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RESEARCH ARTICLE

The speed and metabolic cost of digesting a blood meal depends on temperature in a major disease vector

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ABSTRACT

The energetics of processing a meal is crucial for understanding energy budgets of animals in the wild. Given that digestion and its associated costs may be dependent on environmental conditions, it is necessary to obtain a better understanding of these costs under diverse conditions and identify resulting behavioural or physiological trade-offs. This study examines the speed and metabolic costs – in cumulative, absolute and relative energetic terms – of processing a bloodmeal for a major zoonotic disease vector, the tsetse fly *Glossina brevipalpis*, across a range of ecologically relevant temperatures (25, 30 and 35°C). Respirometry showed that flies used less energy digesting meals faster at higher temperatures but that their starvation tolerance was reduced, supporting the prediction that warmer temperatures are optimal for bloodmeal digestion while cooler temperatures should be preferred for unfed or post-absorptive flies. ^{13}C-Breath testing revealed that the flies oxidized dietary glucose and amino acids within the first couple of hours of feeding and overall oxidized more dietary nutrients at the cooler temperatures, supporting the premise that warmer digestion temperatures are preferred because they maximize speed and minimize costs. An independent test of these predictions using a thermal gradient confirmed that recently fed flies selected warmer temperatures and then selected cooler temperatures as they became post-absorptive, presumably to maximize starvation resistance. Collectively these results suggest there are at least two thermal optima in a given population at any time and flies switch dynamically between optima throughout feeding cycles.

KEY WORDS: Metabolism, Energetics, Behaviour, Climate change, Stable isotopes, Thermal preference, Heat increment of feeding, Specific dynamic action, Tsetse, Diptera

INTRODUCTION

Animals eat to obtain the energy essential for the basic functions of life – growth, survival and reproduction – but processing food has an inherent energetic cost. The increase in metabolic rate that occurs in postprandial animals is called specific dynamic action (SDA) and represents costs stemming from ingestion, digestion, assimilation, protein synthesis, nutrient routing and excretion (McCue, 2006; Secor, 2009). SDA has been observed in hundreds of species representing most invertebrate phyla and all classes of vertebrates, and is believed to occur in all animals (reviewed in Jobling, 1983; McCue, 2006; Wang et al., 2006; Secor, 2009). Surprisingly, information on SDA among one of the largest taxonomic groups – insects – comes from very few studies (Table 1). Furthermore, the SDA of insects remains poorly characterized in terms of the standard metrics used to characterize this phenomenon in other animals (e.g. magnitude, peak time, duration and coefficient). Given insects' multiple roles as disease vectors, pests of agriculture, and as model taxa for evolutionary, climate and conservation-related research, this constitutes a significant limitation for integrating mechanistic understanding into population dynamics modelling, including population persistence and vulnerability to environmental change.

The SDA in vertebrate ectotherms typically accounts for 10–30% of the assimilated energy in a given meal (Secor, 2009) and thus accounts for a large component of their overall energy budget. In species that intermittently consume large meals, SDA is also accompanied by a suite of other behavioural changes and physiological modifications involving gut morphology, blood distribution and acid–base balance (Wang et al., 2006; Secor, 2009). Several studies have examined the effect of temperature on SDA in non-insect ectotherms (Table S1) and consistently report two general outcomes. First, higher temperatures cause the peak postprandial metabolic rates to peak at higher magnitudes but the duration of SDA is shorter. Second, the overall energy devoted to SDA is usually independent of temperature. Studies measuring apparent assimilation efficiencies have suggested that SDA is temperature independent (e.g. in the tsetse fly, Diptera: Glossinidae; Bursell and Taylor, 1980), but we are not aware of any studies that have directly measured the relationship between SDA and temperature in any insect.

Research into the SDA of vertebrates known to digest relatively large meals at relatively infrequent intervals has revealed that SDA is fuelled using a mixture of endogenous and exogenous nutrients (Starck et al., 2004; Waas et al., 2010), but those studies were not able to identify which classes of nutrients (e.g. carbohydrates, lipids and amino acids) provided this energy. Indeed, the postprandial oxidative kinetics of different classes of nutrients have been studied in other animals including humans (Hoekstra et al., 1996; Labayen et al., 2004a,b), rodents (McCue et al., 2014), reptiles (McCue et al., 2015a) and birds (Swennen et al., 2007; McCue et al., 2010, 2011). These studies show that both dietary carbohydrates and proteins are readily used for immediate energy during digestion. Dietary lipids are less extensively used as a metabolic fuel during the postprandial period.

The allocation of key nutrients to different tissues has been studied in crickets injected with isotopically labelled tracers (Zera, 2005; Zera and Zhao, 2006; Zhao and Zera, 2006), but we are only aware of one report of postprandial oxidation of dietary nutrients in insects – a phytophagous grasshopper (Nicholas et al., 2015) – and that line of investigation remains ongoing (J. D. Hatle, personal

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Table 1. Literature survey of studies reporting changes in metabolic rates in terrestrial arthropods during feeding and digestion

Study	Animal	Diet	Ration	Magnitude	Peak time	Duration (days)	SDA coefficient (%)
Aidley, 1976	Moth larva	Maize leaf		~2-fold			3
Bennett et al., 1999	Moth larva	Willow leaf		~4-fold		1	
Bradley et al., 2003	Assassin bug	Blood	~10× body	~2-fold	5–10 days	15	
Bursell and Taylor, 1980	Tsetse fly	Blood	50% of body				17
Fielden et al., 1999	Tick	Blood	~100× body	~15-fold	6 days	11	
Fielden et al., 2004	Flea	Blood	50% of body	~2-fold			
Gray and Bradley, 2003	Mosquito	Blood		~2-fold	1 day	2.5	
Jensen et al., 2010	Wolf spider	Flies	~9% of body	~4-fold	2 h		21
McEvoy, 1984	Moth larva	Ragwort leaf		~2-fold			3
Nespolo et al., 2011	Tarantula	Cricket	18% of body	~6-fold	1 h	1	
Sarfati et al., 2005	Flea	Blood	50% of body	~2-fold		1	
Scrivner et al., 1989	Cockroach	Starch		Decrease			
This study	Tsetse fly	Blood	55% of body	2-fold	1–2 days	1.5–4	5–17
Young and Block, 1980	Mite	Guano/lichen					
Zanotto et al., 1997	Locust	Casein/sucrose					

communication). Consequently, the timing and extent to which blood-feeding insects oxidize different dietary macronutrients remains poorly understood.

Ectotherms may alter their thermal preferences during digestion, and most studies report selection of warmer temperatures – a behaviour known as postprandial thermophily (Wall and Shine, 2008). Postprandial thermophily has been documented in a variety of tetrapods including amphibians (Lillywhite et al., 1973; Witters and Sievert, 2001), turtles (Gatten, 1974; Hammond et al., 1988), lizards (Regal, 1966; Witten and Heatwole, 1978), alligators (Lang, 1979) and snakes (Greenwald and Kanter, 1979; Slip and Shine, 1988; Dorcas et al., 1997; Sievert and Andreadis, 1999; Blouin-Demers and Weatherhead, 2001). While studies of insects report that higher temperatures maximize performance variables including locomotion, growth and digestion (e.g. Porter, 1988; Harrison and Fewell, 1995; Chown and Terblanche, 2007; Lachenicht et al., 2010), only a handful have examined the possibility that thermal preference is influenced by digestive status (but see Miller et al., 2010; Coggan et al., 2011; Clissold et al., 2013).

We therefore designed the present study to investigate the SDA and associated physiological and behavioural responses to digestion in the tsetse fly, an insect known to ingest blood meals constituting over 50% of its body mass (e.g. ranging from 35 to 110% in *Glossina brevipalpis* depending on various factors; reviewed in Leak, 1999), on average every 2 days (Leak, 1999). Previous research on tsetse digestion has shown that fed flies have elevated metabolic rates over unfed flies of a given age class (e.g. Rajagopal and Bursell, 1966; Taylor, 1977; Terblanche et al., 2004), likely reflecting the costs of transformation of the bloodmeal into lipid and proline food reserves and also uric acid, but typically such studies do not examine the full time course of the SDA response. Furthermore, none to our knowledge have examined SDA among different temperatures. The determination of ecological energetics across a range of temperatures may be compounded by changes in activity levels (Halsey et al., 2015) and we therefore consider changes in minimum, average and maximum metabolic rates.

Firstly, to examine the relationship between SDA and temperature, we measured rates of CO_2 production ($\dot{V}_{CO_2}$) at 25, 30 and 35°C in fed and unfed tsetse flies. Thereafter, to describe the extent to which these important nutrients are oxidized, tsetse flies were fed control blood meals or blood meals spiked with trace amounts of either ^{13}C-glucose, ^{13}C-leucine or ^{13}C-palmitic acid. We selected these tracers because glucose is the most common carbohydrate in vertebrate blood, leucine is one of the most common essential amino acids in the bodies of vertebrates and insects, and palmitic acid is one of the most common fatty acids in the bodies of vertebrates and insects (reviewed in McCue et al., 2015c; Welch et al., 2016). We then measured the $^{13}CO_2$ excreted in the breath during digestion to characterize the extent to which flies oxidize these nutrients at different temperatures. Lastly, because tsetse are highly mobile and, like other insects, capable of microhabitat selection (*sensu* Dillon et al., 2012; Sears and Angilletta, 2015), showing complex behavioural responses to diverse climate conditions, including highly specific microhabitat selection (e.g. warthog burrows) to avoid potentially lethal high temperatures, maximize reproductive output and offspring survival (Hargrove, 2004), we used a thermal gradient of ecologically realistic temperatures to determine whether fed flies adjust their body temperature preference differently from unfed flies.

MATERIALS AND METHODS

Animals and feeding

We chose to study *Glossina brevipalpis* for three reasons. First, it is one of the largest *Glossina* species and would generate the CO_2 levels needed for the breath testing faster than smaller species (Terblanche et al., 2004). Second, it is a vector of trypanosomiases that infect livestock but not humans (Leak, 1999; Esterhuizen et al., 2005). Third, its potential responses to climate change are of significant socio-economic importance in southern Africa (Rogers and Randolph, 1991; Rogers, 2000).

Pupae (~n=1000) were obtained from Onderstepoort Veterinary Institute mass-bred cultures, and transferred to a secure facility at Stellenbosch University, where they were maintained in an incubator at 25°C (12 h:12 h light:dark). The pupae were staggered in age so that individual adults would periodically eclose over the course of a 1-month period. Newly emerged adults (age 1–2 days) were allowed to feed for 1 h on defibrinated bovine blood warmed to 35°C through a silicone membrane as previously described (see Terblanche et al., 2004; Terblanche and Chown, 2007). Adults that did not feed were euthanized so that we could accurately track the ages of the flies in the experiments.

On days 3–4, the flies consumed a second blood meal. These second meals contained natural abundance levels of ^{13}C (control) or were spiked with one of three purified ^{13}C-tracer molecules: ^{13}C-1-glucose 1.0 g l^{-1}, ^{13}C-1-L-leucine 0.9 g l^{-1} or ^{13}C-1-palmitic acid 1.0 g l^{-1} (Cambridge Isotope Laboratories, Tewksbury, MA, USA). The tracers were added to 1 litre of freshly defibrinated blood and a magnetic stir bar mixed the blood for ~2 h. The blood was

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then transferred into 20 ml scintillation vials and stored at −80°C until needed for feeding

The masses of the first and second meals were measured on a subset of n=25 flies by weighing them before and immediately after feedings on a microbalance (accuracy±0.1 mg; AB104-S/Fact, Mettler-Toledo International, Greifensee, Switzerland). These values, along with the energetic content of the blood meals (see below), were used to model the SDA responses.

Respirometry and energetics

After the second feeding, subgroups of n=7 fed individuals were placed inside modified 5 ml syringe barrels (hereafter: metabolic chambers) to measure their metabolic rates [rate of CO_2 production ($\dot{V}_{CO_2}$); ml CO_2 h^{-1}] at one of three experimental temperatures (25, 30 or 35°C). The choice of temperatures reflects a realistic, ecologically relevant range of conditions that flies routinely experience under field conditions during natural diurnal temperature fluctuations (see e.g. Hargrove, 2004; Terblanche et al., 2009 for further climate information). At these temperatures, *G. brevipalpis* show continuous gas exchange with no substantive differences in gas exchange pattern type between male and female flies (Basson and Terblanche, 2011). Temperature treatments were randomized among trials and run in triplicate. Temperature was controlled using a programmable circulating and refrigeration bath filled with ethanol (CC410wl, Huber, Berching, Germany) and monitored using iButtons.

$\dot{V}_{CO_2}$ and rates of water loss were measured using a push mode 8-channel multiplexing respirometry system (previously described in e.g. Terblanche et al., 2004, 2009; Basson and Terblanche, 2010, 2011) programmed to cycle among each of the metabolic chambers every 2 h (15 min per channel). In short, CO_2-free dry air [scrubbed using columns containing soda lime, silica gel and Drierite (W. A. Hammond Drierite Company, Xenia, OH, USA)] was pushed at ∼200 ml min^{-1} through the metabolic chambers and the amount of CO_2 in the excurrent gas was measured using a calibrated Li-7000 infra-red gas analyser; data were recorded with standard LiCor software (LiCor, Lincoln, NE, USA). Airflow was maintained at 200 ml min^{-1} using a mass flow control valve (Sidetrak, Sierra International, USA) connected to a mass flow control box (Sable Systems International, Las Vegas, NV, USA) and measured upstream of the CO_2 analyser. Cuvettes were cycled using a multiplexer (RM8 Intelligent Multiplexer, V5, Sable Systems) connected to a desktop PC using a Universal Interface (UI2) and controlled using Expedata software (Sable Systems). Continuous flow was maintained in all non-selected chambers at ∼30 ml min^{-1}. $\dot{V}_{CO_2}$ was determined using standard equations (Lighton, 2008). One metabolic chamber was left empty and served as a baseline and to assess if any analyser drift occurred within the course of a trial, but this was typically non-existent.

Data were extracted using custom-written macros in Expedata (v1.8.5; Sable Systems). The central 13 min of each 15 min recording were used for analysis (i.e. the first and last minute were discarded to eliminate artefacts associated with slight pressure changes). The following variables were extracted for both $\dot{V}_{CO_2}$ (ml h^{-1}) and $\dot{V}_{H_2O}$ (rate of water loss; mg h^{-1}): (1) the average over the entire 13 min, (2) the lowest values for 15 consecutive seconds and (3) the most level section for 5 min. These broadly represent the average metabolic rates for those conditions, a minimum metabolic rate representing the absolute lowest stable and resting rates of energy consumption, and finally, the average rate for a shorter period to determine whether this altered conclusions derived from the other two metabolic rate parameters considered. Each individual's metabolic rate and SDA time course was plotted and these data truncated at the point just before death, reflective of a spike in water loss rate and a sharp decline in metabolic rate. Thus, a set of SDA data for each individual at each temperature was created that only included live flies. Mean values for all parameters pooled across all individuals were created for each 2 h respirometry block and used for determination of energetics.

The standard metabolic rate (SMR) was defined as the minimum metabolic rate of unfed flies that was sustained over a 10 min period, and routine metabolic rate (RMR) above SMR was attributed to activity (*sensu* IUPS, 2001). SDA in the postprandial flies was defined as any $\dot{V}_{CO_2}$ in excess of the RMR measured in flies that had not consumed a second meal. We continued respirometry measurements until >50% of the animals died as indicated by a cessation of CO_2 production (Stevens et al., 2010; Kafer et al., 2012; MacMillan et al., 2012).

The energetic components of SMR, RMR and SDA were calculated assuming 20.13 kJ l^{-1} O_2 (Jobling, 1981; Chappell and Ellis, 1987) and a respiratory exchange ratio of 0.80 (Schimpf et al., 2009; Jensen et al., 2010). Subsamples of the bovine blood (n=3×5 ml) were dried to a constant mass at 70°C to determine water content, and the energy content of the dried blood was then measured using a bomb calorimeter (CAL2K-ECO, South Africa) at 3000 kPa in oxygen. The latter measurement allowed us to calculate the SDA coefficient (a measure of the relative cost of SDA: $Energy_{ingested}/Energy_{SDA}\times100$) as the percentage of the ingested energy that was devoted to SDA.

Isotope tracers

Determinations of the oxidative kinetics of the three ^{13}C tracers were made at two temperatures (i.e. 25 and 35°C) using ∼150 flies. Recently fed flies (≤1 h after feeding) were individually placed inside 40 ml plastic syringes and capped to make them air-tight. At predetermined time points, specific to each of the experimental temperatures, samples of the gas inside each syringe was injected into evacuated Exetainer vials (Labco Limited, Lampeter, UK). To allow CO_2 to accumulate to >2% in the syringes, flies at 35°C were subjected to 4-h intervals and flies at 25°C were subjected to 8-h intervals. After sampling, the air inside each syringe was flushed with room air (i.e. 0.04% CO_2 – a concentration that is unlikely to alter the measured ^{13}C values; McCue and Welch, 2016), and recapped for the next measurement.

We measured the $\delta^{13}C$ values (in terms of the international standard VPDB) in each vial using a HeliFan Plus (Fischer, ANalysen Instrumente, Germany) non-dispersive infrared spectrometer interfaced with a FanAS autosampler as previously described (McCue et al., 2015b). Vials containing CO_2 with known $\delta^{13}C$ were run before and after each batch of samples. Because we were comparing ^{13}C enrichments from flies fed a tracer with control flies, we modelled $\delta^{13}C$ in terms of atom fraction excess (AFE) according to the following equation from Welch et al. (2016):

$$\mathrm{AFE} = \left[\frac{\mathrm{VPDB}\cdot\left(\frac{\delta^{13}C_{\mathrm{tracer}}}{1000}+1\right)}{1+\left[\mathrm{VPDB}\cdot\left(\frac{\delta^{13}C_{\mathrm{tracer}}}{1000}+1\right)\right]}\right] - \left[\frac{\mathrm{VPDB}\cdot\left(\frac{\delta^{13}C_{\mathrm{control}}}{1000}+1\right)}{1+\left[\mathrm{VPDB}\cdot\left(\frac{\delta^{13}C_{\mathrm{control}}}{1000}+1\right)\right]}\right], \quad (1)$$

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where VPDB is a constant (i.e. the absolute ratio of mole fraction ratio of the heavy to light isotopes, 0.0112372; IAEA, 2000). The instantaneous rates of tracer oxidation (T) were then calculated for each time point in terms of nmol h^{-1} according to the following equation:

$$T = \left(\frac{\dot{V}_{CO_2} \cdot \mathrm{AFE}}{m \cdot K}\right), \quad (2)$$

where m is the molar mass of each tracer and K is the volume of CO_2 produced per gram of mixed substrate oxidized using a value of 1.0 l g^{-1} (Gay et al., 1994; McCue et al., 2010; Welch et al., 2016). Cumulative tracer oxidation was estimated by integrating T across time, and the percent dose oxidized was estimated using the average blood meal size that we previously determined under identical rearing and feeding conditions.

Subsamples of the control and ^{13}C-enriched blood that was offered to flies were sent to the Stable Isotope Laboratory in the Department of Archaeology at the University of Cape Town, where the $\delta^{13}C$ was determined in duplicate using an isotope ratio mass spectrometer as previously described (Sealy et al., 2014). A subsample of five flies that were used in the ^{13}C-tracer experiments were also dried and homogenized, and $\delta^{13}C$ measurements were made on their carcasses.

Behavioural thermoregulation assays

The thermal preferences of 15 fed and 15 unfed flies were measured using a custom-built thermal gradient. In short, the apparatus consisted of a clear acrylic structure divided into 10 lanes (resulting in three replicates of 10 flies each), each with a dimension of 6 cm×4 cm×2 m (height×width×length). The floor of the thermal gradient consisted of a 1-cm-thick aluminium plate (2 m×40 cm, length×width). One end of the aluminium plate was immersed into an ethanol bath maintained at −5°C, giving flies a minimum temperature of ∼11°C, and a controllable heat strip located at the other end that maintained a temperature of 40°C, giving flies a maximum temperature of ∼38°C.

Ten thermocouples were embedded into 1 mm holes drilled 5 mm deep into the aluminium plate that were equally spaced every 20 cm along the length of the gradient. The temperatures of each thermocouple were logged every 10 min over the subsequent 4 days of each trial using a USB TC-08 thermocouple datalogger (Pico Technology, UK). The temperatures logged in each position of the gradient were used to derive a linear regression of gradient position to temperature for each day of each trial that described most of the variation (r^2>0.9 in all cases), and was assumed to be equal to fly body temperatures.

A digital webcam mounted above the thermal gradient imaged the flies in the gradient every 30 min and YAWCAM free software (www.yawcam.com) logged images to a desktop computer. The linear position of each fly was recorded for each image that assigned the location of each fly to the nearest corresponding thermocouple. We conducted three 4-day trials using n=10 flies in each trial. Five fed and five unfed flies were placed in alternating lanes near the centre of the thermal gradient.

Statistical analyses

We compared the meal sizes between first and second feedings using a paired t-test. We used unpaired t-tests to compare the $\delta^{13}C$ of the carcasses of flies fed different tracers as well as the $\delta^{13}C$ of the exhaled breath and the instantaneous rates of tracer oxidation (T) at different temperatures at selected time points within a given temperature. These tests were conducted using SigmaPlot 12 (Systat Software, San Jose, CA, USA) and a critical α of 0.05. We previously demonstrated that a closely related fly (i.e. *G. pallidipes*) showed mass-dependent, but not sex-dependent, differences in $\dot{V}_{CO_2}$ during their first two feedings when they are not yet reproductively mature (Terblanche et al., 2004). We used t-tests to compare the $\dot{V}_{CO_2}$ in a subset of male and female flies at identical temperatures to confirm that this was also the case for the *G. brevipalpis*. All of the results described below refer to data pooled from both sexes.

Fly body temperatures from the thermal gradient experiment were compared using repeated-measures ANOVAs performed using the PROC MIXED procedure in SAS (Enterprise Guide 5.1) with Kenward–Roger degrees of freedom to account for the fixed effect of feeding state (fed or unfed) and time, with repeated observations per fly. The repeated-measures model presented used the unstructured covariance matrix, selected from possible alternative covariance matrices on the basis of log likelihood scores, and this method is robust to missing data and unbalanced study design (some trials ran for slightly different amounts of time, resulting in different samples per individual among trials). We tested for the effects of feeding state, time and their interaction, with the expectation that although the feeding state effect may not be significant over the entire period through compensatory adjustments, the feeding state×time effect would be highly significant (e.g. with fed flies selecting warmer temperatures immediately post-feeding).

RESULTS

Bloodmeals

The blood was 81.2±0.1% water and had an energy content of 23.7±1.0 J mg^{-1} dry mass. This means that whole blood consumed by the flies had an energy content of 4.46 J mg^{-1} wet mass. Body mass did not differ between the flies before their first and second feedings. The first meals following eclosion were 45.5±5.1% of body mass and were significantly smaller (t-test, d.f.=23, P=0.002) than the second meals that were 55.2±9.1% of body mass. Thus, for modelling purposes we calculated that the flies consumed 78.4 ±11.4 mg blood meals containing an average of 349.6 J of energy.

Isotope analyses on subsamples of the palmitic acid blood and the control blood revealed that they had similar $\delta^{13}C$ values, suggesting that the palmitic acid tracer was not homogeneously integrated into the blood and may have remained in its native crystalline form that was too large to be ingested by the flies; however, we did not have sufficient replicates of the blood to support this statistically. Nevertheless, we conclude that the palmitic acid tracer was not an effective tracer for two additional reasons. The first is that the $\delta^{13}C$ of the breath of the palmitic acid flies did not differ from that of the control flies (t-test from the first breath samples following feeding: d.f.=30, P=0.084). The second is that the $\delta^{13}C$ of the carcasses of the palmitic acid flies (t-test: d.f.=8, P=0.286) did not differ from those of the control flies. Thus, we excluded the palmitic acid treatment group from further interpretations.

The $\delta^{13}C$ of the leucine blood (21.6±2.5‰) and glucose blood (38.3±2.1‰) were isotopically enriched above the control blood (−14.5±0.3‰; t-tests, P<0.001 in both cases). Similarly, the $\delta^{13}C$ in the carcasses of the leucine flies (−9.2±1.8‰) and glucose flies (−1.3±1.7‰) were higher than in the control flies (−13.7±0.3‰; t-tests, P<0.001 in both cases). These two outcomes, combined with the results of the breath testing, confirm that both the leucine and glucose tracers were integrated into the blood meals.

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Metabolic rates

The SMRs of the unfed flies were directly proportional to the ambient temperature and averaged 0.040, 0.046 and 0.083 ml CO_2 h^{-1} at 25, 30 and 35°C, respectively (Fig. 1A–C) during the first hour. At that time we calculated that the thermal sensitivity (Q_{10}) of SMR was 1.3 between 25 and 30°C, and 3.3 between 30 and 35°C. Across the entire temperature range, the Q_{10} averaged 2.1. At all temperatures, the SMR generally decreased over time in the unfed flies, reaching values nearly 50% of initial at the respective times of death (Fig. 1A–C).

The $\dot{V}_{CO_2}$ of the fed flies were substantially higher than the unfed flies across all temperatures (Fig. 1A–C), with peak values that were 1.5 to 2 times higher than the SMR. The SDA exhibited a circadian pattern, but the timing of the periodic peaks in SDA did not necessarily occur at the same times of day among the three experimental temperatures. The SDA coefficients, a measure of the relative cost of digesting the blood meals, were inversely related to temperature and averaged 16.7, 8.2 and 4.6% at 25, 30 and 35°C, respectively. Thus the flies at 35°C oxidized fewer dietary nutrients during digestion.

The cumulative energy spent on activity was approximately sixfold higher at 25°C (29.4 kJ) than at 35°C (4.5 kJ; Fig. 2A). However, the flies maintained at 25°C also lived longer (~96 h) than those at 30°C (~64 h) and 35°C (~32 h; Fig. 1D). When we consider that the flies at 25°C lived three times longer than those at

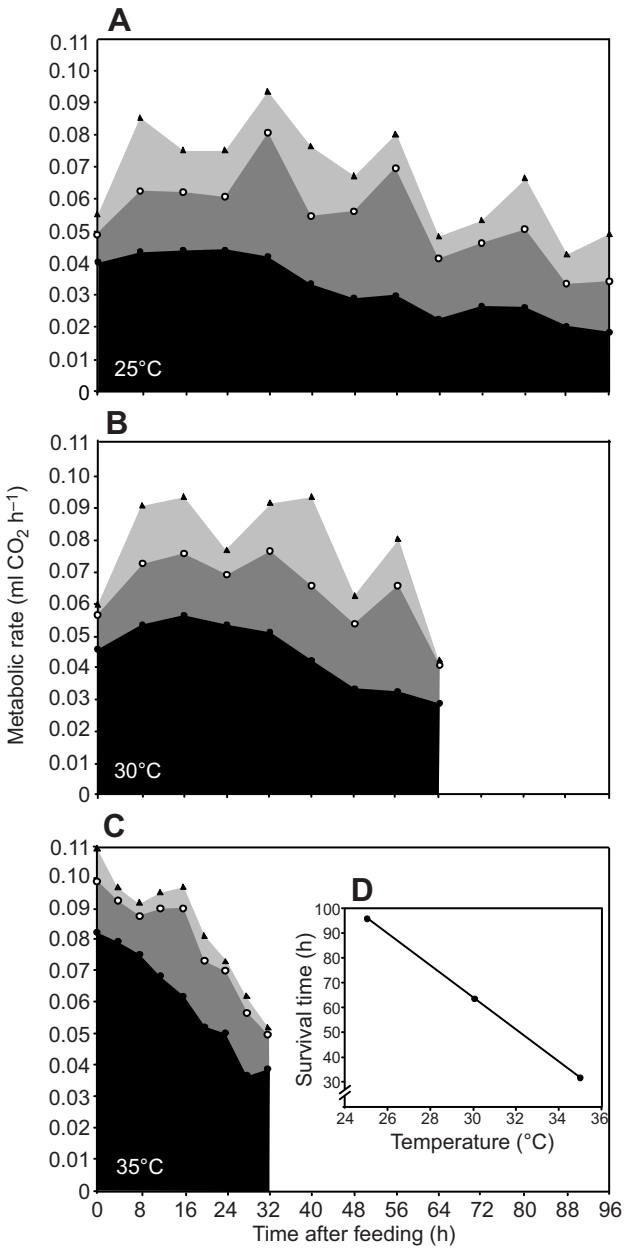

Fig. 1. Mean rates of carbon dioxide production of fed and unfed tsetse flies at different temperatures. (A–C) Closed circles represent standard metabolic rates (SMR; defined as the lowest average metabolic rate sustained over a 15 min period) of unfed flies. Open circles represent the SMR of fed flies. Closed triangles represent the mean metabolic rates of fed flies. All SMR were averaged over 8-h periods (for 25°C in A and 30°C in B) or 4-h periods (for 35°C in C). The difference between the lowest sustained metabolic rates of fed flies and the average metabolic rates of fed flies is considered to be energy associated with activity. (D) Survival time, defined as the time required for >50% of the individuals to die, during respirometry/isotope/pilot experiments. Time points were rounded to the nearest 8 h at 25 and 30°C, and to the nearest 4 h at 35°C. Error bars are excluded for clarity.

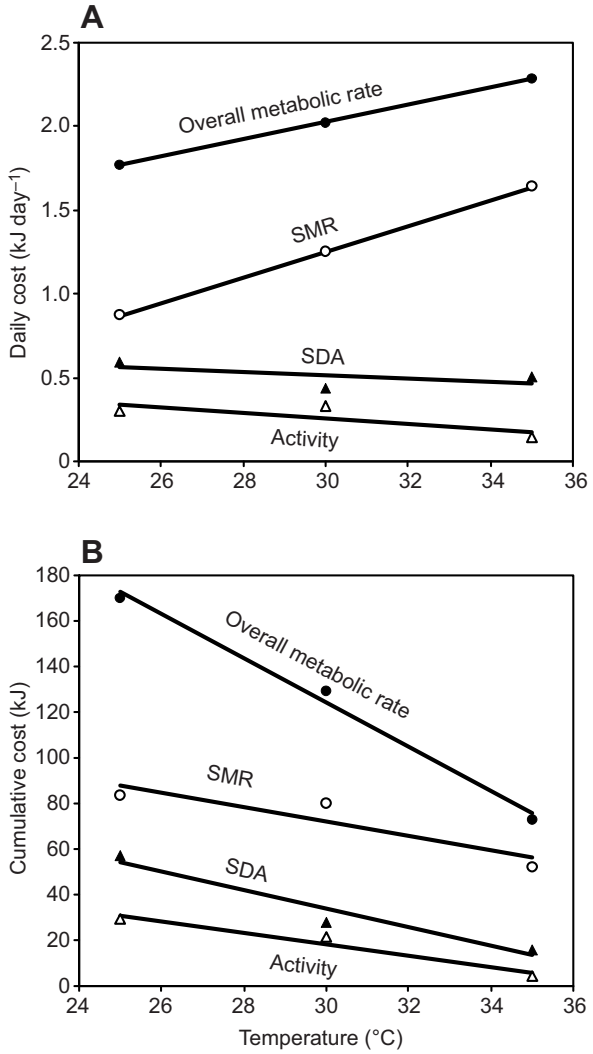

Fig. 2. The metabolic costs of maintenance, digestion and activity at different temperatures. (A) Cumulative costs were calculated by integrating the total CO_2 produced over time for each energy component (Fig. 1) assuming an energy conversion factor of 16.1 kJ l^{-1} of CO_2 (see Materials and methods for details). (B) Daily costs of each energy component calculated by dividing the cumulative costs by the mean survival time at each temperature. SDA, specific dynamic action.

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35°C, the mean daily cost of activity at 25°C (0.31 kJ day^{-1}) was still approximately twice that of flies at 35°C (0.14 kJ day^{-1}; Fig. 2B). Thus, the flies at 35°C reduced their activity levels.

Nutrient oxidation

The $\delta^{13}C$ of the breath of the glucose and leucine flies was sharply increased above background levels by the first sampling points at 25°C (4 h; Fig. 3) and 35°C (2 h; Fig. S1). Thereafter, the $\delta^{13}C$ in the breath decreased, resembling exponential decay functions with more rapid decreases occurring at 35°C. During the final day of the trial, the 25°C flies began to exhibit tracer-specific differences in $\delta^{13}C$ whereby breath of the leucine flies continued to become less isotopically enriched. In contrast, over the same time, the $\delta^{13}C$ increased in the glucose flies (Fig. 3 inset). We determined that the $\delta^{13}C$ measured in the glucose flies between 76 and 100 h (16.0‰) was significantly higher than that measured between 44 and 68 h (9.9‰; *t*-test, d.f.=6, P=0.007). Below we explain how this response is evidence that the flies were using dietary glucose for *de novo* lipogenesis.

The actual rates of tracer oxidation are a product of both the $\delta^{13}C$ and the $\dot{V}_{CO_2}$ (Eqn 2). The highest rates of oxidation for both tracers were observed at 2 h at 35°C and 4 h at 25°C (Fig. 4A,B). But, because of the sampling scheme we used (i.e. 4-h intervals at 35°C and 8-h intervals at 25°C), we could not determine whether the time at which these peaks occurred differed between the two temperatures. In any case, we did not observe significant differences in the peak rates of glucose (*t*-test, d.f.=29, P=0.734) or leucine (*t*-test, d.f.=38, P=0.612) oxidation at the two temperatures. Later, between 8 and 32 h, the effect of the temperature treatments became clearer, with consistently lower rates of tracer oxidation at 35°C. At the point at which the 35°C flies died, we estimate that they oxidized an average of 3.3 and 6.5% of the glucose and leucine, respectively (Fig. 4 insets). These values are substantially lower than cumulative oxidation of glucose (5.2%) and leucine (8.1%) in the 25°C flies over the same time period. The fact that the carcasses of the glucose flies were more enriched in ^{13}C than those of the leucine flies (see above) and that the peak rates of ^{13}C-leucine oxidation were consistently higher than those of ^{13}C-glucose (Fig. 3, Fig. S1) also supports the conclusion that the dietary leucine was oxidized more extensively.

Behavioural thermoregulation

A repeated-measures ANOVA on thermal preferences of both fed and unfed flies revealed a significant time effect ($F_{1,6273}$=1631.98, P<0.0001) and a significant feeding status×time interaction effect ($F_{1,6273}$=8.23, P<0.005). A second repeated-measures ANOVA comparing only fed flies showed a significant effect of time ($F_{1,3136}$=999.54, P<0.0001), and this trend had a negative correlation coefficient (mean±s.e., −0.032±0.001; P<0.0001). Thus, recently fed flies preferred warmer temperatures.

DISCUSSION

We measured the SDA of tsetse flies digesting blood meals at three ambient temperatures (25, 30 and 35°C) that are routinely

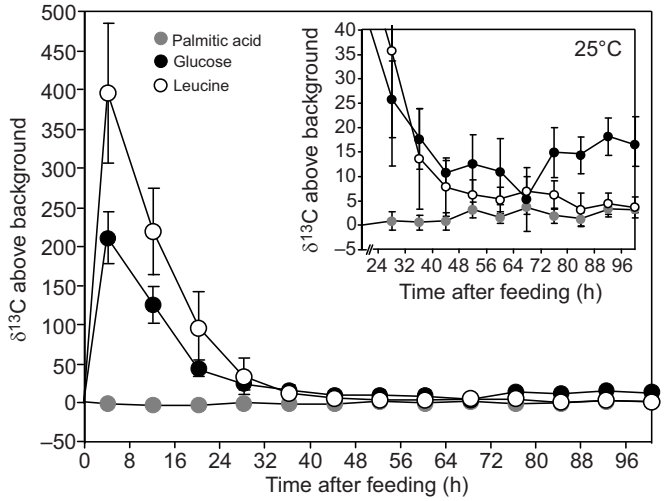

Fig. 3. The increase in ^{13}C content in the exhaled breath of tsetse flies digesting ^{13}C-labeled blood meals at 25°C. Increases in ^{13}C content for each treatment group were calculated by subtracting the ^{13}C content in the breath of flies fed blood meals containing no ^{13}C tracer and are expressed in terms of $\delta^{13}C_{VPDB}$. For further calculations, these values were converted to atom fraction excess (see Eqn 1). The inset illustrates the same data but from 24 h onwards to illustrate the treatment-specific changes in $\delta^{13}C$ during the lattermost stages of digestion. Error bars are ±s.d.

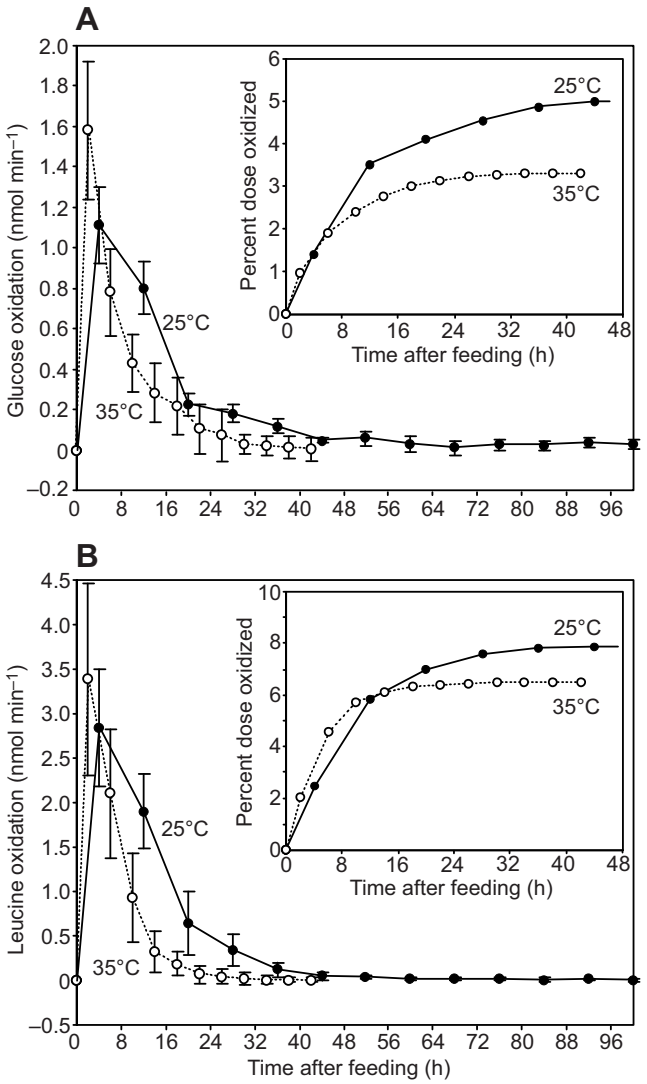

Fig. 4. Rates of ^{13}C-tracer oxidation in tsetse flies digesting blood meals at two temperatures. (A) Meals labelled with ^{13}C-glucose and digested at 25°C (closed circles, n=18) and 35°C (open circles, n=20). (B) Meals labelled with ^{13}C-leucine and digested at 25°C (closed circles, n=19) and 35°C (open circles, n=18). Error bars are ±s.d. Insets in A and B illustrate the mean proportion of each tracer oxidized during the first 42 h of digestion at 25°C (closed circles) and 35°C (open circles).

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experienced by flies during natural diurnal fluctuations under field conditions (see e.g. Hargrove, 2004; Terblanche et al., 2009) and found that the overall cost of digestion was lower at the higher temperatures. While most previous studies report that the cumulative energy of SDA is relatively independent of temperature (reviewed in McCue, 2006; Secor, 2009), some have reported that SDA either increased (e.g. fish; Guderley and Blier, 1988; Khan et al., 2015) or decreased (e.g. leeches; Kalarani and Davies, 1994) at warmer temperatures. In fact, all of the cumulative costs (i.e. maintenance, activity and digestion) were higher at 25°C (Fig. 2A). However, if we consider total survival time and calculate average daily costs, we see a different pattern. For instance, the SMR was higher at the higher temperatures (Fig. 1A–C), as generally expected from Q_{10} effects. However, the daily costs of both SDA and activity were either unaffected by temperature or lower at the higher temperatures (Fig. 2B).

The ^{13}C from leucine and glucose tracers consumed with the blood meals was found in both the breath and the tissues of the flies. The $\delta^{13}C$ in the breath peaked within the first few hours of consuming the leucine and glucose, suggesting that these flies rapidly use nutrients in their meals to fuel at least some of the SDA response. The rates of glucose and leucine oxidation decreased as digestion progressed, but this decrease occurred more rapidly at 35°C. We interpret this latter pattern as evidence that digestion is proceeding more rapidly at warmer temperatures. Future studies using smaller temperature intervals will be useful to characterize the relationship between temperature and digestion at a finer scale.

Overall, the flies oxidized more of the leucine tracer than the glucose tracer at either temperature. Leucine is not a glucogenic amino acid and cannot be used to support *de novo* lipogenesis; however, the flies could synthesize lipids from the dietary glucose. Lipid synthesis is thought to take place around 18 h after feeding (summarized in Leak, 1999), and evidence that these flies were synthesizing lipids from the glucose tracer can be seen in the $\delta^{13}C$ of the breath of the flies at 25°C. For example, while the $\delta^{13}C$ of the flies fed the leucine tracer continually decreased during the final day of the trial, the $\delta^{13}C$ of the flies fed the glucose tracer increased over the same period (Fig. 3 inset). We attribute this pattern to the oxidation of lipids that had been previously synthesized from the dietary ^{13}C-glucose tracer during the first days of digestion. Breath testing of quail (McCue et al., 2013) and sparrows (Khalilieh et al., 2012) fed diets supplemented with ^{13}C-glucose have also shown they are quite effective at converting dietary carbohydrates into lipid stores that are later mobilized during starvation.

Metabolic measurements confirmed that the SDA response was actually smaller at 35°C and ^{13}C breath testing confirmed that digestion occurred more rapidly at 35°C. However, speeding the rate of digestion and minimizing the costs of SDA may not be the only factors shaping the behaviour of these animals, as their starvation tolerance was indirectly proportional to the temperature. Reductions in the preferred temperatures have been reported in fasting insects and ectothermic vertebrates (reviewed in Bicego et al., 2007; Angilletta, 2009). Here we observed that as digestion progresses, the flies selected cooler temperatures. We suggest that these flies may select such temperatures to maximize survival time between meals, but future tests designed to test this possibility would be informative. From field observations and mark–recapture experiments it is clear that young adult flies die off at extremely high rates and are likely taking substantial risks to secure successive blood meals (reviewed in Hargrove, 2004). Furthermore, because fasted or teneral flies are more susceptible to trypanosome infection, understanding the relationships between age, starvation level, preferred temperatures and microsite selection would make an important avenue for mechanistic understanding of the epidemiology. This suggests that the approach we employed may be of further relevance to the field situation, but it could be useful to examine more fully the range of fed/starved conditions at different fly ages, and considering a wider range of potential trade-offs for populations in the wild.

We observed a progressive decrease in the SMR over time at all temperatures to a point at which SMR was reduced by nearly 50%. Although we had not expected to see a change of this magnitude, reductions in SMR of a similar size have been reported in arthropods (Young and Block, 1980; but see Sinclair et al., 2011) and ectothermic vertebrates (Foster and Moon, 1991; Christian et al., 1996; Fuery et al., 1998; McCue, 2007) during prolonged fasting. There are several possible physiological mechanisms to adaptively reduce SMR (reviewed in Storey and Storey, 1990; Hand and Hardewig, 1996) and starvation-induced reductions in the microbial symbionts (*sensu* Carrero-Colon et al., 2006; Arrese and Soulages, 2010; Kohl et al., 2014) could also underlie changes in metabolic rates. Further research will be needed to determine which of these responses may be occurring in these flies. Nevertheless, the fact that these flies are capable of such flexibility in maintenance costs at a given temperature underscores some of the challenges in reporting SMR (reviewed in IUPS, 2001) and its thermal sensitivity (reviewed in Halsey et al., 2015).

We concede that the flies in the metabolic chambers were exposed to dry air, and although that is the typical way that metabolic rates are measured in insects, multiple days of exposure to dry air could have hastened the death of the flies in this study. Indeed, experiments on desiccation resistance in the tsetse fly have shown that flies will lose water at a faster rate in flowing air conditions compared with static air, likely owing to boundary layer effects (Jurenka et al., 2007). Although we could not statistically compare the survival curves of the fed flies in the respirometry trials with those in the ^{13}C breath testing trials, (where no air was flowing over the flies because they were not under continuous observation; *sensu* McCue and De Los Santos, 2013), we did note that the life spans were not perceptibly different. Our comparisons between temperatures are unlikely to be affected by the air flow as all experiments had the same flow rate. While it is possible that handling stress increased the metabolic rate initially (e.g. first 2 h), once again, such effects are likely inconsequential because flies from all experiments were handled equally.

Collectively, these results have profound implications for understanding thermal adaptation and the spatial and temporal energetic ecology in these and other insect disease vectors. Typically, individuals within populations are considered to have a single thermal optimum, which is thought to reflect a composite of multiple underlying traits and processes (reviewed in Angilletta, 2009), and indeed this is also the case in Glossinidae (reviewed in Hargrove, 2004; also see Bursell and Taylor, 1980; Rogers and Randolph, 1986). While animals are often considered to choose suboptimal temperatures through biotic factors such as competitive niche exclusion (Cerda et al., 1998; Mitchell and Angilletta, 2009) or through proximate processes maximizing fitness (Frazier et al., 2006; Martin and Huey, 2008), or for generally spreading risks of adverse weather across individuals or generations in a population (e.g. the maintenance of bet-hedging strategies; Beaumont et al., 2009; de Jong et al., 2011; Starrfelt and Kokko, 2012), it may be useful to possess dynamic thermal traits from an evolutionary perspective. What is increasingly clear is that different cohorts and groups of individuals may target different temperatures for energetic

digestion efficiency and then subsequently for periods between meals to better withstand bouts of limited resource availability (e.g. Coggan et al., 2011).

The results of our physiological and behavioural assays suggest there are two distinct thermal optima – one warm optimal temperature for a fed fly and another cooler optimal temperature for a fasted or post-absorptive tsetse fly. Although we did not examine reproductively mature individuals, future studies would be useful to examine how reproduction influences energy budgets and thermal relationships in this species. These findings are significant for understanding disease vector energetics under field conditions (e.g. Bursell and Taylor, 1980) and for mechanistic modelling of climate change impacts on these and other ectotherm population dynamics (e.g. Kearney et al., 2009).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
M.D.M. and J.S.T. conceived of the experiment. M.D.M., L.B., S.C.-T., E.K. and J.S.T. conducted the experiment. M.D.M., L.B., E.K. and J.S.T. executed data analyses. M.D.M., L.B., S.C.-T. and J.S.T. prepared the manuscript.

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References
Aidley, D. J. (1976). Increase in respiratory rate during feeding in larvae of the armyworm, *Spodoptera exempta*. *Physiol. Entomol.* **1**, 73-75.
Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. New York: Oxford University Press.
Arrese, E. L. and Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Ann. Rev. Entomol.* **55**, 207-225.
Basson, C. H. and Terblanche, J. S. (2010). Metabolic responses of *Glossina pallidipes* (Diptera: Glossinidae) puparia exposed to oxygen and temperature variation: implications for population dynamics and subterranean life. *J. Insect Physiol.* **56**, 1789-1797.
Basson, C. H. and Terblanche, J. S. (2011). Respiratory pattern transitions in three species of *Glossina* (Diptera, Glossinidae). *J. Insect Physiol.* **57**, 433.
Beaumont, H. J. E., Gallie, J., Kost, C., Ferguson, G. C. and Rainey, P. B. (2009). Experimental evolution of bet hedging. *Nature* **462**, 90-93.
Bennett, V. A., Kukal, O. and Lee, R. E. (1999). Metabolic opportunists: feeding and temperature influence the rate and pattern of respiration in the high arctic wollybear caterpillar *Gynaephora groenlandica* (Lymantriidae). *J. Exp. Biol.* **202**, 47-53.
Bicego, K. C., Barros, R. C. H. and Branco, L. G. S. (2007). Physiology of temperature regulation: comparative aspects. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **147**, 616-639.
Blouin-Demers, G. and Weatherhead, P. J. (2001). An experimental test of the link between foraging, habitat selection and thermoregulation in black rat snakes *Elaphe obsoleta obsoleta*. *J. Anim. Ecol.* **70**, 1006-1013.
Bradley, T. J., Brethorst, L., Robinson, S. and Hetz, S. (2003). Changes in the rate of CO_2 release following feeding in the insect *Rhodnius prolixus*. *Physiol. Biochem. Zool.* **76**, 302-309.
Bursell, E. and Taylor, P. (1980). An energy budget for *Glossina* (Diptera: Glossinidae). *Bull. Entomol. Res.* **70**, 187-196.
Carrero-Colon, M., Nakatsu, C. H. and Konopka, A. (2006). Effect of nutrient periodicity on microbial community dynamics. *Appl. Environ. Microbiol.* **72**, 3175-3183.
Cerda, X., Retana, J. and Cros, S. (1998). Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. *Funct. Ecol.* **12**, 45-55.
Chappell, M. A. and Ellis, T. M. (1987). Resting metabolic rates in boid snakes: allometric relationships and temperature effects. *J. Comp. Physiol.* **157**, 227-235.
Chown, S. L. and Terblanche, J. S. (2007). Physiological diversity in insects: ecological and evolutionary contexts. In *Advanced in Insect Physiology*, Vol. 33 (ed. S. J. Simpson), pp. 50-152. San Diego, CA: Academic Press.
Christian, K., Green, B., Bedford, G. and Newgrain, K. (1996). Seasonal metabolism of a small, arboreal monitor lizard, *Varanus scalaris*, in tropical Australia. *J. Zool.* **240**, 383-396.
Clissold, F. J., Coggan, N. and Simpson, S. J. (2013). Insect herbivores can choose microclimates to achieve nutritional homeostasis. *J. Exp. Biol.* **216**, 2089-2096.
Coggan, N., Clissold, F. J. and Simpson, S. J. (2011). Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. *Proc. R. Soc. B Biol. Sci.* **278**, 2745-2752.
Crocker-Buta, S. P. and Secor, S. M. (2014). Determinants and repeatability of the specific dynamic response of the corn snake, *Pantherophis guttatus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **169**, 60-69.
de Jong, I. G., Haccou, P. and Kuipers, O. P. (2011). Bet hedging or not? A guide to proper classification of microbial survival strategies. *Bioessays* **33**, 215-223.
Dillon, M. E., Liu, R., Wang, G. and Huey, R. B. (2012). Disentangling thermal preference and the thermal dependence of movement in ectotherms. *J. Therm. Biol.* **37**, 631-639.
Dorcas, M. E., Peterson, C. R. and Flint, M. E. T. (1997). The thermal biology of digestion in rubber boas (*Charina bottae*): physiology, behavior, and environmental constraints. *Physiol. Zool.* **70**, 292-300.
Esterhuizen, J., Kappmeier-Green, K., Marcotty, T. and Van den Bossche, P. (2005). Abundance and distribution of the tsetse flies, *Glossina austeni* and *G. brevipalpis*, in different habitats in South Africa. *Med. Vet. Entomol.* **19**, 367-71.
Fielden, L. J., Jones, R. M., Goldberg, M. and Rechav, Y. (1999). Feeding and respiratory gas exchange in the American dog tick, *Dermacentor variabilis*. *J. Insect Physiol.* **45**, 297-304.
Fielden, L. J., Krasnov, B. R., Khokhlova, I. S. and Arakelyan, M. S. (2004). Respiratory gas exchange in the desert flea *Xenopsylla ramesis* (Siphonaptera: Pulicidae): response to temperature and blood-feeding. *Comp. Biochem. Physiol. A Comp. Integr. Physiol.* **137**, 557-565.
Foster, G. D. and Moon, T. W. (1991). Hypometabolism with fasting in the yellow perch (*Perca flavescens*): a study of enzymes, hepatocyte metabolism, and tissue size. *Physiol. Zool.* **64**, 259-275.
Frazier, M. R., Huey, R. B. and Berrigan, D. (2006). Thermodynamics constrains the evolution of insect population growth rates: "warmer is better". *Am. Nat.* **168**, 512-520.
Fuery, C. J., Withers, P. C., Hobbs, A. A. and Guppy, M. (1998). The role of protein synthesis during metabolic depression in the Australian desert frog *Neobatrachus centralis*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **119**, 469-476.
Gatten, R. E. (1974). Effect of nutritional status on the preferred body temperature of the turtles *Pseudemys scripta* and *Terrapene ornata*. *Copeia* **1974**, 912-917.
Gay, L. J., Schneiter, P., Schutz, Y., Di Vetta, V., Jequier, E. and Tappy, L. (1994). A non-invasive assessment of hepatic glycogen kinetics and post-absorptive gluconeogenesis in man. *Diabetologia* **37**, 517-523.
Gray, E. M. and Bradley, T. J. (2003). Metabolic rate in female *Culex tarsalis* (Diptera: Culicidae): age, size, activity, and feeding effects. *J. Med. Entomol.* **40**, 903-911.
Greenwald, O. E. and Kanter, M. E. (1979). The effects of temperature and behavioral thermoregulation on digestive efficiency and rate in corn snakes (*Elaphe guttata guttata*). *Physiol. Zool.* **52**, 398-408.
Guderley, H. and Blier, P. (1988). Thermal acclimation in fish: conservative and labile properties of swimming muscle. *Can. J. Zool.* **66**, 1105-1115.
Halsey, L. G., Matthews, P. G. D., Rezende, E. L., Chauvaud, L. and Robson, A. A. (2015). The interactions between temperature and activity levels in driving metabolic rate: theory, with empirical validation from contrasting ectotherms. *Oecologia* **177**, 1117-1129.
Hammond, K. A., Spotila, J. R. and Standora, E. A. (1988). Basking behavior of the turtle *Pseudemys scripta*: effects of digestive state, acclimation temperature, sex, and season. *Physiol. Zool.* **61**, 69-77.
Hand, S. C. and Hardewig, I. (1996). Downregulation of cellular metabolism during environmental stress: mechanisms and implications. *Ann. Rev. Physiol.* **58**, 539-563.
Hargrove, J. W. (2004). Tsetse population dynamics. In *The Trypanosomiases* (ed. I. Maudlin, P. Holms and M. A. Miles), pp. 113-138. Wallingford: CABI Publishing.
Harrison, J. F. and Fewell, J. H. (1995). Thermal effects on feeding behavior and net energy intake in a grasshopper experiencing large diurnal fluctuations in body temperature. *Physiol. Zool.* **68**, 453-473.

Hoekstra, J. H., Van den Aker, J. H. L., Kneepkens, C. M. F., Stellaard, F., Geypens, B. and Ghoos, Y. F. (1996). Evaluation of $^{13}CO_2$ breath tests for the detection of fructose malabsorption. *J. Lab. Clin. Med.* **127**, 303-309.
IAEA (2000). Abundance and fractionation of stable isotopes. In *Environmental Isotopes in the Hydrological Cycle*, Vol. 1 (ed. W. H. Mook), pp. 31-48. Vienna: International Atomic Energy Agency.
IUPS (2001). Glossary of terms for thermal physiology. *Jpn. J. Physiol.* **51**, 245-280.
Jensen, K., Mayntz, D., Wang, T., Simpson, S. J. and Overgaard, J. (2010). Metabolic consequences of feeding and fasting on nutritionally different diets in the wolf spider *Pardosa prativaga*. *J. Insect Physiol.* **56**, 1095-1100.
Jobling, M. (1981). The influences of feeding on the metabolic rate of fishes: a short review. *J. Fish Biol.* **18**, 385-400.
Jobling, M. (1983). Towards an explanation of specific dynamic action (SDA). *J. Fish Biol.* **23**, 549-555.
Jurenka, R., Terblanche, J. S., Klok, C. J., Chown, S. L. and Krafsur, E. S. (2007). Cuticular lipid mass and desiccation rates in Glossina pallidipes: interpopulation variation. *Physiol. Entomol.* **32**, 287-293.
Käfer, H., Kovac, H. and Stabentheiner, A. (2012). Resting metabolism and critical thermal maxima of vespine wasps (*Vespula* sp.). *J. Insect Physiol.* **58**, 679-689.
Kalarani, V. and Davies, R. W. (1994). The bioenergetic costs of specific dynamic action and ammonia excretion in a freshwater predatory leech *Nephelopsis obscura*. *Comp. Biochem. Physiol. A Physiol.* **108**, 523-531.
Kearney, M., Porter, W. P., Williams, C., Ritchie, S. and Hoffmann, A. A. (2009). Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito *Aedes aegypti* in Australia. *Funct. Ecol.* **23**, 528-538.
Khalilieh, A., McCue, M. D. and Pinshow, B. (2012). Physiological responses to food deprivation in the house sparrow, a species not adapted to prolonged fasting. *Am. J. Physiol.* **303**, R551-R561.
Khan, J. R., Pether, S., Bruce, M., Walker, S. P. and Herbert, N. A. (2015). The effect of temperature and ration size on specific dynamic action and production performance in juvenile hapuku (*Polyprion oxygeneios*). *Aquaculture* **437**, 67-74.
Kohl, K. D., Amaya, J. A., Passement, C. A., Dearing, M. D. and McCue, M. D. (2014). Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. *FEMS Microbiol. Ecol.* **90**, 883-894.
Labayen, I., Díez, N., Parra, D., González, A. and Martínez, J. A. (2004a). Basal and postprandial substrate oxidation rates in obese women receiving two test meals with different protein content. *Clin. Nutr.* **23**, 571-578.
Labayen, I., Diez, N., Parra, M. D., Gónzalez, A. and Martínez, J. A. (2004b). Time-course changes in macronutrient metabolism induced by a nutritionally balanced low-calorie diet in obese women. *Int. J. Food Sci. Nutr.* **55**, 27-35.
Lachenicht, M. W., Clusella-Trullas, S., Boardman, L., Le Roux, C. and Terblanche, J. S. (2010). Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). *J. Insect Physiol.* **56**, 822-830.
Lang, J. W. (1979). Thermophilic response of the American alligator and the American crocodile to feeding. *Copeia* **1979**, 48-59.
Leak, S. G. A. (1999). *Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomiasis*. Wallingford, UK: CABI.
Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists*. New York: Oxford University Press.
Lillywhite, H. B., Licht, P. and Chelgren, P. (1973). The role of behavioral thermoregulation in the growth energetics of the toad, *Bufo boreas*. *Ecology* **54**, 375-383.
Machida, Y. (1981). Study of specific dynamic action on some freshwater fishes. *Rep. USA Mar. Biol. Inst. Kochi Univ.* **3**, 1-50.
MacMillan, H. A., Williams, C. M., Staples, J. F. and Sinclair, B. J. (2012). Metabolism and energy supply below the critical thermal minimum of a chill-susceptible insect. *J. Exp. Biol.* **215**, 1366-1372.
Martin, T. L. and Huey, R. B. (2008). Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *Am. Nat.* **171**, E102-E118.
McCue, M. D. (2006). Specific dynamic action: a century of investigation. *Comp. Biochem. Physiol. A Mol. Inegr. Physiol.* **144A**, 381-394.
McCue, M. D. (2007). Snakes survive starvation by employing supply- side and demand-side economic strategies. *Zoology* **110**, 318-327.
McCue, M. D. and De Los Santos, R. (2013). Upper thermal limits of insects are not the result of insufficient oxygen delivery. *Physiol. Biochem. Zool.* **86**, 257-265.
McCue, M. D. and Welch, K. C. Jr. (2016). ^{13}C-Breath testing in animals: theory, applications, and future directions. *J. Comp. Physiol. B* **186**, 265-285.
McCue, M. D., Sivan, O., McWilliams, S. R. and Pinshow, B. (2010). Tracking the oxidative kinetics of carbohydrates, amino acids and fatty acids in the house sparrow using exhaled $^{13}CO_2$. *J. Exp. Biol.* **213**, 782-789.
McCue, M. D., McWilliams, S. R. and Pinshow, B. (2011). Ontogeny and nutritional status influence oxidative kinetics of nutrients and whole-animal bioenergetics in zebra finches, *Taeniopygia guttata*: new applications for 13 C breath testing. *Physiol. Biochem. Zool.* **84**, 32-42.
McCue, M. D., Amaya, J. A., Yang, A. S., Erhardt, E. B., Wolf, B. O. and Hanson, D. T. (2013). Targeted ^{13}C enrichment of lipid and protein pools in the body reveals circadian changes in oxidative fuel mixture during prolonged fasting: a case study using Japanese quail. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **166**, 546-554.
McCue, M. D., Voigt, C. C., Jefimow, M. and Wojciechowski, M. (2014). Thermal acclimation and nutritional history affect the oxidation of different classes of exogenous nutrients in Siberian hamsters, *Phodopus sungorus*. *J. Exp. Zool. A Ecol. Genet. Physiol.* **321**, 503-514.
McCue, M. D., Passement, C. A. and Guzman, R. M. (2015a). Digesting pythons quickly oxidize the proteins in their meals and save the lipids for later. *J. Exp. Biol.* **218**, 2089-2096.
McCue, M. D., Passement, C. A. and Rodriguez, M. (2015b). The magnitude of the naturally occurring isotopic enrichment of ^{13}C in exhaled CO_2 is directly proportional to exercise intensity in humans. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **179**, 164-171.
McCue, M. D., Guzman, R. M., Passement, C. A. and Davidowitz, G. (2015c). How and when do insects rely on endogenous protein and lipid resources during lethal bouts of starvation? A new application for ^{13}C-breath testing. *PLoS ONE* **10**, e0140053.
McEvoy, P. B. (1984). Increase in respiratory rate during feeding in larvae of the cinnabar moth *Tyria jacobaeae*. *Physiol. Entomol.* **9**, 191-195.
McGaw, I. J. and Whiteley, N. M. (2012). Effects of acclimation and acute temperature change on specific dynamic action and gastric processing in the green shore crab. *J. Therm. Biol.* **37**, 570-578.
Miller, G. A., Clissold, F. J., Mayntz, D. and Simpson, S. J. (2010). Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. *Proc. R. Soc. B Biol. Sci.* **276**, 3581-3589.
Mitchell, W. A. and Angilletta, M. J., Jr. (2009). Thermal games: frequency-dependent models of thermal adaptation. *Funct. Ecol.* **23**, 510-520.
Nespolo, R. F., Correa, L., Pérez-Apablaza, C. X., Cortés, P. and Bartheld, J. L. (2011). Energy metabolism and the postprandial response of the Chilean tarantulas, *Euathlus truculentus* (Araneae: Theraphosidae). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **159**, 379-382.
Nicholas, J., Awan, A., McCue, M. D., Williams, C. M., Hahn, D. A. and Hatle, J. D. (2015). Life-extending ovariectomy and dietary restriction each alter leucine metabolism in grasshoppers, but in different ways. *Integr. Comp. Biol.* **55**, E308.
Porter, S. D. (1988). Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. *J. Insect Physiol.* **34**, 1127-1133.
Powell, M. K., Mansfield-Jones, J. and Gatten, R. E. (1999). Specific dynamic effect in the horned frog *Ceratophrys cranwelli*. *Copeia* **1999**, 710-717.
Rajagopal, P. K. and Bursell, E. (1966). The respiratory metabolism of resting tsetse flies. *J. Insect Physiol.* **12**, 287-297.
Regal, P. J. (1966). Thermophilic response following feeding in certain reptiles. *Copeia* **1966**, 588-590.
Robertson, R. F., Meagor, J. and Taylor, E. W. (2002). Specific dynamic action in the shore crab, *Carcinus maenas* (L.), in relation to acclimation temperature and to the onset of the emersion response. *Physiol. Biochem. Zool.* **75**, 350-359.
Rogers, D. J. (2000). Satellites, space, time and the African trypanosomiases. *Adv. Parasitol.* **47**, 129-171.
Rogers, D. J. and Randolph, S. E. (1986). Distribution and abundance of tsetse flies (*Glossina* spp.). *J. Anim. Ecol.* **55**, 1007-1025.
Rogers, D. J. and Randolph, S. E. (1991). Mortality rates and population density of tsetse flies correlated with satellite imagery. *Nature* **351**, 739-741.
Sarfati, M., Krasnov, B. R., Ghazaryan, L., Khokhlova, I. S., Fielden, L. J. and Degan, A. A. (2005). Energy costs of blood digestion in a host-specific haematophagous parasite. *J. Exp. Biol.* **208**, 2489-2496.
Schimpf, N. G., Matthews, P. G. D., Wilson, R. S. and White, C. R. (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *J. Exp. Biol.* **212**, 2773-2780.
Scrivener, A. M., Slaytor, M. and Rose, H. A. (1989). Symbiont-independent digestion of cellulose and starch in *Panesthia cribrata* Saussure, an Australian wood-eating cockroach. *J. Insect Physiol.* **35**, 935-941.
Sealy, J., Johnson, M., Richards, M. and Nehlich, O. (2014). Comparison of two methods of extracting bone collagen for stable carbon and nitrogen isotope analysis: comparing whole bone demineralization with gelatinization and ultrafiltration. *J. Archaeol. Sci.* **47**, 64-69.
Sears, M. W. and Angilletta, M. J. (2015). Costs and benefits of thermoregulation revisited: both the heterogeneity and spatial structure of temperature drive energetic costs. *Am. Nat.* **185**, E94-E102.
Secor, S. (2009). Specific dynamic action: a review of the postprandial metabolic response. *J. Comp. Physiol. B* **179**, 1-56.
Secor, S. M. and Boehm, M. (2006). Specific dynamic action of ambystomatid salamanders and the effects of meal size, meal type, and body temperature. *Physiol. Biochem. Zool.* **79**, 720-735.
Secor, S. M. and Faulkner, A. C. (2002). Effects of meal size, meal type, body temperature, and body size on the specific dynamic action of the marine toad, *Bufo marinus*. *Physiol. Biochem. Zool.* **75**, 557-571.
Sievert, L. M. and Andreadis, P. (1999). Specific dynamic action and postprandial thermophily in juvenile northern water snakes, *Nerodia sipedon*. *J. Therm. Biol.* **24**, 51-55.

Sinclair, B. J., Bretman, A., Tregenza, T., Tomkins, J. L. and Hosken, D. J. (2011). Metabolic rate does not decrease with starvation in *Gryllus bimaculatus* when changing fuel use is taken into account. *Physiol. Entomol.* **36**, 84-89.
Slip, D. J. and Shine, R. (1988). Thermophilic response to feeding of the diamond python, *Morelia s. spilota* (Serpentes: Boidae). *Comp. Biochem. Physiol. A Physiol.* **89**, 645-650.
Starck, J. M., Moser, P., Werner, R. A. and Linke, P. (2004). Pythons metabolize prey to fuel the response to feeding. *Proc. R. Soc. B Biol. Sci.* **271**, 903-908.
Starrfelt, J. and Kokko, H. (2012). Bet-hedging-a triple trade-off between means, variances and correlations. *Biol. Rev.* **87**, 742-755.
Stevens, M. M., Jackson, S., Bester, S. A., Terblanche, J. S. and Chown, S. L. (2010). Oxygen limitation and thermal tolerance in two terrestrial arthropod species. *J. Exp. Biol.* **213**, 2209-2218.
Storey, K. B. and Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Q. Rev. Biol.* **65**, 145-174.
Swennen, Q., Verhulst, P.-J., Collin, A., Bordas, A., Verbeke, K., Vansant, G., Decuypere, E. and Buyse, J. (2007). Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: comparison of adult cockerels of lines selected for high or low residual feed intake. *Poult. Sci.* **86**, 1960-1971.
Taylor, P. (1977). The respiratory metabolism of tsetse flies, *Glossina* spp., in relation to temperature, blood-meal size and pregnancy cycle. *Physiol. Entomol.* **2**, 317-322.
Terblanche, J. S. and Chown, S. L. (2007). The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. *Physiol. Entomol.* **32**, 175-180.
Terblanche, J. S., Klok, C. J. and Chown, S. L. (2004). Metabolic rate variation in *Glossina pallidipes* (Diptera: Glossinidae): gender, ageing and repeatability. *J. Insect Physiol.* **50**, 419-428.
Terblanche, J. S., Clusella-Trullas, S., Deere, J. A., Van Vuuren, B. J. and Chown, S. L. (2009). Directional evolution of the slope of the metabolic rate–temperature relationship is correlated with climate. *Physiol. Biochem. Zool.* **82**, 495-503.
Toledo, L. F., Abe, A. S. and Andrade, D. V. (2003). Temperature and meal size effects on the postprandial metabolism and energetics in a boid snake. *Physiol. Biochem. Zool.* **76**, 240-246.
Waas, S., Werner, R. A. and Starck, J. M. (2010). Fuel switching and energy partitioning during the postprandial metabolic response in the ball python (*Python regius*). *J. Exp. Biol.* **213**, 1266-1271.
Wall, M. and Shine, R. (2008). Post-feeding thermophily in lizards (*Lialis burtonis* Gray, Pygopodidae): laboratory studies can provide misleading results. *J. Therm. Biol.* **33**, 274-279.
Wang, T., Zaar, M., Arvedsen, S., Vedel-Smith, C. and Overgaard, J. (2003). Effects of temperature on the metabolic response to feeding in *Python molurus*. *Comp. Biochem. Physiol.* **133A**, 519-527.
Wang, T., Hung, C. C. Y. and Randall, D. J. (2006). The comparative physiology of food deprivation: from feast to famine. *Ann. Rev. Physiol.* **68**, 223-251.
Welch, K. C., Jr., Perronet, F., Voigt, C. C., Hatch, K. and McCue, M. D. (2016). Combining respirometry with stable isotopes to investigate fuel use in animals. *Ann. N. Y. Acad. Sci.* **1365**, 15-32.
Whiteley, N. M., Robertson, R. F., Meagor, J., El-Haj, A. J. and Taylor, E. W. (2001). Protein synthesis and specific dynamic action in crustaceans: effects of temperature. *Comp. Biochem. Physiol.* **128A**, 595-606.
Witten, G. J. and Heatwole, H. (1978). Preferred temperature of the agamid lizard *Amphibolurus nobbi nobbi*. *Copeia* **1978**, 362-364.
Witters, L. R. and Sievert, L. M. (2001). Feeding causes thermophily in the Woodhouse's toad (*Bufo woodhousii*). *J. Therm. Biol.* **26**, 205-208.
Young, S. R. and Block, W. (1980). Some factors affecting metabolic rate in an Antarctic mite. *Oikos* **34**, 178-185.
Zaidan, F., III and Beaupre, S. J. (2003). Effects of body mass, meal size, fast length, and temperature on specific dynamic action in the timber rattlesnake (*Crotalus horridus*). *Physiol. Biochem. Zool.* **76**, 447-458.
Zanotto, F., Gouveia, S., Simpson, S. and Calder, D. (1997). Nutritional homeostasis in locusts: is there a mechanism for increased energy expenditure during carbohydrate overfeeding? *J. Exp. Biol.* **200**, 2437-2448.
Zera, A. J. (2005). Intermediary metabolism and life history trade-offs: lipid metabolism in lines of the wing-polymorphic cricket, *Gryllus firmus*, selected for flight capability vs. early age reproduction. *Integr. Comp. Biol.* **45**, 511-524.
Zera, A. J. and Zhao, Z. (2006). Intermediary metabolism and life-history trade-offs: differential metabolism of amino acids underlies the dispersal-reproduction trade-off in a wing-polymorphic cricket. *Am. Nat.* **167**, 889-900.
Zhao, Z. and Zera, A. J. (2006). Biochemical basis of specialization for dispersal vs. reproduction in a wing-polymorphic cricket: morph-specific metabolism of amino acids. *J. Insect Physiol.* **52**, 646-658.