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Metabolic rate is canalized in the face of variable life history and nutritional environment

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Summary

1. Despite its central importance in organismal physiology, we have poor understanding of how metabolic rate is influenced by two key factors – food nutritional content and an organism's physiological characteristics.

2. We examined how variation in nutrients and physiological aspects of life history affect standard metabolic rate in *Gryllus firmus* cricket morphs that differ dramatically in flight capability and early-age fecundity.

3. Newly moulted female morphs were fed one of 13 diets that differed in concentrations of protein and carbohydrate. Carbon dioxide production, respiratory exchange ratios (RERs), nutrient intake and mass and lipid levels were measured.

4. CO_2 production and RERs increased to a similar degree in both morphs as food macronutrient content increased. In contrast, no difference in whole-organism O_2 consumption was observed across the protein–carbohydrate landscape or between morphs. Both morphs similarly increased food intake as nutrient concentration – particularly protein – decreased, but differed in mass and lipid gains, across the diets.

5. Modulation of the substrate used for respiration coupled with compensating aspects of morph-specific metabolism appears to buffer the effects of variable nutrient intake and life history on standard metabolic rate. That is, respiration rate can be highly canalized in the face of dramatic variation in both the external nutritional environment and internal aspects of intermediary metabolism.

Key-words: feeding behaviour, life-history trade-offs, nutritional physiology, respirometry

Introduction

An animal's metabolic rate (sensu Burton *et al.* 2011) is a key aspect of animal energetics (Glazier 2005; Karasov & Martínez del Rio 2007; Swallow *et al.* 2009). Researchers use metabolic rate to provide information about current energetic state, and an overriding assumption is that metabolic rate is linked to many features of organismal function and life history (Burton *et al.* 2011). Despite decades of study at the interspecific level (Glazier 2005), and more recently at the intraspecific level (Cruz-Neto & Bozinovic 2004; Steyermark *et al.* 2005; Bozinovic, Muñoz & Cruz-Neto 2007; Burton *et al.* 2011; Konarzewski & Książek 2013), we still have poor understanding of the factors that influence metabolic rate (Glazier 2015). There is good reason to suspect nutritional inputs affect metabolic rate (Cruz-Neto & Bozinovic 2004; Burton *et al.* 2011; Simpson & Raubenheimer 2012; Huang *et al.* 2013; Konarzewski & Książek 2013), especially given the clear evolutionary correlation between food habits and mass-corrected basal metabolic rate in endotherms (Cruz-Neto & Bozinovic 2004; Bozinovic, Muñoz & Cruz-Neto 2007). Life history could also influence metabolic rate (or vice versa; Crnokrak & Roff 2002; Książek, Konarzewski & Łapo 2004), although some studies have generated equivocal results (Djawdan *et al.* 1996; Djawdan, Rose & Bradley 1997; Chappell *et al.* 2009).

Most investigations of the interplay between nutrition and metabolic rate have either focused on animals fed *ad libitum* without quantifying food intake, or relied on starvation or food deprivation protocols. In the real world, animals are likely to experience nutritional variation along multiple continua – especially nutrient concentration (low to high) and nutrient ratio (balanced to imbalanced) (Behmer 2009; Simpson & Raubenheimer 2012). Proper understanding of how feeding and metabolic rates are linked requires a sophisticated approach that maps metabolic rates over an animal's full nutritional landscape and

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that precisely quantifies intake (Cruz-Neto & Bozinovic 2004; Simpson & Raubenheimer 2012).

Animals require a suite of nutrients that vary in energetic and functional characteristics (Raubenheimer & Simpson 1999; Karasov & Martínez del Rio 2007). Multiple studies have shown that two major nutrients – protein (p) and dietary carbohydrates (c) – have large phenotypic effects, and their intake is prioritized over other nutrients (Behmer 2009; Simpson & Raubenheimer 2012). Organisms show preferences for specific ratios of protein-to-carbohydrate, and feeding behaviour and measures such as mass gain, life span and reproductive output are affected by the degree of matching between a given diet and the preferred ratio and concentration (Maklakov *et al*. 2008; Roeder & Behmer 2014). Because protein and carbohydrates have interactive effects on feeding behaviour and performance, interactive effects on metabolic rates are also likely, particularly with respect to allocation between storage, energy and tissue construction (Raubenheimer & Simpson 1999; Warbrick-Smith *et al*. 2006; Behmer 2009; Zera & Harshman 2009; Simpson & Raubenheimer 2012).

Wing-polymorphic insects have been a powerful model system for addressing questions about associations between genetics, diet quality, physiology and metabolism because morphs have highly divergent physiological specialization (Zera & Zhao 2006). Adult female sand crickets (*Gryllus firmus*) occur as one of two genetically distinct morphs that trade off flight capability with reproduction; long-winged, flight-capable individuals [long-winged LW (f)] possess metabolically active, pink flight muscles and can fly, while short-winged crickets (SW) have white, underdeveloped flight muscle, but 100–400% larger ovaries by day 5 of adulthood (Zera, Sall & Grudzinski 1997; Zera, Potts & Kobus 1998; Zera & Brink 2000; Zera & Harshman 2001). The morphs differ in feeding strategy, as well as in mass gain patterns, allocation patterns and numerous aspects of intermediary metabolism (Zera & Brink 2000; Zera 2005; Zera & Zhao 2006; Clark *et al*. 2013; Clark, Zera & Behmer 2015a). In both morphs, mass gains are a function of food protein content, suggesting that protein and carbohydrate will affect other whole-animal characteristics (Clark *et al*. 2013). The LW(f) morph's pink flight muscle is more metabolically active than the white SW flight muscle (Zera, Sall & Grudzinski 1997), and the morphs may also differ in overall energy expenditure (Crnokrak & Roff 2002). These factors raise the possibility that the morphs could differ dramatically in standard metabolic rate. However, because of the numerous factors involved, it is not possible to predict *a priori* the degree or direction of difference in metabolic rate between the morphs.

To address the hypothesis that food protein–carbohydrate content affects metabolic rates, feeding and nutrient utilization, we used an experimental design that employs a large array of diets that vary in both total macronutrient concentration and protein–carbohydrate ratio. We tested this diet array in wing-polymorphic crickets, to assess how whole-animal metabolic rates interact with the genetically based life-history trade-off between dispersal and reproduction. We expected that diet quality and morph-specific physiological differences would influence whole-animal standard metabolic rates, mass gain and body composition. Based on prior findings, we also predicted that crickets would practice partial compensatory feeding when eating imbalanced diets or diets with low total macronutrient content (Clark, Zera & Behmer 2015a). We discuss our results in the context of the evolution and physiological ecology of metabolic rates.

Materials and methods

CRICKET MAINTENANCE AND DIETS

Crickets originated from large, outbred, artificially selected laboratory populations (>200 breeders per generation) that are nearly true-breeding for the flight-capable morph (LW) or the flight-incapable morph (SW). Details about selection and rearing of these populations can be found in Clark, Zera & Behmer (2015a) and references therein.

Immediately after juvenile female crickets moulted into adults (day 0; n = 80 LW, 50 SW), they were weighed and placed in individual plastic cages (as described in Clark *et al*. 2013). Each cage contained a pre-weighed, spill-resistant dish of one of 13 dry synthetic diets that varied in protein (p), carbohydrate (c) and total macronutrient content (Table 1; Fig. 1, panel (a); Clark *et al*. 2013 for diet preparation details). The self-selected p : c ratio for both cricket morphs is ~p3 : c4 (Clark *et al*. 2013), so this ratio was used as a centring food rail for diets in the current experiment. Five p : c ratios were represented, characterized relative to cricket preferences: (i) balanced, (ii) carbohydrate-biased, (iii) protein-biased, (iv) very carbohydrate-biased and (v) very protein-biased. For each p : c ratio, two or three total macronutrient levels were used, such that food macronutrient content ranged from dilute (21%) to concentrated (63%).

RESPIROMETRY MEASUREMENTS AND PHYSIOLOGICAL CHARACTERIZATION

We used flow-through respirometry to measure cricket carbon dioxide (CO_2) production, which is commonly used as an indirect estimate of an organism's metabolic rate (Lighton 2008). Oxygen consumption rates are directly proportional to metabolic rates, while the relationship between CO_2 production and metabolic rate depends on whether protein, carbohydrate or lipid is being utilized (Karasov & Martínez del Rio 2007; Sinclair *et al*. 2011; Harrison, Woods & Roberts 2012). Crickets may shift between carbohydrate, lipid and protein oxidation as a function of diet (Sinclair *et al*. 2011). Thus, it was important to have an estimate of O_2 consumption as well as CO_2 production. Because the flow rate and O_2 analyser we employed could not reliably distinguish the O_2 signal from background noise during flow-through measurements (Lighton 2008), we used a novel 'alternating flow' approach to estimate O_2 consumption. This approach (detailed below) combined the standard flow-through measurements of CO_2 production with a short-duration stop-flow procedure to measure CO_2 production and O_2 consumption, allowing estimation of respiratory exchange ratios (RERs). We then used the RER values to calculate O_2 consumption rates from CO_2 production during the standard 'flow-through' measurement period.

Within one hour of lights-on on day 5, crickets were removed from cages, placed into small glass metabolic chambers (3 cm

Table 1. Dietary treatments expressed as protein : carbohydrate (p : c) ratios, with contents expressed as a percentage of dry mass (e.g. p4 : c17 = 4% protein and 17% carbohydrate, yielding total macronutrient content = 21%). The p : c ratio of each diet is described relative to the nutritional requirements of our crickets. Treatment sample sizes for flight-capable [long-winged(f)] and flightless short-winged crickets on each treatment are given, and letters indicating the position of the diets are also depicted in panel (a) of Fig. 1

Diet protein : carbohydrate content		Total macronutrients (% dry mass)	LW(f)	SW
(a) p4 : c17	Very carbohydrate-biased	21	5	4
(b) p9 : c12	Balanced		3	3
(c) p14 : c7	Very protein-biased		3	4
(d) p9.75 : c21.75	Carbohydrate-biased	31·5	2	4
(e) p17.25 : c14.25	Protein-biased		5	4
(f) p8 : c34	Very carbohydrate-biased	42	5	4
(g) p13 : c29	Carbohydrate-biased		5	4
(h) p18 : c24	Balanced		4	4
(i) p23 : c19	Protein-biased		4	4
(j) p28 : c14	Very protein-biased		4	3
(k) p16.25 : c36.25	Carbohydrate-biased	52·5	4	4
(l) p28.75 : c23.75	Protein-biased		1	4
(m) p27 : c36	Balanced	63	2	4
Total			47	50

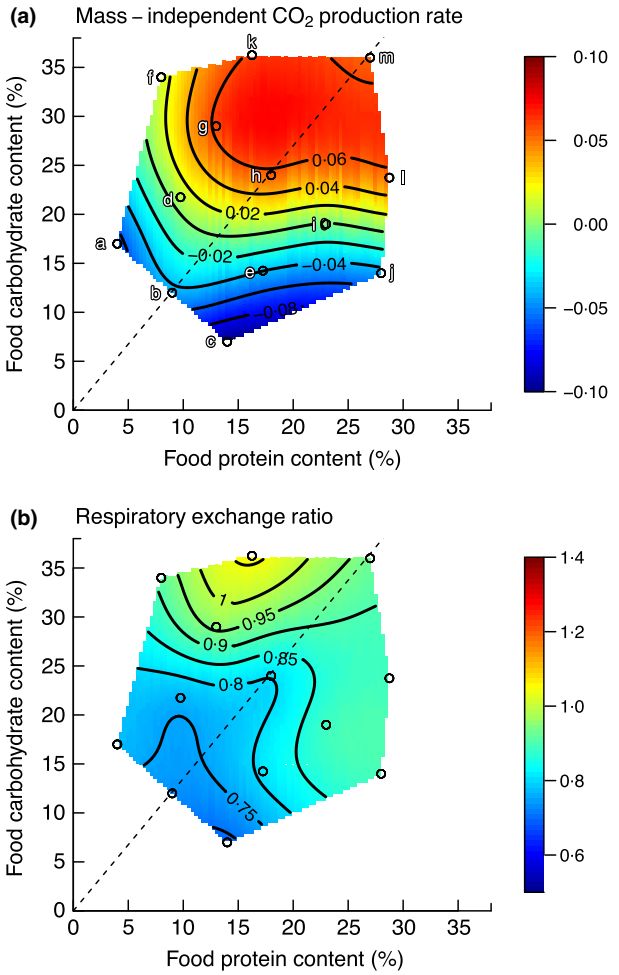

Fig. 1. Cricket respiration rates. Residuals of the best-fit line of the regression of carbon dioxide production against day zero adult cricket mass were used to construct a response surface for 'mass-independent' CO_2 production, shown in panel (a) as a function of food protein and carbohydrate content, for a total of 47 flight-capable [long-winged(f)] and 50 flightless, short-winged individuals. Data from both morphs have been combined into one plot because morphs showed similar patterns of CO_2 production in response to diet treatments. The letters in panel (a) and open circles denote locations, in protein–carbohydrate nutrient space, of the 13 diet treatments; the dashed line indicates the average self-selected protein-to-carbohydrate ratio (Clark *et al.* 2013). Panel (b) shows the corresponding respiratory exchange ratio (RER) between CO_2 and O_2 across diets. Food carbohydrate content is significantly and positively associated with CO_2 production and RER (Tables 2 and S1 for statistics).

diameter, 9·5 cm long) and acclimated for one hour prior to measurements, as crickets tend to move around for the first 30 min after handling. Feeding in insects increases respiration rate, which, in locusts, returns to pre-feeding levels by 10 min after feeding ends (Gouveia *et al.* 2000). Thus, the 1-h acclimation period should also eliminate confounding effects of feeding *per se* on respiration rates. Six metabolic chambers at a time were connected to a Multiplexer (Sable Systems Intelligent Multiplexer V5, Las Vegas, NV, USA). Following acclimation, cricket respiratory rates were measured at 28 ± 1 °C (temperature maintained by a Sable Systems Pelt5 controller). Dry, CO_2-free air from a tank was passed through the chambers. Air flow rate was regulated by a Sable Systems MFC2 Mass Flow Control Unit connected to two Tylan 2900 series flow valves each configured to maintain flow rates of 100 mL min^{-1}, monitored by a mass flow meter (Sable Systems Mass Flow System). One air stream was directed through the chambers for respiratory measurements, while the other provided continuous air to crickets during non-measurement periods (purge input; ~20 mL min^{-1} per chamber). Excurrent air passed through a magnesium perchlorate scrubber column to remove water vapour, before CO_2 content was measured with a CA-10a CO_2 analyser (Sable Systems). Air from the CO_2 analyser travelled through an ascarite column to remove CO_2 before passage through a single fuel-cell O_2 analyser (Sable Systems FC-10). Both analysers interfaced with Sable Systems hardware and software (ExpeData; Sable Systems) such that a PC recorded one air sample per second from each analyser. Each cricket's average CO_2 production was measured over the final 6 min (360 samples) from a 500-sample (08:20) recording. Baseline air measurements were taken between successive crickets to correct for analyser drift.

As mentioned above, cricket O_2 consumption rates are difficult to reliably distinguish from background fluctuations in O_2 readings at a flow rate of 100 mL min^{-1} (Fig. S1, Supporting Information, for example traces). However, the reduced air flow rate from the purge input created clear, strong initial spikes in CO_2 production and O_2 utilization at the beginning of each recording, allowing precise calculation of RERs. RERs were then used to calculate O_2 consumption rates from the measured CO_2 production rates

during the 'flow-through' period. It should be noted that this stop-flow-like condition could not be used to measure respiration rate because air flow rates from the purge input were unknown. O_2 measurements were baseline- and lag-corrected before calculations (Fig. S1).

After respirometry, crickets were weighed to the nearest 0·1 mg and frozen for characterization of muscle morphology. A subset of LW females are cryptically flightless and possess white, histolysed (non-functional) flight muscle [designated LW(h), as in previous publications]. All LW(h) crickets were excluded from analysis ($n = 34$) as our focus was on comparisons between the well-studied flight-capable [LW(f)] vs. flightless (SW) morphs. There is no association between diet treatment and early flight muscle histolysis in LW females (Clark, Zera & Behmer 2015a). Sample sizes reported in Table 1 include only the remaining flight-capable LW (f) crickets retained in the analysis.

LW(f) and SW crickets were then dried for 3 days at 60 °C and re-weighed, and total lipids were extracted with chloroform in a series of three 24-h washes (as in Clark, Zera & Behmer 2015a). Lipid content was calculated as the difference between dry mass and lean mass (mass after lipid extraction). This provided information about total lipid content, without distinguishing between somatic and ovarian lipid, in contrast to a previous experiment (Clark, Zera & Behmer 2015a). Four crickets did not eat during feeding trials and were removed from analysis. Leftover diet was kept at room temperature for 24 h to stabilize humidity and then re-weighed to determine the amount eaten. Total calorie ingestion was calculated as the product of the total amount of food eaten, the diet's total macronutrient content and the average calorie content of protein and carbohydrate (4 kcal g^{-1}).

STATISTICAL ANALYSIS

Diet effects were characterized using linear models of response surfaces, with the cricket's initial mass as a size covariate, as described previously (Clark, Zera & Behmer 2015a). Response surface models included linear and quadratic terms for diet protein and carbohydrate content and a protein-by-carbohydrate interaction term. Structuring the response surface shape based on the diet treatments rather than amount of food consumed allows for direct characterization of responses to treatments, which enables straightforward comparisons between the morphs. The bulk cellulose used to dilute the diets may be costly to ingest and process, so even if crickets on different treatments consume the same total amounts of protein and carbohydrate, they do so in different physiological contexts. To test for differences between cricket morphs, 'morph' and five 'morph × ____' interaction terms were added to a given model. This full model was compared to the reduced model lacking 'morph' terms, with a partial *F*-test. Where significant, 'morph' terms were retained in the final model; otherwise, the reduced model is reported. Nonparametric response surface figures were visualized as thin-plate splines. Cricket final masses and CO_2 production were log-transformed to examine the overall intraspecific scaling of respiratory rates across dietary treatments. Residuals from the regression of CO_2 production and final mass were then used as a measurement of mass-independent respiratory rate for comparison between morphs and across dietary treatments. An equivalent procedure was used for O_2 consumption.

Results

DIET BUT NOT MORPH INFLUENCES ON RESPIRATION AND METABOLIC RATE

There were no morph differences in mass-independent CO_2 production, RERs and calculated O_2 consumption, so for all three analyses, we report reduced models that omit all 'morph' terms (Tables 2 and S1). On a log–log scale, resting CO_2 production scaled positively with final cricket wet mass, with a slope significantly greater than one (Fig. S2; slope: 1·55 ± 0·19 SEM; 95% slope confidence range 1·17–1·92; intercept = −6·51 ± 0·55; $F_{1,95} = 67{\cdot}3$, $P < 0{\cdot}0001$; adj. $R^2 = 0{\cdot}41$). The residuals from this regression, which represent mass-independent CO_2 production, were positively and significantly associated with the amount of digestible carbohydrate in the diet, as indicated by a significant positive linear carbohydrate term in the response surface model (Fig. 1, Tables 2 and S1). There were no significant effects of protein, or interactions between main effects, on mass-independent CO_2 production (Fig. 1, Tables 2 and S1). Mass-independent CO_2 production was uniformly high across four concentrated diets, p13 : c29, p18 : c24, p16.25 : c36.25 and p28.75 : c23.75 (diets g, h, k and l, Fig. 1a), and lowest on the low-macronutrient, protein-biased diet p14:c7 (diet c).

Dietary carbohydrate was also significantly and positively associated with RER, as indicated by a significant linear carbohydrate response surface term (Fig. 1b, Tables 2 and S1). On high-carbohydrate, high-nutrient diets (e.g. diet k, p16.25 : c36.25), mean RERs were near 1·0, indicating utilization of carbohydrate as the main metabolic fuel. On low-carbohydrate diets (e.g. diet c, p14 : c7), mean RERs were close to 0·7. The 'protein' terms were not significant in the linear response surface model for RERs.

Finally, calculated O_2 consumption was positively associated with final cricket wet mass, but with a slope not significantly different from one on a log–log scale (Fig. S2; slope: 0·86 ± 0·20 SEM; 95% slope confidence range 0·47–1·25; intercept = −4·42 ± 0·58; $F_{1,95} = 18{\cdot}9$, $P < 0{\cdot}0001$; adj. $R^2 = 0{\cdot}16$). Residuals from this regression were not significantly associated with any response surface model terms, and the model showed extremely low goodness-of-fit (adj. $R^2 = -0{\cdot}03$; Tables 2 and S1). The quantity of stored molecules, such as water, lipids and carbohydrates, can affect estimates of metabolic rate, so differences in the accumulation of storage compounds across treatments could obscure associations between the diet treatments and O_2 consumption (Djawdan, Rose & Bradley 1997). However, regression models where final cricket mass was subdivided into constituent components in various ways (e.g. separate model terms that allowed lean dry mass and lipid mass to vary independently of each other; a model using dry mass only) did not measurably improve any aspect of the analysis (Clark, Zera & Behmer 2015b data file in Dryad and script file in Appendix S1).

FOOD CONSUMPTION, MASS GAIN AND LIPID LEVELS

Whether analysed from the perspective of total food consumed or total macronutrients ingested, feeding patterns were similar between LW(f) and SW morphs, so the reduced response surface models are reported (Table 3).

Table 2. Statistical results for response surface models testing the effects of diet protein and carbohydrate content, and morph type [short-winged vs. long-winged(f)] on CO_2 production, respiratory exchange ratio and calculated O_2 consumption

Model terms	Mass-independent CO_2 production	Respiratory exchange ratio	Mass-independent O_2 consumption
Full model	**$F_{6,90}$ = 2·52** **P = 0·03**	**$F_{6,90}$ = 4·60** **P = 0·0004**	$F_{6,90}$ = 0·54 P = 0·77
Intercept	$F_{1,90}$ = 1·76 P = 0·19	**$F_{1,90}$ = 13·7** **P = 0·0004**	$F_{1,90}$ = 0·43 P = 0·52
Initial cricket mass (covariate)	$F_{1,90}$ = 0·83 P = 0·36	**$F_{1,90}$ = 8·83** **P = 0·004**	$F_{1,90}$ = 0·13 P = 0·72
Protein	$F_{1,90}$ = 1·31 P = 0·25	$F_{1,90}$ = 2·03 P = 0·16	$F_{1,90}$ = 0·67 P = 0·42
Carbohydrate	**$F_{1,90}$ = 10·50** **P = 0·002**	**$F_{1,90}$ = 15·95** **P = 0·0001**	$F_{1,90}$ = 0·78 P = 0·38
Protein^2	$F_{1,90}$ = 1·27 P = 0·26	$F_{1,90}$ = 0·01 P = 0·93	$F_{1,90}$ = 1·69 P = 0·20
Carbohydrate^2	$F_{1,90}$ = 1·45 P = 0·23	$F_{1,90}$ = 0·21 P = 0·66	$F_{1,90}$ = 0·17 P = 0·68
Protein × carbohydrate	$F_{1,90}$ = 0·18 P = 0·67	$F_{1,90}$ = 0·62 P = 0·43	$F_{1,90}$ = 0·55 P = 0·46
Model adjusted R^2	0·09	0·18	−0·03
Morph differences: partial F-test against model without 'morph'	$F_{6,84}$ = 1·98 *P = 0·078*	$F_{6,84}$ = 0·48 P = 0·82	$F_{6,84}$ = 0·98 P = 0·44

Initial cricket mass was included in models as a covariate.
Bold indicates significance at the $\alpha = 0{\cdot}05$ level.
Italics indicate marginal significance ($\alpha < 0.10$).

Crickets appeared to adjust food intake in response to diet protein content, as indicated by a marginally significant negative quadratic protein term (Fig. 2a, Tables 3 and S1). Both morphs consumed more food when diet protein levels and total nutrient density were intermediate to low (e.g. they ate 767 ± 50 mg on p17.25 : c14.25, compared to 518 ± 55 mg on p27 : c36; Fig. 2a). Consumption tapered off across diets containing >20% protein. Despite ingesting greater amounts of low-protein foods, crickets did not fully compensate for nutrient dilution; total macronutrient intake (and therefore calorie intake) was more than double on the most nutrient-dense foods compared to nutrient-dilute foods (e.g. 373 ± 29 mg macronutrients on p16.25 : c36.25; 327 ± 33 mg on p27 : c36; vs. 141 ± 10 mg on p9 : c12). Total caloric intake was a significant negative quadratic function of protein content and positive linear function of food carbohydrate content (Tables 3 and S1).

Final mass was associated with diet protein and carbohydrate content, as indicated by significant linear protein and carbohydrate terms (Fig. 3a, Tables 3 and S2). At day 0 of adulthood, LW(f) crickets were significantly heavier than SW crickets ($\bar{x}_{SW}$ = 597 ± 13 mg; $\bar{x}_{LW}$ = 676 ± 15 mg wet mass; t = 3·93, d.f. = 93, P = 0·0002). However, by day 5, SW crickets had gained more mass on a percentage basis compared to LW(f) crickets on 11 out of 13 diet treatments, thereby achieving similar final masses (final wet mass averaged across diets: $\bar{x}_{SW}$ = 829 ± 20 mg; $\bar{x}_{LW}$ = 859 ± 22 mg). Mass gains on the remaining two diets were similar between the morphs (p9.75 : c21.75 and p28.75 : c23.75). Final masses were highest for LW(f) crickets on protein-biased diet p17.25 : c14.25 and for SW crickets on carbohydrate-biased, high-nutrient diet p16.25 : c36.25. Crickets given protein-biased diets also tended to gain more mass compared to those fed low concentration, carbohydrate-biased diets (e.g. diet a, p4 : c17).

Morph lean masses and lipid levels indicated morph- and nutrient-specific responses to the dietary treatments. In the lean mass response surface model, both the morph-by-protein and linear protein terms were significant (Tables 3 and S2). SW crickets had higher final lean masses (partial F-test, $F_{6,84}$ = 5·87, $P < 0{\cdot}0001$), and for both morphs, lean masses were higher on very protein-biased foods relative to most other treatments (e.g. on diet p28 : c14, $\bar{x}_{SW}$ = 296 ± 20 mg; $\bar{x}_{LW}$ = 234 ± 28 mg; Fig. 3c,d). Morph type and dietary protein and dietary carbohydrate content also influenced lipid levels (significant linear response to carbohydrate; significant morph-by-protein^2 interaction; Tables 3 and S2). Across all diets, LW(f) crickets had consistently higher lipid levels (>10 mg more except on diet k, p16.25 :c36.25; than SW crickets Fig. 3c, Table 2; partial F-test, $F_{6,84}$ = 6·89, $P < 0{\cdot}0001$). On the most nutrient-dense diet (p27 : c36), LW(f) crickets had over double the lipid content of LW(f) crickets on the most nutrient-dilute, very protein-biased food (e.g. 136 ± 1 mg lipids on diet p27 : c36; 51 ± 9 mg lipids on diet p14 : c7). SW crickets had peak lipid levels on the high-nutrient, carbohydrate-biased diet p16.25 : c36.25.

Discussion

Life-history traits and nutrition have been cited, but rarely or thoroughly vetted, as factors that influence metabolic

Table 3. Statistical results for response surface models testing the effects of diet protein and carbohydrate content, and morph type [short-winged vs. long-winged(f)] on days 0–5 feeding, calorie intake, mass gain, lean mass and lipid level in crickets

Model terms	Feeding	Calorie intake	Mass gain	Lean mass	Lipid levels
Full model	**$F_{6,90}$ = 5·84** **P < 0·0001**	**$F_{6,90}$ = 23·9** **P < 0·0001**	**$F_{6,90}$ = 29·1** **P < 0·0001**	**$F_{12,84}$ = 24·5** **P < 0·0001**	**$F_{12,84}$ = 22·3** **P < 0·0001**
Intercept	**$F_{1,90}$ = 7·93** **P = 0·006**	*$F_{1,90}$ = 7·00* *P = 0·01*	**$F_{1,90}$ = 14·5** **P = 0·0003**	$F_{1,84}$ = 1·20 P = 0·27	$F_{1,84}$ = 0·03 P = 0·86
Initial mass (covariate)	**$F_{1,90}$ = 23·81** **P < 0·0001**	**$F_{1,90}$ = 20·7** **P < 0·0001**	**$F_{1,90}$ = 127·4** **P < 0·0001**	**$F_{1,84}$ = 151·9** **P < 0·0001**	**$F_{1,84}$ = 50·9** **P < 0·0001**
Morph				**$F_{1,84}$ = 9·74** **P = 0·002**	$F_{1,84}$ = 2·25 P = 0·14
Protein	*$F_{1,90}$ = 3·03* *P = 0·08*	**$F_{1,90}$ = 37·2** **P < 0·0001**	**$F_{1,90}$ = 18·9** **P < 0·0001**	**$F_{1,84}$ = 20·5** **P < 0·0001**	$F_{1,84}$ = 2·21 P = 0·14
Carbohydrate	$F_{1,90}$ = 0·92 P = 0·34	**$F_{1,90}$ = 81·2** **p < 0·0001**	**$F_{1,90}$ = 4·42** **P = 0·04**	$F_{1,84}$ = 0·02 P = 0·89	**$F_{1,84}$ = 79·5** **P < 0·0001**
Protein^2	*$F_{1,90}$ = 2·94* *P = 0·09*	**$F_{1,90}$ = 5·30** **P = 0·02**	$F_{1,90}$ = 1·63 P = 0·21	$F_{1,84}$ = 2·79 P = 0·10	$F_{1,84}$ = 1·95 P = 0·17
Carbohydrate^2	$F_{1,90}$ = 0·81 P = 0·37	$F_{1,90}$ = 1·39 P = 0·24	$F_{1,90}$ = 1·79 P = 0·18	$F_{1,84}$ = 0·16 P = 0·69	$F_{1,84}$ = 0·45 P = 0·50
Protein × carbohydrate	$F_{1,90}$ = 0·19 P = 0·66	$F_{1,90}$ = 0·20 P = 0·65	$F_{1,90}$ = 0·92 P = 0·34	$F_{1,84}$ = 1·66 P = 0·20	$F_{1,84}$ = 0·60 P = 0·44
Morph × protein				**$F_{1,84}$ = 8·20** **P = 0·005**	$F_{1,84}$ = 0·27 P = 0·61
Morph × carbohydrate				$F_{1,84}$ = 0·07 P = 0·78	$F_{1,84}$ = 1·43 P = 0·24
Morph × protein^2				$F_{1,84}$ = 0·45 P = 0·51	**$F_{1,84}$ = 4·76** **P = 0·03**
Morph × carbohydrate^2				$F_{1,84}$ = 0.04 P = 0·84	$F_{1,84}$ = 0·56 P = 0·46
Morph × protein × carbohydrate				$F_{1,84}$ = 1·83 P = 0·18	$F_{1,84}$ = 0·40 P = 0·53
Model adjusted R^2	0·23	0·59	0·64	0·75	0·73
Morph differences: partial F-test against model without 'morph'	$F_{6,84}$ = 1·49 P = 0·19	$F_{6,84}$ = 1·23 P = 0·30	$F_{6,84}$ = 1·83 P = 0·10	**$F_{6,84}$ = 5·87** **P < 0·0001**	**$F_{6,84}$ = 6·89** **P < 0·0001**

Initial cricket mass was included in models as a covariate.
Bold indicates significance at the α = 0·05 level.
Italics indicate marginal significance (α < 0.10).

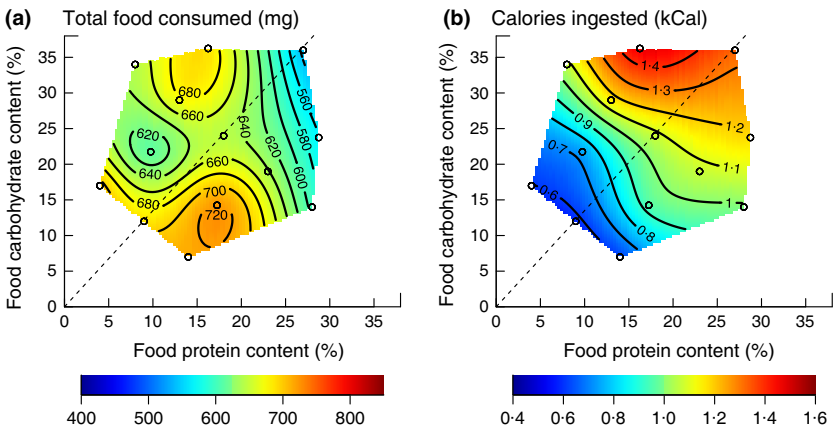

Fig. 2. Food consumption (a) and calorie ingestion (b) by flight-capable [long-winged (f), N = 47] and flightless (short-winged, N = 50) crickets each given one of 13 diets containing different amounts of protein and carbohydrate. Symbol meanings are the same as in Fig. 1. Feeding appears dependent on diet protein content (marginally significant quadratic protein term), while calorie ingestion was a function of both dietary protein and carbohydrate content (significant quadratic protein term and linear carbohydrate term; statistics in Tables 3 and S2).

rates (Cruz-Neto & Bozinovic 2004; Burton *et al.* 2011; Konarzewski & Książek 2013). Our results, however, show that standard metabolic rate can in fact be completely dissociated from both a major life-history trade-off and food quality. The two cricket morphs, which differ dramatically in life history, exhibited statistically indistinguishable standard metabolic rates. In other words, the physiological mechanisms generating the life-history trade-off between the morphs occur in the absence of any measurable differential effect at the level of whole-animal standard metabolic rate. Moreover, crickets appeared to have shifted metabolic fuels for respiration (carbohydrate vs. protein), across the diets. This resulted in invariant whole-animal standard metabolic rates across the diets even as diet

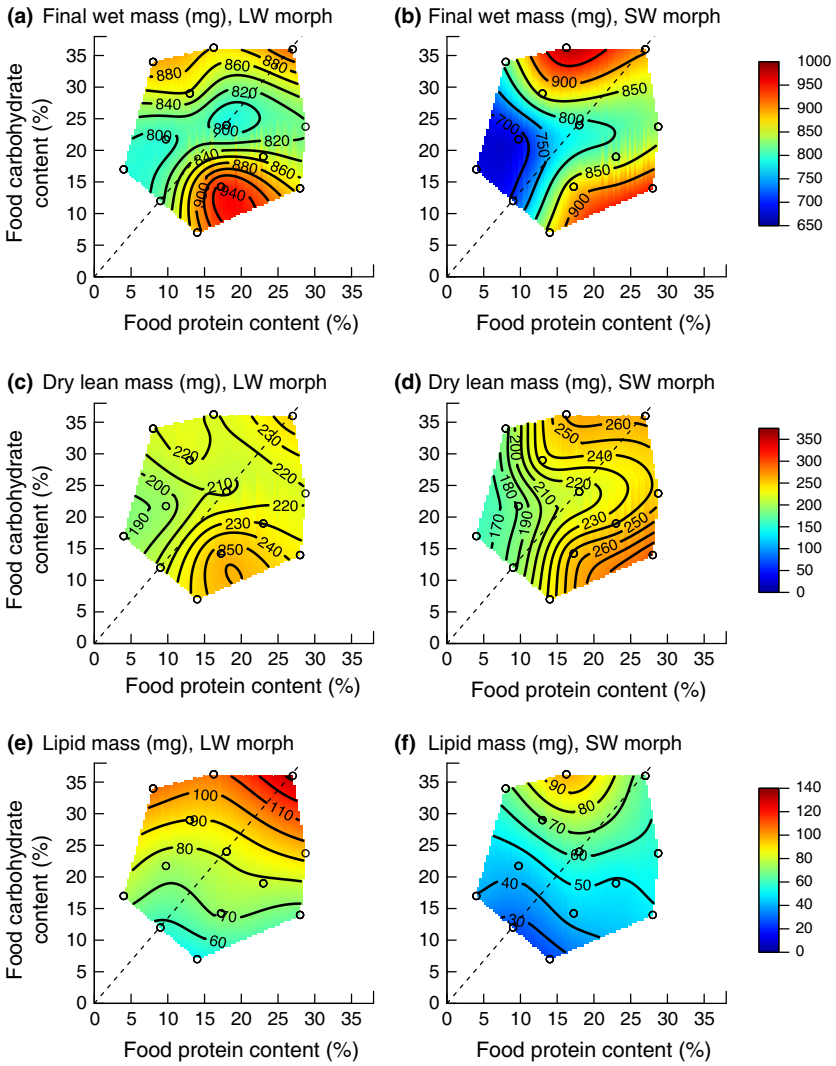

Fig. 3. Response surfaces for wet mass gains (panels a and b), final lean mass (dry mass; panels c and d) and lipid levels (panels e and f), across diets for flight-capable [long-winged(f), $N = 47$] vs. flightless (short-winged, $N = 50$) crickets given one of 13 diets with different amounts of protein and carbohydrate. See Fig. 1 for symbol meanings. Final lean masses and lipid levels differed across diets and between cricket morphs (Tables 3 and S2 for statistics). Mass gains were similar between morphs, but were a function of diet protein and carbohydrate content (significant linear protein and carbohydrate terms). Individual panels are shown for each morph to facilitate comparison with lean mass and lipid findings.

protein–carbohydrate content continued to affect food intake, mass gain and lipid levels.

Evolutionary biologists have long been interested in determining the mechanistic nature of connections between fundamental life-history characteristics (Zera & Harshman 2001), particularly how various processes are linked to metabolic energetic demand (Burton *et al.* 2011; Glazier 2015). The cricket morphs differ in features that should influence metabolic rates, including feeding strategy (Clark *et al.* 2013; Clark, Zera & Behmer 2015a) and numerous aspects of physiology and intermediary metabolism. For example, flight muscles of LW(f) individuals exhibit 10 times greater resting metabolic rate than the underdeveloped flight muscles of the SW morph or ovaries of that morph (Zera, Sall & Grudzinski 1997). Additionally, biosynthesis of yolk protein and oxidation of fatty acid are greater in the SW morph, while amino acid oxidation and lipid biosynthesis are greater in the LW(f) morph (Zhao & Zera 2002, 2006; Zera & Zhao 2006).

Because of these morph differences, we expected to see morph-specific consequences for standard metabolic rate. That this was not observed indicates a remarkable morph-specific counterbalancing of energy allocation to various aspects of morph physiology resulting in no observed trade-off at the level of whole-organism metabolic rate. This finding has several important consequences. On the one hand, it indicates that phenotypes that do not differ in whole-organism metabolic rate can nevertheless differ dramatically in aspects of intermediary metabolism that contribute significantly to metabolic rate. On the other hand, measuring various phenotype-specific aspects of intermediary metabolism does not necessarily allow inferences to be drawn regarding differences in overall energy metabolism between the phenotypes. Measuring both whole-organism respiration rate and aspects of intermediary metabolism is required to obtain a comprehensive understanding of important components of intermediary metabolism that have been modified to produce life-history adaptations, and their contribution to whole-organism energetics.

Related experimental studies of life-history physiology have included the following: direct selection on resting metabolic rate in mice, with measures of anatomic and energetic correlates (reviewed in Konarzewski & Książek

2013); selection for herbivorous or carnivorous diets in bank voles (reviewed in Swallow *et al*. 2009); or selection in *Drosophila* on longevity, starvation resistance or stress resistance (Djawdan, Rose & Bradley 1997; Van Voorhies, Khazaeli & Curtsinger 2004; Khazaeli, Van Voorhies & Curtsinger 2005; Baldal, Brakefield & Zwaan 2006; Rion & Kawecki 2007). Aside from some organ- and tissue-specific responses in mice, no direct, mechanistic associations have been found between metabolic rates as a whole and changes in these major life-history characteristics. In *Drosophila*, for instance, laboratory selection experiments have not found connections between metabolic rate and longevity (Djawdan *et al*. 1996; Van Voorhies, Khazaeli & Curtsinger 2003, 2004), stress resistance (Djawdan, Rose & Bradley 1997) or starvation resistance (Djawdan, Rose & Bradley 1997; Harshman & Schmid 1998; Baldal, Brakefield & Zwaan 2006; Rion & Kawecki 2007). However, selection for increased starvation resistance did cause a reduction in activity levels of metabolic enzymes and in overall movement in the absence of effects on metabolic rate (Harshman & Schmid 1998; Schwasinger-Schmidt, Kachman & Harshman 2012).

A number of investigators have argued that behavioural mechanisms, rather than resting metabolism, may be a more common direct target of selection on energetics (Swallow *et al*. 2009). Activity such as locomotion or flying can comprise a substantial fraction of an animal's metabolic budget (Steyermark *et al*. 2005), and locomotor behaviour can be altered substantially by selection pressures (Rion & Kawecki 2007; Schwasinger-Schmidt, Kachman & Harshman 2012).

Crnokrak & Roff (2002) reported higher residual CO_2 production rates in days 5–7 LW compared to SW *G. firmus*. They measured CO_2 production in non-quiescent crickets and corrected rates for differences in activity levels and body size, concluding that residual respiration rate was higher in the LW morph. While this difference may have important implications for morph energetics, it is difficult to ascertain which factors generated the observed morph difference. Specifically, a difference may occur because respiration does not scale linearly with activity, or scales differently with activity for each morph. The morph difference could also be associated with metabolic substrate switching. The findings of Crnokrak & Roff (2002) bring up two important caveats for the present study. First, we only measured respiration rates during one short time window on day 5 – important morph differences in respiration could occur at other times that were not measured. Secondly, we have not yet quantified activity differences between the morphs, and their contribution to morph-specific respiration.

In addition to morph similarities, metabolic rate was strongly canalized across the nutrient landscape, due to a shift in metabolic fuel use in response to food carbohydrate content. Resting CO_2 production and RERs increased as diet carbohydrate content increased. Since our synthetic diets contain almost no lipid, it is most likely that crickets on low-carbohydrate foods have lower RERs than crickets on high-carbohydrate foods because they switched to utilizing dietary protein or stored lipid for energy. The observed shift towards lower CO_2 production on nutrient-dilute foods matches previous observations that food deprivation lowers CO_2 production in crickets (Nespolo, Castañeda & Roff 2005; Sinclair *et al*. 2011). However, Sinclair and colleagues determined that this could be explained by a switch in fuel use from carbohydrates to lipids. It remains an open question whether this switch serves as a mechanism for dissipating a nutrient eaten in excess of requirements (Simpson *et al*. 2004; Warbrick-Smith *et al*. 2006), as opposed to a morph-specific adaptation for using a substrate for energy production that is in lower demand for biosynthesis (Zera & Harshman 2009).

An unusual finding of the present study was the high final body mass – CO_2 scaling exponent for *G. firmus* (1·55), which is higher than most of those reported elsewhere (Withers 1992, Chown *et al*. 2007). This high scaling exponent could be due to some constitutive size-based difference in metabolism, or alternatively could be associated with diet-induced differences in mass and lipid gains. Crucially, the residuals of this mass–CO_2 scaling relationship were only associated with food carbohydrate content. Further, food carbohydrate content was positively associated with RER, mass gains and lipid gains. A consistent positive association between food carbohydrate content, mass gains, RER and CO_2 suggests that unusually elevated CO_2 production in larger crickets, or low CO_2 production in smaller crickets, is due to respiratory substrate switching in response to food carbohydrate content. Thus, this high scaling exponent should be interpreted cautiously relative to other reports of intraspecific metabolic scaling exponents (Glazier 2005, Chown *et al*. 2007), where either no diet information is reported, or nutritionally uniform diets were used.

Nutrition and metabolic rates are undoubtedly important in shaping an organism's fitness, but the detailed interactions between diet and metabolic rates are just beginning to be understood (Cruz-Neto & Bozinovic 2004; Burton *et al*. 2011). To date, most studies exploring links between metabolic rates and fitness have been conducted in limited contexts, where animals have access to abundant, high-quality food (Zera & Harshman 2001; Burton *et al*. 2011). There is growing recognition, however, that metabolic rates should be characterized in settings that mirror the environmental heterogeneity that organisms experience, including heterogeneity in nutrient content. Recent studies in mice (Huang *et al*. 2013) and spiders (Jensen *et al*. 2010) reveal that organisms adjust specific aspects of respiration in response to changes in nutrient content, but efforts to fully tie details of animal nutrition to whole-animal metabolic consequences remain in their infancy. Instead of diet quality driving resting metabolic rates, other aspects of an organism's physiology, such as metabolic control mechanisms outlined by Glazier (2015), may form the underlying connections between feeding ecology and metabolic rate. The present results suggest that variation in metabolic rates may be best considered as

a consequence of factors other than variation in food nutrient content, especially given our demonstration that standard metabolic rates can be decoupled from both key life-history traits and nutrient variation.

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Data accessibility

Data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.68s75 (Clark, Zera & Behmer 2015b).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Sample cricket respirometry traces.
Fig. S2. Log–log plot of mass and carbon dioxide production.
Fig. S3. Log–log plot of mass and oxygen consumption.
Table S1. Response surface model coefficients for measures without morph differences.
Table S2. Morph-specific response surface model coefficients for lean mass and lipid levels.
Appendix S1. R script with detailed analyses of body composition and respiration.