drones. In these cases, the areas between the curves and the baselines are proportional to the total heat Q_T dissipated during the experiments. Fig. 1a renders a Q_T for drones of 2190 J from the 1st larval day till to the hatching of the adult drone. The first part of this curve till to the moment of capping amounts to 510 J. The drone *p*–*t* curve in Fig. 2a starts later – sometime after capping – leaving about 13 days for further development. This curve renders a Q_T of 1230 J, the corresponding part in Fig. 1a, a value of 1530 J. This difference remains within the limit of scatter seen in Fig. 1, so that both approaches support one another. The Q_T value determined for the worker bee (Fig. 2b) amounts to 450 J, the point-wise evaluated one (not shown) to 750 J. The significantly lower figures for workers than for drones were expected due to the strongly increased metabolism of the drones. The differences between the point-wise and the continuous determinations for both castes may result from the fact that always-fresh animals with higher metabolic activities entered the experiments in the discontinuous monitoring.

As honeybee queens are reared in much lower numbers than drones or workers and the removal of queen cells is much more disturbing to the honeybees, only two orientating experiments were performed with two queens between the 2nd pupal day (P2) and hatching (H), rendering a heat output increase from 0.7 mW (P2) to 1.85 mW (H) within 4 days. These values approximately correspond to those of drones, but the 2.6-fold multiplication occurred quicker because queen development is significantly shorter (15 days) than that of workers (20 days) or of drones (23 days).

3.3. Energy content during ontogenesis

Table 3 gives a compilation of bomb calorimetric data for honeybee workers and drones obtained from a different group of animals later in the year. Numerical deviations from the results of the isoperibol calorimetric experiments are thus explainable, principle structures remain the same. Time is given in days after hatching from the egg in the usual nomenclature of L, VP, P and A for larvae, prepupae, pupae and adults. Due to the small dry mass of early stages, only individuals from larval day 3 on rendered enough material for combustion experiments. Nevertheless, varying numbers of larvae or pupae had to be pooled to provide enough dry mass for burning. The table shows the already known rapid mass increase (fresh weight) in the first larval days, but more important the change in dry mass proportion (biomass) and in energy content. While the fresh weight increases from L3 to L7 by a factor of 4.5, dry mass rises 6.2 times and the mass specific energy density by 1.4 times. Thus, the energy content increase during the transition from L3 to L7 by a factor of $6.2 \times 1.4 = 8.7$, that means about nine-fold. The energy density of 18.4 for L3 indicates that biomass mainly consists of carbohydrates (16.7 kJ g^{-1}) and proteins (18.8 kJ g^{-1}) and of only small quantities of fat (39.7 kJ g^{-1}), a picture that changes significantly towards L7 and P4 for workers and that is even more pronounced for drones. These changes are also reflected in the reduction of the heat production of drones after the 5th day (L5) although their mass is still increasing: mass gain is due to formation of fat reservoirs and thus of metabolically less active tissue.

Table 4 compiles the results of the elemental analysis of the samples. It shows the mass contents in percent for carbon, hydrogen, nitrogen, and ash, which were directly determined, and that for oxygen as difference to 100%. Moreover, it ren-

Table 3
Fresh and dry weight, biomass percentage in the fresh weight, total energy content and mass specific energy density of an animal during the development of honeybee workers and drones

Day	Sample size	Fresh weight (mg)	Dry weight (mg)	Biomass (%)	Energy content (J)	Energy density (J mg^{-1})
Workers						
3 (L3)	10	32 ± 3	6.2 ± 0.6	19.4	114	18.4 ± 1.6
7 (L7)	12	145 ± 11	38.3 ± 2.8	26.4	958	25.0 ± 1.0
14 (P4)	9	130 ± 4	27.4 ± 1.0	21.1	723	26.4 ± 1.1
22 (A)	7	116 ± 4	21.5 ± 1.1	18.5	499	23.2 ± 1.2
Drones						
4 (L4)	9	121 ± 19	23.6 ± 4.5	19.5	590	25.0 ± 2.5
9 (VP)	3	320 ± 26	90 ± 6	28.1	2934	32.6 ± 1.2
15 (P4)	6	337 ± 12	81 ± 3	24.0	2438	30.1 ± 3.5
24 (A)	5	256 ± 15	57 ± 2	22.3	1186	20.8 ± 0.7

Energy values are determined by combustion calorimetry. Data are means ± S.D. Energy density values are significantly different (Mann–Whitney *U*-test, $\alpha < 0.05$) within the two groups between developmental stages except for VP and P4 of drones.

Table 4
Relative elementary composition and ash content, C:N ratio, C-mol formula and energy content of honeybee samples during the ontogenesis (W: worker bees, D: drones)

Day	Description	Mass (g)	C (%)	H (%)	N (%)	O (%)	Ash (% dm)	C:N ratio	C-mol formula	Energy density ($J\,mg^{-1}$)
7	WL	0.147	47.2	6.4	5.2	38.1	3.1	9.07	$CH_{1.63}O_{0.61}N_{0.09}$	18.29
9	DL	0.342	48.4	6.5	5.5	36.5	3.1	8.80	$CH_{1.61}O_{0.61}N_{0.10}$	19.13
19	WP	0.134	47.7	6.2	9.9	30.5	5.7	4.82	$CH_{1.57}O_{0.57}N_{0.18}$	19.54
21	DP	0.299	46.0	5.8	10.0	33.3	4.9	4.60	$CH_{1.51}O_{0.62}N_{0.19}$	17.89
21	WA	0.144	46.2	5.8	8.8	34.9	4.3	5.25	$CH_{1.51}O_{0.64}N_{0.16}$	17.67
23	DA	0.226	46.4	6.1	11.1	31.0	5.4	4.18	$CH_{1.58}O_{0.59}N_{0.20}$	18.87
Means ± S.D.			47.0 ± 1.0	6.1 ± 0.3	8.4 ± 2.5	38.5 ± 2.0	4.4 ± 1.1	6.1 ± 2.2	$CH_{1.57}O_{0.61}N_{0.15}$	18.57 ± 0.73

Energy density is calculated from CHO determination using the equation q ($kJ\,g^{-1}$) = 33.76 × C + 144.05 × (H − (O/8)), 14–26 animals were pooled per group for elemental analysis. The nomenclature of days equals that used in Tables 1–3 and Figs. 1 and 2: WL and DL, worker and drone larvae just before capping; WP and DP, worker and drone pupae just before hatching; WA and DA, just emerged worker and drone adults.

ders the “C:N ratio” as measure of the protein content in the sample and a C-mol based elemental formula $CH_xO_yN_z$ for the samples as a mean “honeybee formula”. Finally, an energy density is given calculated from the elemental composition.

4. Discussion

4.1. General observations

Isoperibol calorimetry is nowadays a well-established method to determine metabolism and locomotor activities in animals and to study environmental influences on them. Terrestric species are better suited for calorimetric investigations than aquatic ones kept in water. Water interferes with the calorimetric signal due to its high heat capacity and the large enthalpy change connected with evaporation. Among all terrestric animals insects are most frequently used, as they are easy to breed, to handle and with a comfortable size for most calorimeters. And among them, social insects like honeybees, bumblebees, wasps and hornets are preferred [3,12,26,27].

The present paper concerns honeybee workers and drones and their extremely quick development from eggs to adults with special emphasis on their energy storage and turnover. Uncapped honeybee larvae get a nourishing fluid secreted by a special gland of the nursing worker bees. This fluid is rich of carbohydrates and proteins and has an energy content of about 7.7 $kJ\,ml^{-1}$ [18]. During their intensive brood care worker larvae receive droplets of 3 μl (23 J) per visit and are able to increase their body mass from 0.32 mg (L1) to 185 mg (L6) (Table 1), which corresponds to a factor of 580 in 1 week. The corresponding values for drone larvae are 1.0 mg (L1), 402 mg (L7) and 400-fold, respectively (Table 2). Till to capping the mass growth curve can be described by a sigmoid function with a maximum growth rate of a factor 10 per day for worker bees and 5 for drones. Such high rates are essential to produce large numbers of progeny in a short time and thus to compete for the harsher conditions in temperate climates. These growth rates are among the highest in the animal kingdom and are even higher than those of intensively growing plants (unpublished results).

The strong decrease in metabolic rate after capping seen in Figs. 1 and 2 is in good agreement with expectation. In this period a break-down and digestion of larval structures occurs, followed by a complete reorganisation of the body (metamorphosis) till to the adult bee, a process seen in the once more increasing rate.

It is known from the literature that honeybee larvae use glycogen as main energy reservoir and less fat than other insect larvae [28]. Moreover, drone larvae store more fat than worker larvae. Thus, one could expect to find values for the mass specific energy content that lie between those for carbohydrates and for fat and higher ones for drones than for workers. Both anticipations were correct (Table 3).

p–t curves taken continuously for worker and drone larvae show short-term oscillations in the heat output during the first days of larval and the last days of pupal development. They are connected with locomotor activities and render information about the ontogenetic state and the well being of the animals. Although significant in the graphs, they are unimportant in their contribution to the overall heat output. In a comparable manner Harak and colleagues investigated calorimetrically the periodically occuring rhythmic body movements in pupae of the mealworm *T. molitor* and the greater wax moth *G. mellonella* [2]. The results show that these movements contribute with no more than 1% to the total heat output and are thus negligible on the background of usual biological scatter. Combination with respirometric monitoring revealed that such activities are tightly coupled with tracheal ventilation. Also *Blattella germanica* long-term monitoring exhibited regular peaks in the *p–t* curves owing to irregular sudden locomotion of the animals [1].

Several moultings occurring during the larval period are not detectable in the *p–t* curves. The impressive exothermic peaks during the transition to pupae or adults are due to the force necessary to break and shed the old cuticula and for young worker bee to open the capped cell and to escape from it in the last state.

In a recent paper, long-term calorimetric registration of insects was presented for the greater wax moth *G. mellonella* with highly resolved graphs for the larval–pupae and the pupae–adult ecdysis [5]. The first transition exhibited a strong exothermic peak followed by some less resolved exothermic structures and a deep endothermic trough. A similar sequence is seen in the second transition to the adult animal. Corresponding moulting events are also detectable in *p*–*t* curves of developing mealworms during hatching [2]. In Fig. 2a and b of the present paper the first exothermic peaks are strongly expressed while the endothermic troughs are nearly missing. This might be due to the high humidity around larvae and pupae in the capped cells and the significantly reduced possibility for evaporation.

Another typical structure for insect development is the U-shaped curve of heat production (or respiration) rate during larval–pupal development. Already indicated in Fig. 1a for the metabolic rate it is strongly indicated below for the mass specific rate. Fig. 2 exhibits the full U in the lower graph (b) and the right part of the U in the upper one (a) in which the first larval days lack. Similar results for honeybees were published already – among others – by Melampy and Willis in 1939 [29] and for further insects like *G. mellonella* [5] and *T. molitor* [2,21].

The chemical composition of the honeybee body changes not only with the individual development, but also with the year. During summer carbohydrate (honey) and protein (pollen) reserves are stored in the combs and no fat and protein depots can be found in the individual bee. The picture changes in wintertime when such depots exist in the bee and mainly carbohydrates are stored in the combs [30]. In worker larvae, a composition of 33.4% glycogen and 18% fat and 48.6% of further carbohydrates, proteins and less important components is found in the dry mass with an energy density of 20.85 kJ g^{-1} [28]. The corresponding figures for drone larvae are 25.5% glycogen, 21.3% fat and a rest of 53.2% leading to 21.26 kJ g^{-1} [28]. These energetic values are near to that of the food sap in the first 3 days (20.6 kJ g^{-1}) and that in the following days (18.7 kJ g^{-1}). Such data resulting from determinations of the body composition as carbohydrate, protein, and fat, may be compared with those of elemental analysis (Table 4), which is a rather well known technique in microbiological calorimetry [16,17,31,32] but seems strange for honeybees. It just enables an easy comparison with the degree of reduction in glucose (CH_2O in a similar C-mol based form) the main food of bees.

4.2. Some energetic considerations

4.2.1. Honey collection

An old rule of thumb for beekeepers state that a bee has to fly six times around the earth to collect enough nectar for 1 kg of honey. A honeybee flying with a power of 50 mW at 30 km h^{-1} needs 8000 h for this distance and consumes 1450 kJ of fuel. One kilogram of honey with a sugar content of 80% represents an energy amount of 16 MJ that has to be compared with the energy input of 1450 kJ: a collecting efficiency of more than 90% is the astonishing result [33].

4.2.2. Feeding of worker bees and energy investment in progeny

Food sap given to the developing larvae contains 18% protein ($\times$18.8 kJ g^{-1} = 3.38 kJ ml^{-1}), 5.5% fat ($\times$39.7 kJ g^{-1} = 2.18 kJ ml^{-1}) and 12.5% carbohydrates ($\times$16.7 kJ g^{-1} = 2.09 kJ ml^{-1}) in the mean. Two to four microlitres (with an energy concentration of 7.7 kJ ml^{-1}) of this sap are initially placed at the bottom of the larval cell corresponding to an energy source of 15.4–30.8 J. This is a rather small amount of energy considering the rapid larval growth and an energy content of 114 J on L3 and a maximum content of 958 J directly after capping (L7), which means that larvae have to be continuously supplied with energy as long as the cells are uncapped.

Another calculation may follow data published by Winston [34] stating that 142 mg of honey and 125–145 mg pollen (with 30 mg protein) have to be invested in a worker larva. The honey corresponds to 1780 J, the pollen/protein to 560 J, in total 2340 J, an efficiency of 41% for rearing the brood. The lifetime of a bee amounts to 15–38 days in summer with a mean of 30 days. Thus, 1300 bees die per day in a 40,000 member colony and have to be replaced by new ones to maintain at least a steady state of the system (normally, they will produce an overplus of bees for the production of swarms). This figure corresponds well to observed laying frequencies of honeybee queens with 1200–2000 eggs per day. Combining these values render 185 g honey (or 3 MJ) that are used for progeny daily. As only a few hundred drones with a lifetime of 50 days live in the same hive, they can be neglected in this calculation. During the whole season a mother queen produces about 320,000 workers consuming 320,000 × 1780 J or 570 MJ (honey) for their development. This amount of energy originates from 35 kg honey in a colony that collects between 60 and 80 kg honey per season so that one half is consumed for rearing the brood.

Until now, no direct measurements have been performed to determine the amount of energy used for rearing brood and to calculate the efficiency. Assuming that 958 J have to be fed in form of sap with 7.7 J μl^{-1} to the larva at an energy efficiency of 30%, about 420 μl of sap have to be transported in 3 μl quantities during 140 visits to the cell till to capping (larval day L6). This is in an astonishing agreement with old data of Lindauer (1952). He states that nursing bees visit the larval cells 59 times for cleaning, 1926 times for inspection and 143 times for feeding [35]. There is no further energy resources left in the capped worker cell, so that the mass of the worker larva decreases directly after.

4.2.3. Energy needed for heating the hive

The main aim of a beehive is to guarantee an optimal temperature in the brood area, independent of the ambient temperature. Honeybees are able to produce heat by intensive shivering of their flight muscles when extra heat is needed. The dissipation rate is as high as during flight: 50–160 mW bee^{-1} [24,25,34]. The energy flow calculated above for rearing progeny amounts to 3 MJ per day or 35 W. At an efficiency of 40%, 14 W are fixed in biomass and 21 W are dissipated as heat. This value corresponds to a mass specific value of 5 mW g^{-1} for the whole colony which is at the lower end of a list for indirect calorimetric data of intact bee colonies [7]. Taking this list (Table 3 in [7]), omitting one extremely high value and selecting the experimental temperature range from 20 to 30 °C (11 values, median 24 °C) the data span from 7.1 to 75.0 mW g^{-1} with a mean of 26.6 ± 22.4 mW g^{-1} (S.D.) and a median of 16.3 mW g^{-1}. The highest turnover rate of 75.0 mW g^{-1} was determined by Kronenberg and Heller [36], while the median corresponds to a result of Ritter [37]. The 5 mW g^{-1} calculated above indicate that further heat has to be added from other processes going on in the hive. But they are difficult to estimate since they are strongly dependent on the level of activity and the number of animals participating in it. A very rough calculation can be performed using the "heating sugar values" of Büdel [30] for beehives of different sizes. Following his determinations a "usual" bee colony needs an additional amount of 15 kg sugar per year that is only used for heating. It corresponds to a continuous heating of 12 W rendering a total heat output of 33 W or 7.9 mW g^{-1}. Of course such values vary considerably during the year depending on different parameters, among them ambient temperature, presence of brood and state of the colony.

4.2.4. Mass reduction and water loss of capped worker brood

Directly after capping worker brood starts to loose weight. In spite of the sealed cell and the high humidity in the beehive it might be supposed that this loss is due to water evaporation only. With an enthalpy uptake of 2256 J g^{-1} and total loss of 70 mg (Table 1) 158 J are consumed during the 15 days of capping, rendering a mean heat signal of 0.12 mW that is too small to be detected in the calorimetric output. On the other hand, Table 3 shows that dry mass decreases from 38.3 mg (for L7) to 21.5 mg (for a freshly hatched adult) and fresh mass from 145 to 116 mg so that a loss of 16.8 mg originates from organic material and only 12.2 mg from water. The latter transform to an evaporation enthalpy of 27.5 J and a mean power uptake of 0.021 mW, by far within the thermal noise of the measurement. This means that the determined exothermal metabolic heat output is near to the true value and not reduced by endothermal evaporation.

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