

## CALORESPIROMETRIC DETERMINATION OF THE EFFECTS OF TEMPERATURE, HUMIDITY, LOW O<sub>2</sub> AND HIGH CO<sub>2</sub> ON THE DEVELOPMENT OF *MUSCA DOMESTICA* PUPAE

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Heat and CO<sub>2</sub> rates are measured on *M. domestica* pupae as a function of age and temperature. Heats of combustion of flies, pupae, and puparia are also measured. Heat and CO<sub>2</sub> rates decrease for 3 d post-pupation and increase exponentially over 4 d prior to eclosure. Heat and CO<sub>2</sub> rates are low and increase slowly from 5 to 23°C, then rapidly increase from 23 to 26°C, then more slowly up to 38°C where pupae are stressed. Respiratory substrate varies with age and temperature, is a mix of lipids and carbohydrates, and has an average carbon oxidation state of -0.3 at room temperature. Humidity, elevated CO<sub>2</sub>, or reduced O<sub>2</sub> did not affect pupal metabolism.

**Keywords:** age, calorimetry, humidity, metabolism, *Musca domestica* pupae, temperature

### Introduction

Evolutionary fitness [1, 2] and physical activity [3] are related to the metabolic activity of organisms. The metabolic rate of an organism may be measured as the rates of CO<sub>2</sub> or heat production, or of O<sub>2</sub> consumption [4–6, 7]. Calorespirometric data on both heat and CO<sub>2</sub> rates allows calculation of anabolic rates, calculation of efficiencies of conversion of substrate into anabolic products, and provides information on the nature of the substrate carbon source [6, 7]. In this study, heat and CO<sub>2</sub> production rates of *Musca domestica* (Diptera: Muscidae) pupae were measured during exposure to varied humidity, temperature, decreasing O<sub>2</sub> partial pressure, and to increasing CO<sub>2</sub> partial pressure. Heat and CO<sub>2</sub> rates were also measured as a function of development age.

Previous work on insect pupae has demonstrated the value of respirometric measurements in studying insect development. Gas exchange rates of Mediterranean fruit fly (*Ceratitis capitata*) pupae measured as a function of temperature were used to calculate respiratory quotients and specific heat rates [8]. Respiratory quotients varied inversely with temperature with values as large as 2.7 at low temperatures. Despite these high values, it was concluded that the substrate was carbohydrate. The calculated heat rates varied from 0.80 μW mg<sup>-1</sup> at 35°C to 8.0 μW mg<sup>-1</sup> at 35°C and increased with increasing age of the pupae. In other studies, the effects of exposure to cold tempera-

tures on metabolism of *M. domestica* pupae at 26–28°C have been measured as O<sub>2</sub> consumption rates [9]. During development at 28°C, O<sub>2</sub> consumption rates decreased and then increased, producing a U-shaped pattern; a similar response of metabolic heat rate occurs during development of pupae of honeybee and *Galleria mellonella* [10]. Pupae of different developmental age vary in their ability to survive exposure to low temperatures (7°C), and exposure to extreme high temperature (44 to 46°C) for more than two hours affected the number of *M. domestica* pupae that survived and emerged as flies [11]. While Noorman and Den Otter [12] showed that production of cuticular hydrocarbons from flies newly emerged from their puparia were temperature and humidity dependent in *M. domestica*, no studies concerning humidity effects on metabolic rates in pupae were found in the literature. Respirometric measurements on two species of adult insects, *Locusta migratoria* and *Manduca sexta*, were used to show different tolerances to anoxic environments [13]. Calorimetric methods have also been used to determine the effects of elevated CO<sub>2</sub> and reduced O<sub>2</sub> atmospheres on metabolic heat rates of *Platynota sultana* pupae [14, 15]. Calorimetry of insect metabolism is reviewed in Lamprecht and Schmoltz [16].

Very few previous calorimetric studies on insects have measured both gas exchange and heat rates. However, as shown by recent studies [6, 17–20], simultaneous measurement of heat and

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CO<sub>2</sub> rates is a much more powerful method for obtaining insight into ectotherm metabolism than measurement of heat or gas exchange rates alone. This study further explores the utility of calorimetric methods for obtaining metabolic information about insect pupae under various environmental stresses, and thus provides a comprehensive analysis of metabolism during pupal development of *M. domestica*.

## Methods and material

*M. domestica* pupae were purchased from Carolina Biological Supply Company (Burlington, NC) and maintained in a ventilated container held in the lab at a constant temperature (25 to 26°C, as measured inside the container) with moisture provided by a damp paper tissue. Pupal metabolism was measured in four Hart Scientific (Pleasant Grove, UT), model 7707, and two Calorimetry Sciences Corp. Model 4100, differential, heat-conduction calorimeters operated in isothermal mode at various temperatures from 5 to 41°C. Each calorimeter measures heat rates from three sample ampules simultaneously. Instrument baseline corrections were made by subtracting heat rates measured with empty ampules from heat rates measured with pupae in the ampules. The pupae were randomly selected from each of the purchased lots, weighed, and placed into the 1 mL calorimeter ampules. Pupae introduced into the calorimeters were allowed to reach thermal steady state, requiring about 30 min, then heat rate data were collected for about 15 min. After the first heat rate measurement ( $R_{q1}$ ), a 40  $\mu$ L vial of 0.4 M NaOH was placed in each ampule and the heat rate was measured again after the samples reached the second thermal steady state ( $R_{qNaOH}$ ). The NaOH vial was then removed from the ampule and the third thermal steady state measurement ( $R_{q2}$ ) was obtained. Specific metabolic heat rates ( $R_q$ ) were calculated by

$$R_q = [(R_{q1} + R_{q2})/2]/FW \quad (1),$$

where  $FW$  is the sample fresh mass. During the second measurement of the heat rate, the CO<sub>2</sub> produced by aerobic metabolism reacted with the NaOH solution forming Na<sub>2</sub>CO<sub>3</sub> (*aq*) and additional heat. The rate of the additional heat liberated in the CO<sub>2</sub> + 2OH<sup>-</sup> → CO<sub>3</sub><sup>2-</sup> reaction ( $\Delta H = -108.5$  kJ mol<sup>-1</sup> CO<sub>2</sub>) is proportional to the rate of CO<sub>2</sub> production [21]. Specific CO<sub>2</sub> production rates ( $R_{CO_2}$ ) were calculated by

$$R_{CO_2} = [(R_{qNaOH}/FW) - R_q]/108.5 \quad (2).$$

To determine the temperature dependence of the metabolic rates, experiments employed three ampules per calorimeter, and were repeated three times at each temperature. Thus, nine replicates were obtained at

~3°C intervals over the entire temperature range. To observe heat rates in the optimum range (200 to 500  $\mu$ W), small samples ( $n=6$ ) were used at 41°C and large samples ( $n=18$ ) at 5°C, with determinations at intermediate temperatures using between 16 and 8 pupae. Pupae were measured within three days of receipt and were used for only one measurement to avoid any effects of previous conditions on subsequent measurements.

To determine the effects of humidity on metabolic activity, vials of water and saturated salt solutions were placed in ampules with pupae and heat rates were measured at 30°C. Saturated solutions of KBr, NaBr, and MgCl<sub>2</sub> were used to maintain 82, 57 and 32% relative humidity, respectively [22]. The effects of 100% relative humidity were determined by adding a vial of distilled H<sub>2</sub>O. To measure the effects of ~0% relative humidity, anhydrous CaSO<sub>4</sub> was added to the bottom of the ampule. A circular plastic screen fitted above the CaSO<sub>4</sub> prevented the pupae from direct contact.

The developmental stage of the pupae is not synchronized within a population and it is not possible to nondestructively determine the precise developmental age of a pupa, i.e. from the external appearance of the pupa. Therefore, changes in metabolic activity as a function of developmental age were determined by measuring  $R_q$  and  $R_{CO_2}$  on samples taken from a given group of pupae on 4 consecutive days prior to the day of eclosure of most of the population. The population aged one day at the temperature in the container (25 to 26°C) between each measurement. The results indicate the average daily change in metabolic activity during development. Three fresh pupae were measured in each of the 18 ampules available. Average fresh pupae weights per ampule were 60.7, 61.7, 59.6, and 64.6 mg for days 1, 2, 3, and 4, respectively. During some measurements, flies emerged from their puparia. Calorimetric measurements from ampules in which flies emerged were excluded from the data.

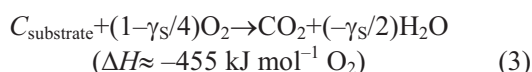
The response of pupal metabolism to varied concentrations of CO<sub>2</sub> and O<sub>2</sub> was determined by using different sample sizes. Because the ampules are airtight and do not allow air exchange with the external environment, pupal metabolism generates an increasing CO<sub>2</sub> concentration and decreasing O<sub>2</sub> concentration within the ampules. Over a fixed period of time, large samples alter gas composition in the ampules faster and thus more than small samples.  $R_q$  of small samples of 1, 2, and 3 pupae were measured at 25°C, and large samples of 12 pupae were measured at 23 and 26°C.

Heats of combustion of flies, pupae, and puparia were determined with a Parr semi-micro oxygen bomb calorimeter calibrated with 0.2 g aliquots of benzoic acid. Samples were dried in a vacuum oven at

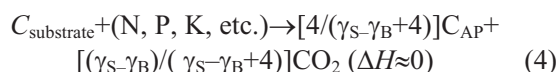
70–80°C for 24 h, ground with a mortar and pestle, and pressed into pellets for combustion. C and N determinations were done on aliquots of the same samples by Prof. Bruce L. Webb of the Department of Plant and Animal Sciences, BYU.

### Data analysis

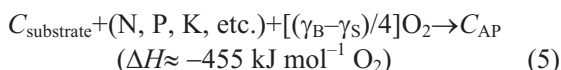
Respiratory metabolism can be divided into two types of overall reactions, oxidation reactions (catabolism and oxidative anabolism, reactions 3 and 5) and disproportionation reactions (anabolism and anaerobic catabolism, reaction 4).



where  $\gamma_S$  is the average chemical oxidation state of carbon in the substrate that is oxidized to  $\text{CO}_2$ .



where  $\gamma_B$  is the average chemical oxidation state of carbon in the anabolic products ( $C_{\text{AP}}$ ) of the reaction (other than  $\text{CO}_2$ ) and N, P, K represent compounds or ions of these elements. If the anabolic products are more oxidized than the substrate, as in organisms in which lipid and/or protein is a major substrate, then the anabolic reaction must be represented as



which we shall refer to as oxidative anabolism. These three overall equations can be used to interpret calorespirometric data in terms of the relative rates of reactions, including growth or development rate, and substrate carbon conversion efficiency. The quantitative model used to calculate  $f_3$ , the fraction of total  $\text{CO}_2$  produced by reaction 3,

$$f_3 = (R_q/R_{\text{CO}_2})/[455(1-\gamma_S/4)] \quad (6)$$

is described in previous publications [6, 19]. Note that the only unknown in Eq. (6) is  $\gamma_S$ , the substrate carbon oxidation state.

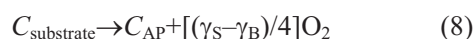
The value of  $\gamma_S$  is nearly always  $\leq 0$  in multi-cellular organisms, i.e. the substrate is carbohydrate with  $\gamma_S = 0$  and  $\Delta H_3 = -470 \text{ kJ Cmol}^{-1}$ , protein with  $\gamma_S \approx -1$  and  $\Delta H_3 = -543 \text{ kJ Cmol}^{-1}$ , or lipid with  $\gamma_S \approx -1.4$  and  $\Delta H_3 = -611 \text{ kJ Cmol}^{-1}$ , or some combination of these three substances [25]. Therefore, ratios of  $R_q/R_{\text{CO}_2} < 455 \text{ kJ mol}^{-1}$  indicate carbohydrate as the substrate and the presence of an anaerobic component of respiration, i.e. anaerobic anabolism or anaerobic catabolism. Values of  $R_q/R_{\text{CO}_2} > 455 \text{ kJ mol}^{-1}$  indicate the absence of anaerobic anabolism or other anaerobic reactions, the presence of oxidative anabolism and

oxidation of a catabolic substrate more reduced than carbohydrate as the source of  $\text{CO}_2$  [6].

An equation for the substrate carbon conversion efficiency ( $\epsilon$ ) can be obtained by expressing the heat rate for the weighted sum of Eqs 3 and 4, or 3 and 5 [6], as

$$R_q = -\Delta H_3 R_{\text{CO}_2} - [\epsilon/(1-\epsilon)] \Delta H_B R_{\text{CO}_2} \quad (7)$$

where  $\Delta H_B$  is the enthalpy change per Cmol for the reaction.



In Eq. (7),  $\Delta H_3 = (1-\gamma_S/4) 455 \text{ kJ mol}^{-1} \text{ CO}_2$ .

$\Delta H_B$  is dependent on the oxidation states of both substrate ( $\gamma_S$ ) and anabolic products ( $\gamma_B$ ) [26],

$$\Delta H_B = 455 [(\gamma_S - \gamma_B)/4] \text{ kJ mol}^{-1} \quad (9)$$

Equation (9) represents an endothermic reaction if  $\gamma_S > \gamma_B$  and an exothermic reaction if  $\gamma_S < \gamma_B$ . Calculation of  $\epsilon$  requires measured values of  $R_q$  and  $R_{\text{CO}_2}$  and values of  $\gamma_S$  and  $\Delta H_B$  or  $\gamma_B$ . The values of  $\gamma_B$  and  $\Delta H_B$  can be obtained either from measurements of the composition or heat of combustion of the organism [26].

For either anaerobic or oxidative anabolism, the anabolic rate ( $R_{\text{AP}}$ ) can be calculated from  $R_q$  and  $R_{\text{CO}_2}$  by the relation [6],

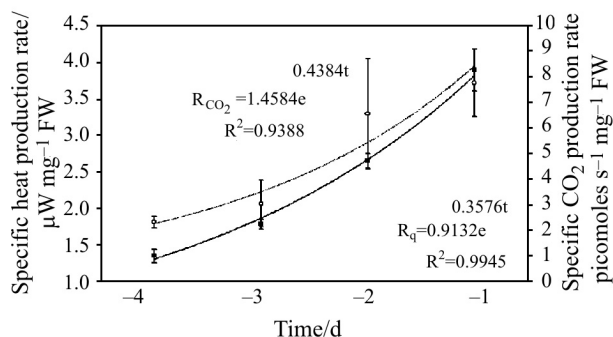
$$R_{\text{AP}} \Delta H_B = 455(1-\gamma_S/4)R_{\text{CO}_2} - R_q \quad (10)$$

Equation (10) gives the anabolic rate ( $R_{\text{AP}} \Delta H_B$ ) as the rate of storage (or loss, in which case  $R_{\text{AP}} \Delta H_B$  would be negative) of chemical energy (i.e. enthalpy) in anabolic products, or if the value of  $\Delta H_B$  is known, as the rate of incorporation of carbon into anabolic products,  $R_{\text{AP}}$ , which is also the rate of growth or development.

## Results and discussion

No significant changes in pupal metabolism resulted from short-term exposure to varied humidity, elevated concentrations of  $\text{CO}_2$ , or the reduced concentrations of  $\text{O}_2$  tested. Exposure to relative humidities of 100, 82, 57, 32 and 0% did not alter specific heat rates at 30°C. Specific heat and  $\text{CO}_2$  rates of large sample sizes were indistinguishable from specific heat rates from small sample sizes. Therefore, we concluded that neither short-term exposure to concentrations of  $\text{CO}_2$  up to about 10%,  $\text{O}_2$  concentrations down to about 10%, nor humidity influence pupal metabolism in this species.

Specific heat and  $\text{CO}_2$  production rates at 25°C of samples taken from a single batch of pupae as a function of time over four consecutive days preceding eclosure are reported in Fig. 1. As is usually the case



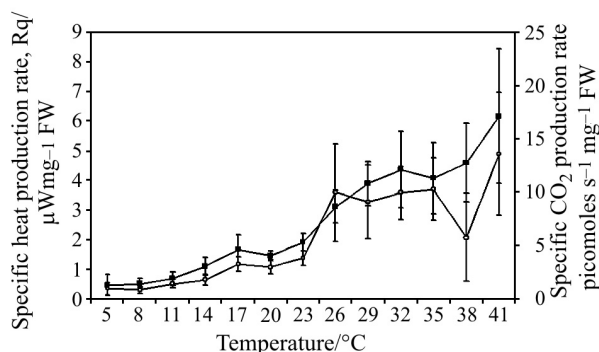
**Fig. 1** Mean specific heat (■) and CO<sub>2</sub> (○) production rates at 25°C (± standard deviation) of *M. domestica* pupae as functions of average developmental age of the sample. Eighteen ampules were used with 3 pupae included in each. Ampules in which flies emerged from puparia were excluded from calculation. On Day -4,  $n=54$ , Day -3,  $n=51$ , Day -2,  $n=54$ , and on Day -1,  $n=45$ , where  $n$  is the number of pupae

for growing or developing organisms, both  $R_q$  and  $R_{CO_2}$  fit increasing exponential curves in time. This response is expected because development of the fly inside the puparia is expected to progress exponentially in time after the initial disintegration of the larvae inside the puparia, thus giving rise to the U-shaped curves seen for pupal development [10]. Average weight of pupae used on each of the four days was  $61.6 \pm 2.1$  mg per ampule, showing there is no significant change in pupal mass during development.

The  $R_q$  produced by pupae as a function of temperature is presented in Fig. 2. At temperatures  $\leq 23^\circ\text{C}$ , little heat is generated in comparison to heat rates at temperatures above  $23^\circ\text{C}$ . Based on an expected exponential increase with temperature, the heat rate profile appears to be divisible into three temperature regions, the first from 5 to  $18^\circ\text{C}$ , the second between 21 and approximately  $32^\circ\text{C}$ , and the third between 35 and  $41^\circ\text{C}$ .

The  $R_{CO_2}$  of the same samples are also presented in Fig. 2 as a function of temperature. The profile of the  $R_{CO_2}$  curve vs. temperature closely resembles the heat rate vs. temperature profile. At temperatures between 5 and  $23^\circ\text{C}$ , CO<sub>2</sub> is produced very slowly. A sharp increase in  $R_{CO_2}$  occurred between 23 and  $26^\circ\text{C}$ , above which the rate remained fairly constant as temperature increased up to  $35^\circ\text{C}$ . The high  $R_{CO_2}$  values at 38 and  $41^\circ\text{C}$  are probably the result of cellular response to temperature stress, such as oxidation of abnormal metabolic substrate and loss of membrane integrity.

The increased noise in the  $R_q$  and  $R_{CO_2}$  values above  $23^\circ\text{C}$  is due to variation in the ages of the pupae in the samples. Smaller numbers of pupae were placed in the ampules at the higher temperatures, and thus the sample has a statistically larger chance of differing from the average age of the population. Developmentally younger pupae produce significantly lower



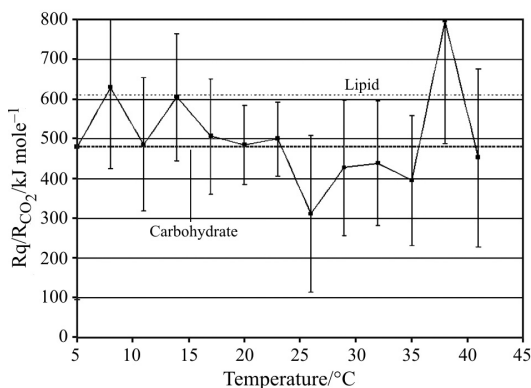
**Fig. 2** Mean basal specific heat (■) and CO<sub>2</sub> (○) production rates as functions of temperature. The number of pupae per replicate varied from 18 pupae at  $5^\circ\text{C}$  to 6 pupae at  $41^\circ\text{C}$ . The averages of 9 determinations at each temperature with uncertainties as the standard deviation are shown

$R_q$  and  $R_{CO_2}$  values than older pupae. Because pupae were not sampled according to their developmental age when measuring the  $R_q$  and  $R_{CO_2}$  values shown in Fig. 2, pupae of varied developmental age were measured and this variation produced the observed increase in standard deviation as samples became smaller with increasing temperature.

The ratio  $R_q/R_{CO_2}$ , calculated from data in Figs 1 and 2 is given as a function of developmental age in Table 1 and is plotted as a function of temperature in Fig. 3. The ratio appears to decrease with age at  $25^\circ\text{C}$ , but is constant with age at 20 and  $30^\circ\text{C}$ . The response of the ratio to temperature clearly differs with age. The horizontal lines in Fig. 3 are drawn at 470 and  $611 \text{ kJ mol}^{-1} \text{ CO}_2$ , the ratios for combustion of carbohydrates and lipids, respectively [25]. The measured  $R_q/R_{CO_2}$  ratio is  $>470 \text{ kJ mol}^{-1} \text{ CO}_2$  and no changes of statistical significance occur from 5 to  $23^\circ\text{C}$ . At temperatures from 26 to  $35^\circ\text{C}$ , the ratio is slightly below  $470 \text{ kJ mol}^{-1} \text{ CO}_2$ . The  $R_q/R_{CO_2}$  values across the temperature range from 5 to  $35^\circ\text{C}$  indicate that lipid and/or protein constitutes a major part the substrate [6]. The very large value at  $38^\circ\text{C}$  indicates a stress response and further that the catabolic substrate at this temperature must be essentially all lipid. Figure 3 shows that only at temperatures between about 26 and  $35^\circ\text{C}$  is it possible that a significant fraction of respiratory CO<sub>2</sub> is produced by anaerobic anabolism.

Some insight into the nature of the substrate is provided by the fraction  $f_3$  of CO<sub>2</sub> produced by oxidation of substrate by O<sub>2</sub>, i.e. by reaction 3, see Fig. 4 and Table 1. Assuming carbohydrate ( $\gamma_S=0$ ) as the substrate gives  $f_3$  values that are mostly  $>1$ , an impossibility. Assuming lipid ( $\gamma_S=-1.36$ ) as the substrate gives  $f_3$  values between 0 and 1, but closer examination shows many of the values of  $f_3$  in Table 1 and Fig. 4, particularly at temperatures between 20 and  $35^\circ\text{C}$ , to be unreasonable. If lipid is the substrate, all





**Fig. 3** The mean ratio of  $R_q$  to  $R_{CO_2}$  ( $\pm$  standard deviation) as a function of temperature including values for the heats of combustion for lipid,  $\Delta H_3 = -611 \text{ kJ mol}^{-1} \text{ CO}_2$ , and for carbohydrate,  $\Delta H_3 = -470 \text{ kJ mol}^{-1} \text{ CO}_2$  [25]

the  $\text{CO}_2$  has to come from oxidative catabolism and  $f_3$  values should be close to unity. Further, calculation of  $\varepsilon$  values with Eq. (7) and  $R_{AP}$  values with Eq. (10) assuming either carbohydrate or lipid substrate produces unreasonable or negative values at many of the conditions of temperature and age. Thus we conclude that the substrate cannot be either solely carbohydrate or solely lipid at all the conditions studied.

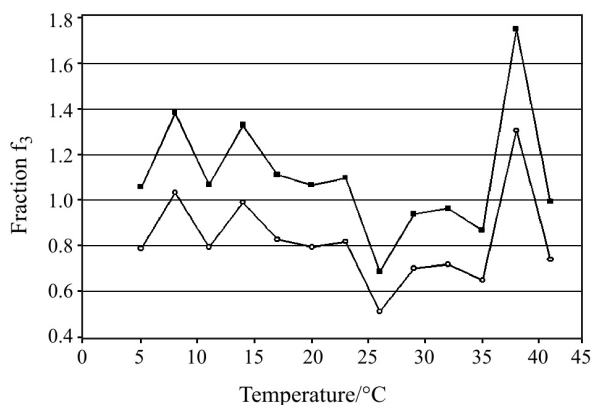
To further examine the nature of the substrate, Eqs (7) and (9) can be combined and rearranged to obtain

$$\varepsilon/(1-\varepsilon) = \{[(R_q/R_{CO_2})/455] - 1 + (\gamma_S/4)\} / [(\gamma_B - \gamma_S)/4] \quad (11)$$

and Eqs (9) and (10) can be combined to obtain

$$R_{AP} = [455(1 - \gamma_S/4) R_{CO_2} - R_q] / [455(\gamma_S - \gamma_B)/4] \quad (12)$$

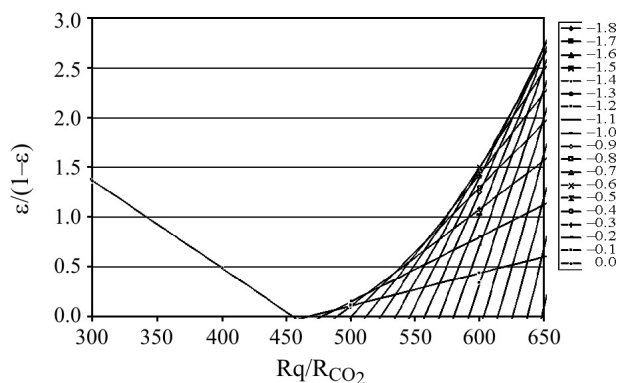
Equations (11) and (12) allow limits to be placed on  $\gamma_S$  if  $\gamma_B$  is known from heat of combustion or composition data on the emerging organism. The value of  $\varepsilon/(1-\varepsilon)$  must be  $\geq 0$  and less than approximately 9 which corresponds to  $\varepsilon = 0.9$ , i.e., a 90% substrate carbon use efficiency for development.  $R_{AP}$  must be  $\geq 0$  and must be maximum in the temperature range where rapid development is known to occur, i.e., 25 to 35°C. Figure 5 is a nomograph limited to  $\varepsilon/(1-\varepsilon) \geq 0$  constructed with Eq. (11) covering the entire range of possible  $\gamma_S$  values and all the  $R_q/R_{CO_2}$  values observed at non-stress temperatures. Note that Eq. (11) goes to infinity at  $\gamma_S = \gamma_B$  but otherwise describes a maximum curve of the saddle points for  $R_q/R_{CO_2} > 455 \text{ kJ mol}^{-1}$ . Thus, values of  $R_q/R_{CO_2}$  less than  $455 \text{ kJ mol}^{-1}$  can only be achieved with carbohydrate substrate. Values of  $R_q/R_{CO_2} > 455 \text{ kJ mol}^{-1}$  can only be achieved with substrates with  $\gamma_S < 0$ . Since  $R_q/R_{CO_2}$  is observed to change systematically with temperature and age, the maximum value of  $\varepsilon$  can only be achieved by varying the substrate along the curve at the maximum  $\varepsilon/(1-\varepsilon)$  shown in Fig. 5. Therefore we conclude that  $\gamma_S$  varies from about  $-0.6$  to near 0 at tempera-



**Fig. 4** The fraction of  $\text{CO}_2$  produced by oxidation reactions ( $f_3$ ) as calculated from equation 6 assuming carbohydrates with  $\gamma_S = 0$  ( $\bullet$ ) or with lipid with  $\gamma_S = -1.36$  ( $\circ$ ) as the substrate

tures between 5 and 23°C, and at temperatures between 25 and 35°C,  $\gamma_S \approx 0$ . It is clear from Figs 3 and 5 and Eq. (12) that a  $\gamma_S$  value can be chosen so as to produce positive values of  $R_{AP}$  from 5 to 35°C. However,  $R_{AP}$  values from 5 to 23°C must be small or zero and values at  $25 < T < 35^\circ\text{C}$  would be much larger.

Table 1 gives values of  $R_{AP}$  calculated with the values of  $\gamma_S$  given in the table. Note that  $R_{AP}$  also goes to infinity when  $\gamma_S = \gamma_B$ . The data indicate that growth rate is at a maximum at temperatures around 25°C in agreement with the data in Fig. 3 at 26°C. The difference in absolute values of  $R_q/R_{CO_2}$  between Fig. 3 and Table 1 are caused by the difference in development age of the pupae measured. The temperature study was done with pupae in the first three days post-pupation, and the age study used pupae in the last four days of the pupal stage. At 25°C, the  $R_q/R_{CO_2}$  ratio goes through a minimum between days 3 and 4 of the pupal stage. In agreement with the conclusions from the temperature study, pupae kept at 18°C did not eclose within ten days while pupae at 25 to 26°C always eclosed in seven days or less.



**Fig. 5** Nomograph of Eq. (11) relating the substrate carbon conversion efficiency ( $\varepsilon$ ) to the calorespirometric ratio  $R_q/R_{CO_2}$  and to the substrate oxidation state

**Table 1** The calorespirometric ratio ( $R_q/R_{CO_2}$ ) and the fraction of  $CO_2$  produced by aerobic oxidation reactions ( $f_3$ ) in *M. domestica* pupae

Developmental age at 25–26°C	$R_q/R_{CO_2}/kJ\ mol^{-1}\ a$			$f_3$ with $\gamma_S=0^b$			$f_3$ with $\gamma_S=-1.36^b$			$R_{Ap}/\ nanomol\ s^{-1}\ mg^{-1}\ FW^c$		
	20°C	25°C	30°C	20°C	25°C	30°C	20°C	25°C	30°C	20°C $\gamma_S=-0.6$	25°C $\gamma_S=-0.6$	
Days												
-4	509	578	595	1.12	1.27	1.30	0.84	0.95	0.98	-0.6	$\gamma_S=-0.3$ 58	6.4
-3	516	533	599	1.13	1.17	1.32	0.85	0.87	0.93	-0.4	$\gamma_S=-0.3$ 45	9.4
-2	523	491	604	1.15	1.08	1.33	0.86	0.80	0.99	-0.0	$\gamma_S=-0.3$ 4	13.7
-1	530	453	608	1.16	1.00	1.34	0.87	0.74	1.00	0.7	$\gamma_S=0$ 1	20.0
0	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure	enclosure

<sup>a</sup> From fitting  $R_q$  and  $R_{CO_2}$  data to an exponential function of the form rate =  $ce^{kt}$ .<sup>b</sup> See Eq. (6)<sup>c</sup> Calculated with  $\gamma_B=-0.27$ .**Table 2** Heat of combustion and chemical analyses of *M. domestica* flies, pupae and puparia

	Heat of combustion <sup>a</sup> / $kJ\ g^{-1}$	Ash/mass%	C/mass%	N/mass%	$\Delta H_{comb}/$ $kJ\ Cmol^{-1}$	$\gamma_B^b$
Flies	22.06±0.12 (6)	4.3	50.6	12.1	-573	-0.60
Pupae	21.98±0.21 (3)	4.9	47.6	10.2	-554	-0.87
Puparia	19.97±0.52 (3)	2.0	42.9	8.9	-559	-0.91

<sup>a</sup>Not corrected for ash or nitric acid formation. Uncertainties are the standard deviation with the number of determinations given in parentheses.<sup>b</sup>Calculated assuming  $\gamma_B = 4 - \{[4(\Delta H_{comb}^0/kJ\ C\ mol^{-1})]/[-455\ kJ\ C\ mol^{-1}]\}$

Further insight into the substrate oxidation state is provided by the heats of combustion in Table 2. These data show the puparia and pupae are both more reduced than flies, indicating that highly reduced compounds are the catabolic substrate during pupation. The increase in nitrogen content of flies compared to pupae shows the catabolic substrate is lipid, not protein. Calculated from the heat of combustion for *M. domestica* flies,  $\Delta H_B = +53 \text{ kJ Cmol}^{-1}$  if the substrate is carbohydrate,  $-20 \text{ kJ Cmol}^{-1}$  if it is protein, and  $-88 \text{ kJ Cmol}^{-1}$  if it is lipid.

The heat of combustion of *M. domestica* measured in this study is similar to the heats of combustion of similar organisms. The heats of combustion of Ephemera mayflies, *Agelastica alui*, arthropods (except crustaceae), *Otiorynchus singularis*, spiders, *Trypoxylon politum* pupae, and in general of all detritus consumers are all in the range 20.2 to 27.6 kJ g<sup>-1</sup>.

## Conclusions

This study shows that effects of temperature, humidity, CO<sub>2</sub> concentration, O<sub>2</sub> concentration and age on development rate of pupae can be determined using calorespirometry. Although no effects of humidity, CO<sub>2</sub> and O<sub>2</sub> appeared in this species, it is clear that the methods used could detect such effects if present. Respiration rate is often suppressed by CO<sub>2</sub> as seen in studies of other species [14, 15, 17], but not in *M. domestica* under the conditions used here. Our results agree with previous determinations of temperature limits on development [9, 11]. Assuming the average oxidation state of carbon in *M. domestica* is the same as in other insects, we conclude the substrate at  $T \leq 23^\circ\text{C}$  in the pupae is a mix of carbohydrate, protein, and lipid, which has an average carbon oxidation state of  $\approx -0.3$ . At  $25 < T < 35^\circ\text{C}$ , the substrate is apparently carbohydrate. The major substrate component(s) also appears to change from protein/lipid to carbohydrate during the pupal stage. This laboratory grown population of *M. domestica* appears to be narrowly adapted [27] to development at temperatures near 25 to 26°C. This work demonstrates that calorespirometry can provide significant insight into the relation between metabolism and the ecophysiology of insect pupae.

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