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RESEARCH ARTICLE

Nutritional physiology of life-history trade-offs: how food protein–carbohydrate content influences life-history traits in the wing-polymorphic cricket *Gryllus firmus*

 Rebecca M. Clark¹, Anthony J. Zera² and Spencer T. Behmer^{1,*}
ABSTRACT

Although life-history trade-offs result from the differential acquisition and allocation of nutritional resources to competing physiological functions, many aspects of this topic remain poorly understood. Wing-polymorphic insects, which possess alternative morphs that trade off allocation to flight capability versus early reproduction, provide a good model system for exploring this topic. In this study, we used the wing-polymorphic cricket *Gryllus firmus* to test how expression of the flight capability versus reproduction trade-off was modified across a heterogeneous protein–carbohydrate nutritional landscape. Newly molted adult female long- and short-winged crickets were given one of 13 diets with different concentrations and ratios of protein and digestible carbohydrate; for each cricket, we measured consumption patterns, growth and allocation to reproduction (ovary mass) versus flight muscle maintenance (flight muscle mass and somatic lipid stores). Feeding responses in both morphs were influenced more by total macronutrient concentration than by protein–carbohydrate ratio, except at high-macronutrient concentration, where protein–carbohydrate balance was important. Mass gain tended to be greatest on protein-biased diets for both morphs, but was consistently lower across all diets for long-winged females. When long-winged females were fed high-carbohydrate foods, they accumulated greater somatic lipid stores; on high-protein foods, they accumulated greater somatic protein stores. Food protein–carbohydrate content also affected short-winged females (selected for early reproductive onset), which showed dramatic increases in ovary size, including ovarian stores of lipid and protein, on protein-biased foods. This is the first study to show how the concentration and ratio of dietary protein and carbohydrate affects consumption and allocation to key physiological features associated with the reproduction–dispersal life-history trade-off.

KEY WORDS: Feeding behavior, Dispersal, Reproductive physiology, Nutrient allocation, Sand cricket

INTRODUCTION

Since the 1980s, a major question in life-history studies has been the extent to which trade-offs are influenced by nutrient input (van Noordwijk and de Jong, 1986; Zera et al., 1998; Boggs, 1992; Boggs, 2009; Zera and Harshman, 2001). Multiple studies have attempted to examine this issue, but the inferences drawn from these studies must be interpreted with caution because of three overlapping problems: (1) only a few diets were used, and these

were often poorly defined with regard to specific nutrient content; (2) typically, there was no attempt to quantify or control for changes in consumption between experimental diets, which is important given the ability of animals to practise compensatory feeding (Karasov and Martínez del Río, 2007; Behmer, 2009); and (3) variation in calories was often confounded with variation in the amount of specific nutrients (see Carvalho et al., 2005; Bass et al., 2007; Simpson and Raubenheimer, 2007; Lee et al., 2008; Fanson et al., 2009; Grandison et al., 2009; Tatar, 2011; Piper et al., 2014). For example, in many past experiments, diet treatments differed in caloric content and simultaneously in the amounts and ratios of specific macronutrients. Thus, it was not possible to untangle the effects of these variables on life-history traits, or to determine the effect of phenomena such as caloric restriction per se on life-history traits. Because of such confounding effects, and underlying methodological problems, we still know relatively little about how nutrient inputs affect life-history trade-offs.

Exploring how variation in food nutrient content is linked to the expression of life-history trade-offs requires two things: a demonstrated physiological trade-off that underlies a life-history trade-off, and a detailed nutritional framework to investigate specific effects of nutrient variation. Wing-polymorphic insects exhibit a physiological trade-off that underlies a classic life-history trade-off. Wing polymorphism involves discrete phenotypes that differ (trade-off) in flight capability and egg production. The physiological basis of the trade-off has been extensively studied (reviewed in Zera and Denno, 1997; Mole and Zera, 1993; Zera and Mole, 1994; Zera et al., 1994; Zera et al., 1997; Zhao and Zera, 2001; Zera and Larsen, 2001; Zera, 2005; Zera and Zhao, 2006; Zhao and Zera, 2006). In the wing-polymorphic sand cricket (*Gryllus firmus* Scudder 1902), the mechanisms underlying this trade-off include shifts in juvenile hormone expression, lipid metabolism and amino acid metabolism. Long-winged [LW(f)] adult female *G. firmus* maintain large flight muscles and triglyceride stores to fuel dispersal during early adulthood, but delay egg production. By contrast, short-winged (SW) crickets, which never fully develop flight muscles or wings, and which accumulate lower lipid reserves, exhibit greater ovarian growth and begin laying eggs sooner. Both functions require specific nutritional inputs (including energetic and structural components), and therefore the expression of the trade-off between flight and reproduction is likely to depend on the nutritional context. However, physiological studies of this trade-off in *G. firmus* have almost exclusively been conducted on a single diet, and the influence of nutrient variability has largely been ignored.

All animals, including those that exhibit life-history trade-offs, require a broad suite of nutrients, so a detailed nutritional framework is vital to explicitly link life-history trade-offs to specific nutrients, in the context of overall food nutrient content. The geometric framework (GF) does this, by investigating how animals

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simultaneously regulate and utilize multiple nutrients (Simpson and Raubenheimer, 2012). Mostly, though, the GF has focused on protein and digestible carbohydrates because they strongly impact animal growth and reproduction, and most animals actively, and tightly, regulate their intake (Behmer, 2009; Simpson and Raubenheimer, 2012). While proteins and carbohydrates have equal energetic value, they are utilized differently, with carbohydrates serving as an energy source and proteins providing amino acids that are assembled into structural tissues, enzymes and proteins involved in almost every physiological process. Importantly, animals regulate intake in response not only to the total amount of protein and carbohydrate in food (nutrient concentration) but also with respect to the ratio of the two nutrients (Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 2012). The GF allows these important issues to be investigated, which have been largely ignored in physiological studies of life-history trade-offs.

We recently applied the GF to crickets to understand interactions between nutrition and life-history trade-offs (Clark et al., 2013). Using five diets that differed in their protein–carbohydrate ratios, but had similar total macronutrient content (42%), we observed two key differences in the feeding strategies of the two adult female morphs. First, in a ‘choice’ assay, LW(f) crickets, compared with SW crickets, self-selected a diet that was more carbohydrate biased. Second, in a ‘no-choice’ assay, LW(f) females decreased total consumption as the protein–carbohydrate ratio of the available food became increasingly imbalanced, whereas SW females consumed similar total amounts of food regardless of food protein–carbohydrate ratio. This suggests that an important aspect of morph-specific adaptations for dispersal versus egg production is the differential acquisition of nutrients required for morph-specific functions, but we do not know yet how these intake strategies affect allocation. Testing the hypothesis that protein–carbohydrate content affects the trade-off between flight ability and reproduction necessitates a broader investigation of dietary quality, incorporating both differences in protein–carbohydrate ratios and concentrations. Crickets are opportunistic feeders (Capinera et al., 2004), so the nutritional content of their food can be highly variable. Animals, including insects, are known to employ compensatory mechanisms when eating foods that are nutritionally imbalanced (Simpson and Raubenheimer, 1993; Simpson et al., 2002; Cook et al., 2010) or have low-nutrient concentration (Yang and Joern, 1994; Slansky and Wheeler, 1989; Lee et al., 2004). Furthermore, multiple studies have revealed that reproductive output and lifespan are also responsive to protein and carbohydrate concentrations and ratios (Maklakov et al., 2008; Lee et al., 2008; Roeder and Behmer, 2014).

In the current study, we first characterized body conditions of newly molted SW and LW(f) adult *G. firmus* female crickets. Specifically, we compared the morphs’ initial body mass and allocation to somatic versus reproductive tissues. Next, we assessed the role of food protein–carbohydrate variation in the nature of the flight versus reproduction life-history trade-off over the first 5 days of adulthood. To do so, we gave LW(f) and SW females one of 13 diets with different concentrations and ratios of protein and carbohydrate for 5 days, and measured feeding patterns, mass gain, allocation to flight versus reproductive tissues, and lipid and protein profiles. We were particularly interested in determining the extent to which key morph differences in aspects of nutrient acquisition (e.g. patterns of consumption, dietary optima), documented in our pilot study (Clark et al., 2013), could be generalized across the more expansive nutritional environment of the present study. Finally, we sought to determine the extent to which the magnitude of the trade-off between nutrient allocation to components of flight (flight

muscle mass and somatic lipid) versus reproduction (mass, lipid content and protein content of ovaries) was canalized across the nutritional landscape. Alternatively, this would allow us to identify specific regions of nutritional space in which the dispersal–fecundity trade-off was either magnified or ameliorated. These results would not only identify how a life-history trade-off responds to nutrient heterogeneity but also set the stage for an analysis of the biochemical mechanisms underlying this response.

RESULTS

Body condition of newly molted adults

The two morphs differed in initial dry mass (*t*-test: $t_{32}=6.92$, $P<0.001$), with SW crickets being smaller than their LW(f) counterparts (Fig. 1A). The morphs also differed in their overall initial body composition (MANOVA: $F_{4,29}=14.0$, $P<0.001$; Fig. 1A), which consisted of dry mass measures of: (1) ovaries, (2) flight muscles, (3) somatic lipids (recovered from the carcass, excluding the flight muscles and ovaries) and (4) the remaining carcass (excluding flight muscle, ovaries and somatic lipids). Comparisons of individual body compartments (Fig. 1A) revealed that SW crickets possessed correspondingly lower flight muscle mass (*t*-test: $t_{29}=5.01$, $P<0.001$), lower absolute amounts of somatic lipids (*t*-test: $t_{32}=6.16$, $P<0.001$) and lower lipid-free somatic mass (*t*-test: $t_{32}=4.35$, $P<0.001$). Initial ovary mass was similar between the morphs (*t*-test: $t_{32}=1.18$, $P=0.250$), and represented a small fraction of the total dry mass in both morphs ($4.3\pm 0.3\%$).

We also examined protein allocation to different non-reproductive (somatic) tissues. For these day zero crickets, there were differences between the two morphs in the protein content of flight muscle and non-reproductive tissue (MANOVA: $F_{2,32}=28.4$, $P<0.001$; Fig. 1B). Most notably, LW(f) crickets had higher total amounts of protein in both flight muscle (*t*-test, $t_{30}=5.7$, $P<0.001$) and somatic tissue (*t*-test, $t_{32}=5.6$, $P<0.001$). The higher absolute protein levels were associated with the initial size differences between the morphs. However, on a percentage basis, the SW crickets contained more somatic protein ($57.7\pm 1.3\%$) than LW(f) crickets ($50.2\pm 0.9\%$), and this difference was significant (*t*-test: $t_{31}=4.9$, $P<0.001$). There was no statistical difference between the two morphs (*t*-test, $t_{24}=0.81$, $P=0.43$) in the percentage of protein found in the flight muscle ($70.3\pm 1.9\%$).

Results from experimental manipulation of food protein–carbohydrate content

Throughout the rest of the results, we present findings from a 5 day feeding experiment where crickets were given one of 13 diets containing different ratios and total amounts of protein and digestible carbohydrate (Table 1). Results from the 5 day feeding trials were analyzed as linear models of two-dimensional response surfaces, with cricket body mass as a covariate. To test for differences between the morphs, we used partial *F*-tests to select between reduced models containing only linear protein (p) and carbohydrate (c) terms plus their two-way combinations (p, c, p^2 , c^2 , $p\times c$) and models that contained these terms plus crossed combinations of all p and c terms with ‘morph’.

Consumption results on the different diets

Over the 5 day feeding trials, SW and LW(f) crickets had similar patterns of total food consumption across all 13 diets (partial *F*-test: $F_{6,157}=0.96$, $P=0.46$), so statistical results (Table 2) and coefficients (in the Results) from the reduced model only are reported. Separate figures are shown to facilitate understanding of the connection between food consumption and macronutrient intake (Fig. 2). In the

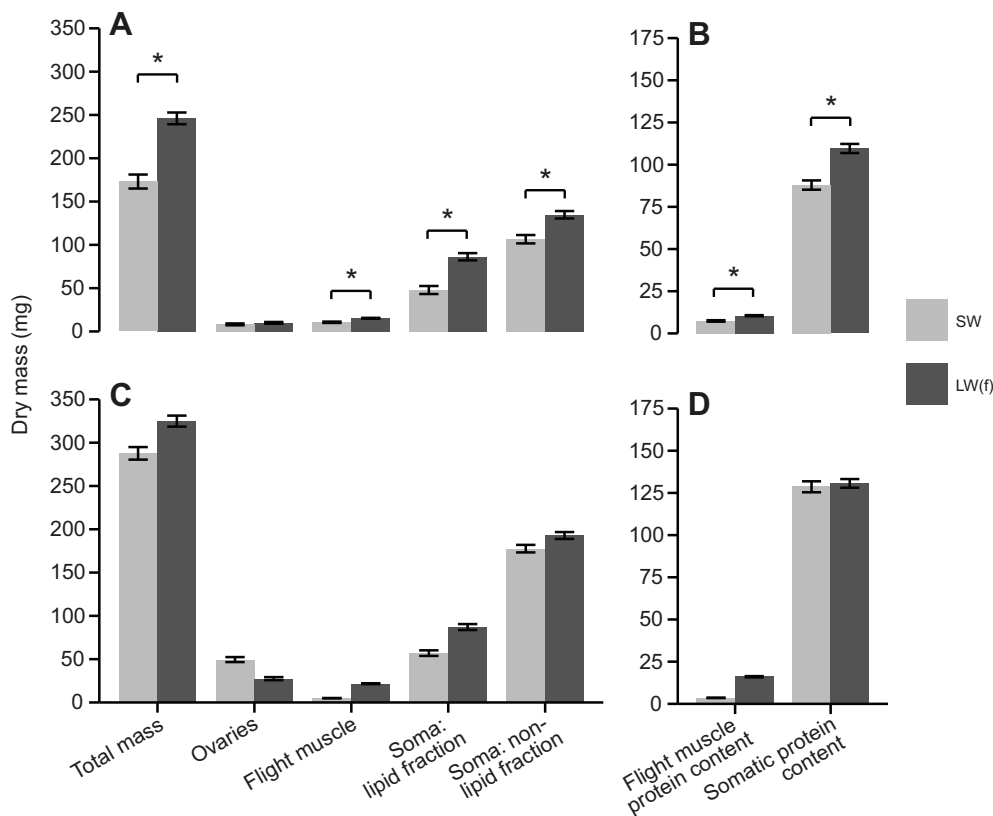


Fig. 1. Mean (\pm s.e.m.) cricket body allocation patterns, during early adulthood, for flightless (SW) and flight-capable [LW(f)] female crickets. (A,B) Total mean (\pm s.e.m.) cricket mass, and the mass of flight muscle, ovaries, lipid versus non-lipid carcass fractions, and flight muscle and somatic protein content at day zero. Asterisks indicate significant differences [MANOVA with *post hoc t*-tests, $P < 0.05$, $N = 17$ SW and 16 LW(f) crickets]. (C,D) After 5 days, allocation patterns shift and differ between morphs (see subsequent figures and Results for details and statistics). Day 5 data are averages from across all 13 dietary treatments and are from 79 SW and 91 LW crickets.

reduced model, which omitted all ‘morph’ terms, we found a significant quadratic carbohydrate term ($c^2 = -112.57 \pm 44.59$), a significant linear protein term ($p = -52.44 \pm 21.99$), a significant intercept (369.62 ± 87.21), and a significant covariate (initial mass, 0.49 ± 0.13); the protein \times carbohydrate interaction term was non-significant. Collectively, this indicates strong effects of both carbohydrate and protein content on total food consumption (Table 2). Crickets ate the most on diets that had two features: (1) p:c ratios that were balanced or carbohydrate biased and (2) low-macronutrient concentration (e.g. diet p9.75:c21.75). Crickets also tended to consume more food when diets were low in protein.

We also analyzed total macronutrient consumption (the combined intake of protein plus carbohydrate). Total macronutrient intake is presented and statistically analyzed in two ways: (1) as a response surface (Fig. 2B) and (2) as protein–carbohydrate intake arrays (Fig. 2C), which aids in visual analysis of intake patterns. Despite a general similarity in the shape of the response surfaces for each morph (Fig. 2B), patterns of total macronutrient consumption differed between the two morphs, as indicated by a significant morph \times protein \times carbohydrate interaction effect (partial F -test: $F_{6,157} = 2.27$, $P = 0.039$; Table 2; supplementary material Tables S1, S2). A significant negative quadratic carbohydrate term

Table 1. Dietary treatments expressed as protein:carbohydrate (p:c) ratios, with content expressed as a percentage of dry mass

Diet protein:carbohydrate content	Description	Total macronutrients (% dry mass)	LW(f)	SW
p4:c17	Very carbohydrate biased	21	9	6
p9:c12	Balanced		7	6
p14:c7	Very protein biased		6	6
p9.75:c21.75	Carbohydrate biased	31.5	7	6
p17.25:c14.25	Protein biased		8	6
p8:c34	Very carbohydrate biased	42	9	6
p13:c29	Carbohydrate biased		5	6
p18:c24	Balanced		5	6
p23:c19	Protein biased		9	6
p28:c14	Very protein biased		5	7
p16.25:c36.25	Carbohydrate biased	52.5	5	6
p28.75:c23.75	Protein biased		9	6
p27:c36	Balanced	63	7	6
Total			91	79

Macronutrient content is given as, for example, p4:c17=4% protein and 17% carbohydrate, with total macronutrient content=21%. The p:c ratio of each diet is also described relative to the nutritional requirements of our crickets.

Treatment sample sizes for flight-capable [LW(f)] and flightless (SW) crickets in each treatment are also given.

Table 2. Statistical results for response surface models testing the effects of protein and carbohydrate concentration and morph type [SW versus LW(f)] on day 0–5 feeding, caloric intake, mass gain, flight muscle mass and ovary mass in crickets

Model terms	Amount of food consumed	Macronutrient intake	Mass gain	Flight muscle mass	Ovary mass
Full model	$F_{6,163}=5.9$ $P<0.001$	$F_{12,157}=16.0$ $P<0.001$	$F_{12,157}=31.6$ $P<0.001$	$F_{12,156}=161$ $P<0.001$	$F_{12,156}=12.9$ $P<0.001$
Intercept	$F_{1,163}=17.8$ $P<0.001$	$F_{1,157}=18.8$ $P<0.001$	$F_{1,157}=43.6$ $P<0.001$	$F_{1,156}=34.1$ $P<0.001$	$F_{1,156}=18.3$ $P<0.001$
Initial cricket mass (covariate)	$F_{1,163}=13.9$ $P<0.001$	$F_{1,157}=9.29$ $P=0.003$	$F_{1,157}=213.2$ $P<0.001$	$F_{1,156}=111.2$ $P<0.001$	$F_{1,156}=2.9$ $P=0.09$
Morph		$F_{1,157}=0.24$ $P=0.62$	$F_{1,157}=7.0$ $P=0.009$	$F_{1,156}=327$ $P<0.001$	$F_{1,156}=18.5$ $P<0.001$
Protein	$F_{1,163}=5.69$ $P=0.02$	$F_{1,157}=19.4$ $P<0.001$	$F_{1,157}=22.9$ $P<0.001$	$F_{1,156}=0.21$ $P=0.65$	$F_{1,156}=41.7$ $P<0.001$
Carbohydrate	$F_{1,163}=5.48$ $P=0.02$	$F_{1,157}=13.3$ $P<0.001$	$F_{1,157}=2.3$ $P=0.13$	$F_{1,156}=0.33$ $P=0.57$	$F_{1,156}=6.5$ $P=0.01$
Protein ²	$F_{1,163}=0.16$ $P=0.69$	$F_{1,157}=0.05$ $P=0.83$	$F_{1,157}=0.03$ $P=0.87$	$F_{1,156}=0.38$ $P=0.54$	$F_{1,156}=0.37$ $P=0.55$
Carbohydrate ²	$F_{1,163}=6.38$ $P=0.01$	$F_{1,157}=5.7$ $P=0.02$	$F_{1,157}=6.0$ $P=0.02$	$F_{1,156}=0.27$ $P=0.60$	$F_{1,156}=5.4$ $P=0.02$
Protein×carbohydrate	$F_{1,163}=0.02$ $P=0.90$	$F_{1,157}=5.3$ $P=0.02$	$F_{1,157}=4.6$ $P=0.03$	$F_{1,156}=1.93$ $P=0.17$	$F_{1,156}=5.7$ $P=0.02$
Morph×protein		$F_{1,157}=0.56$ $P=0.46$	$F_{1,157}=0.17$ $P=0.68$	$F_{1,156}=6.9$ $P=0.01$	$F_{1,156}=8.4$ $P=0.004$
Morph×carbohydrate		$F_{1,157}=6.6$ $P=0.01$	$F_{1,157}=2.2$ $P=0.14$	$F_{1,156}=0.019$ $P=0.89$	$F_{1,156}=3.4$ $P=0.07$
Morph×protein ²		$F_{1,157}=1.3$ $P=0.26$	$F_{1,157}=0.31$ $P=0.58$	$F_{1,156}=0.27$ $P=0.61$	$F_{1,156}=0.40$ $P=0.52$
Morph×carbohydrate ²		$F_{1,157}=0.13$ $P=0.72$	$F_{1,157}=1.04$ $P=0.31$	$F_{1,156}=0.79$ $P=0.37$	$F_{1,156}=0.72$ $P=0.40$
Morph×protein×carbohydrate		$F_{1,157}=5.4$ $P=0.02$	$F_{1,157}=1.6$ $P=0.21$	$F_{1,156}=1.24$ $P=0.27$	$F_{1,156}=4.2$ $P=0.04$
Model adjusted R^2	0.15	0.52	0.68	0.92	0.46
Morph differences: partial F -test between models with/without six 'morph' terms	$F_{6,157}=0.96$ $P=0.46$	$F_{6,157}=2.27$ $P=0.039$	$F_{6,157}=4.99$ $P=0.001$	$F_{6,156}=191$ $P<0.001$	$F_{6,156}=14.4$ $P<0.001$

Initial cricket mass was included in models as a covariate, and protein and carbohydrate model terms were standardized to a scale from -1 to 1. Model term significance was assessed with partial F -tests. Bold indicates significance at the $\alpha=0.05$ level and italicized terms are marginally significant ($\alpha<0.10$).

(supplementary material Table S2) and the intake array (Fig. 2C) show that SW crickets achieved similar macronutrient intake on all but the three diets with the lowest nutrient content, which is indicated by the cluster of intake points lying roughly equidistant from the origin. LW(f) crickets, in contrast, tended to ingest macronutrients in proportion to macronutrient concentration in each diet (significant linear protein and carbohydrate terms; supplementary material Table S1). Inspection of the intake array for LW(f) crickets (Fig. 2C) shows that across the five isocaloric foods (42% macronutrient concentration), protein + carbohydrate intake was highest on the balanced diet compared with either protein-biased or carbohydrate-biased diets. This produced a curved line on the intake array for these diets (Fig. 2C), which repeats a pattern shown previously for LW(f) crickets (Clark et al., 2013). Overall, macronutrient intake was twice as high for high-concentration diets (>300 mg total p+c consumed), compared with low-concentration diets (160 mg total p+c consumed).

Physiological consequences of diets on mass gain, tissue gain and nutrient stores

All experimental crickets were weighed at day zero (adult molt), and the SW experimental crickets were significantly smaller than the LW(f) crickets (t -test: $t_{289}=6.49$, $P<0.001$; SW=597±10 mg, LW(f)=667±9 mg live mass). At the end of the 5 day feeding period, a significant morph effect and a significant protein×carbohydrate interaction were observed for mass gain (Table 2; supplementary material Tables S1, S2). Averaged across all the treatments, SW

crickets gained more mass than LW(f) crickets, and for both morphs the greatest gains occurred on high-concentration, high-protein diets (e.g. p28:c14; Fig. 3A).

As the focus of this paper is on life-history trade-offs, we were particularly interested in how mass of the flight muscles and ovaries, after feeding for 5 days on the different diets, diverged between the morphs, and may have changed in a nutrient-dependent manner. In terms of flight muscle, we found a significant morph×protein interaction (Table 2; supplementary material Tables S1, S2). For SW crickets, flight muscle mass was similar (on average 5.0±0.2 mg) across all the diets (Fig. 3B); however, compared with day zero SW crickets, flight muscle mass decreased by ~50% (Fig. 1A; model coefficients <1; see supplementary material Table S2). In contrast, flight muscle mass for LW(f) crickets increased on all treatments after 5 days of feeding as an adult, but increases were not constant across the different diets – instead, flight muscle mass increased as food protein concentration increased (Fig. 3B; supplementary material Table S1).

Both cricket morphs had increased ovary mass by day 5 of adulthood (Fig. 1), but more importantly we found that ovary mass changed in a significant morph×protein×carbohydrate manner (Table 2). Two observations reveal the nature of this interaction. First, on equivalent diets, the ovary mass of SW crickets was always greater compared with that of LW(f) crickets (higher intercept estimate; supplementary material Tables S1, S2). Second, the range of ovary masses across the protein–carbohydrate nutritional landscape was much wider for SW crickets (21–86 mg) compared

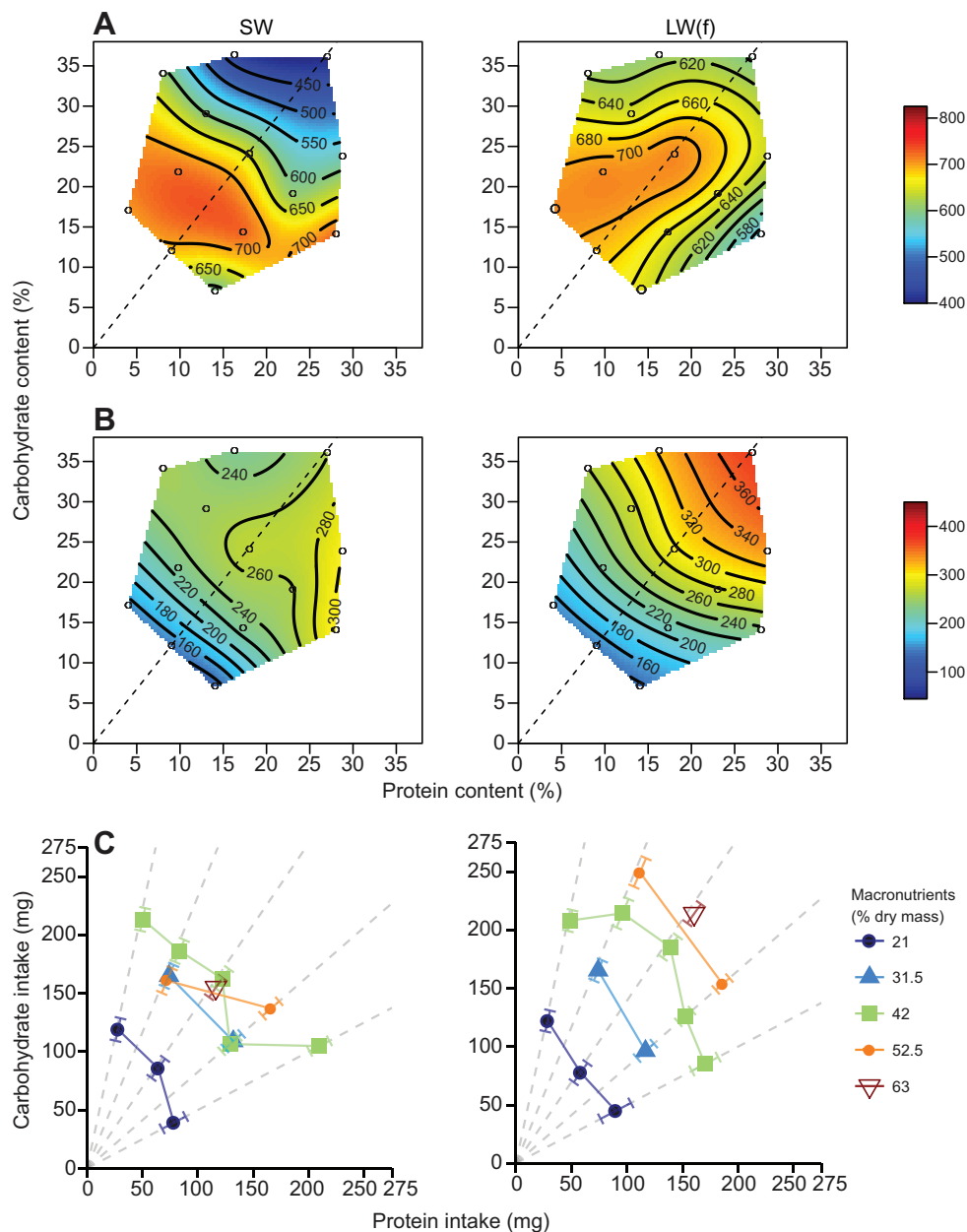


Fig. 2. Mean cricket consumption patterns across diets with different protein-carbohydrate content. Each nutrient is expressed as a percentage of dry mass. SW ($N=79$) and LW(f) ($N=91$) crickets were given access to one of 13 diets (open circles) containing different amounts of protein and carbohydrate for the first 5 days of adulthood. The total amount of food consumed (A) and macronutrients ingested (B) (both in mg) are mapped as non-parametric thin-plate spline response surfaces to allow detailed visualization of responses across the nutrient landscape. The dashed line indicates the average self-selected ratio of protein to carbohydrate from a prior experiment (Clark et al., 2013). Associated parametric statistics are given in Table 2. Macronutrient ingestion is also presented as a bi-coordinate plot of the mean (\pm s.e.m.) protein and carbohydrate ingested for each diet (C). Dashed lines indicate food ratios, and the colored, solid lines connect intake points across each of the five macronutrient concentrations offered.

with LW(f) crickets (14–38 mg). This was associated with larger coefficient estimates for the SW morph for protein and carbohydrate (significant linear protein and quadratic carbohydrate effects; supplementary material Tables S1, S2). Peak ovary size for both morphs occurred on diets that had high protein paired with moderate carbohydrate content (Fig. 3C), although peak ovary mass for SW crickets was approximately two times (197%) greater than for LW(f) crickets. Furthermore, there was a smaller magnitude of difference in ovary mass between the morphs on diets with high-carbohydrate content (>28%), or at very low-carbohydrate content (7%), compared with treatments with carbohydrate content between these two values.

Next, we analyzed lipid amounts in the soma (carcass minus flight muscle and ovaries) and ovaries, for both morphs, across the different diets (Table 3). Total lipids (for all tissues combined except flight muscle) were similar between morphs, but were dependent on diet carbohydrate content, as indicated by a significant linear carbohydrate effect ($c=20.4\pm 3.6$), but not diet protein content; the

intercept and initial cricket mass terms were significant (intercept= -41.9 ± 12.7 ; initial mass= 0.19 ± 0.02). However, when analyzed on a tissue-specific level, important morph- and diet-dependent differences revealed how lipids were distributed across somatic versus reproductive tissues, and over the nutrient landscape (Table 3). First, we found significant linear morph and carbohydrate effects for somatic (carcass) lipid contents (Fig. 4A; supplementary material Tables S1, S2). LW(f) crickets had higher somatic lipid amounts than SW crickets across the full nutrient landscape, with peak values of 117 ± 7 mg on the balanced, highest macronutrient diet (p27:c36). In contrast, for the SW crickets, peak lipid amounts of 79 ± 8 mg occurred on the very carbohydrate-biased diet (p8:c34). Second, analysis of ovary lipids revealed a significant morph \times protein effect (Table 3; supplementary material Tables S1, S2). Ovary lipid amounts were consistently higher in SW crickets across all of the diets, and peaked strongly on the high-macronutrient, protein-biased diet p28.75:c23.75, reaching a total of 13 ± 3 mg. Ovary lipids were also maximal for LW(f) crickets on this

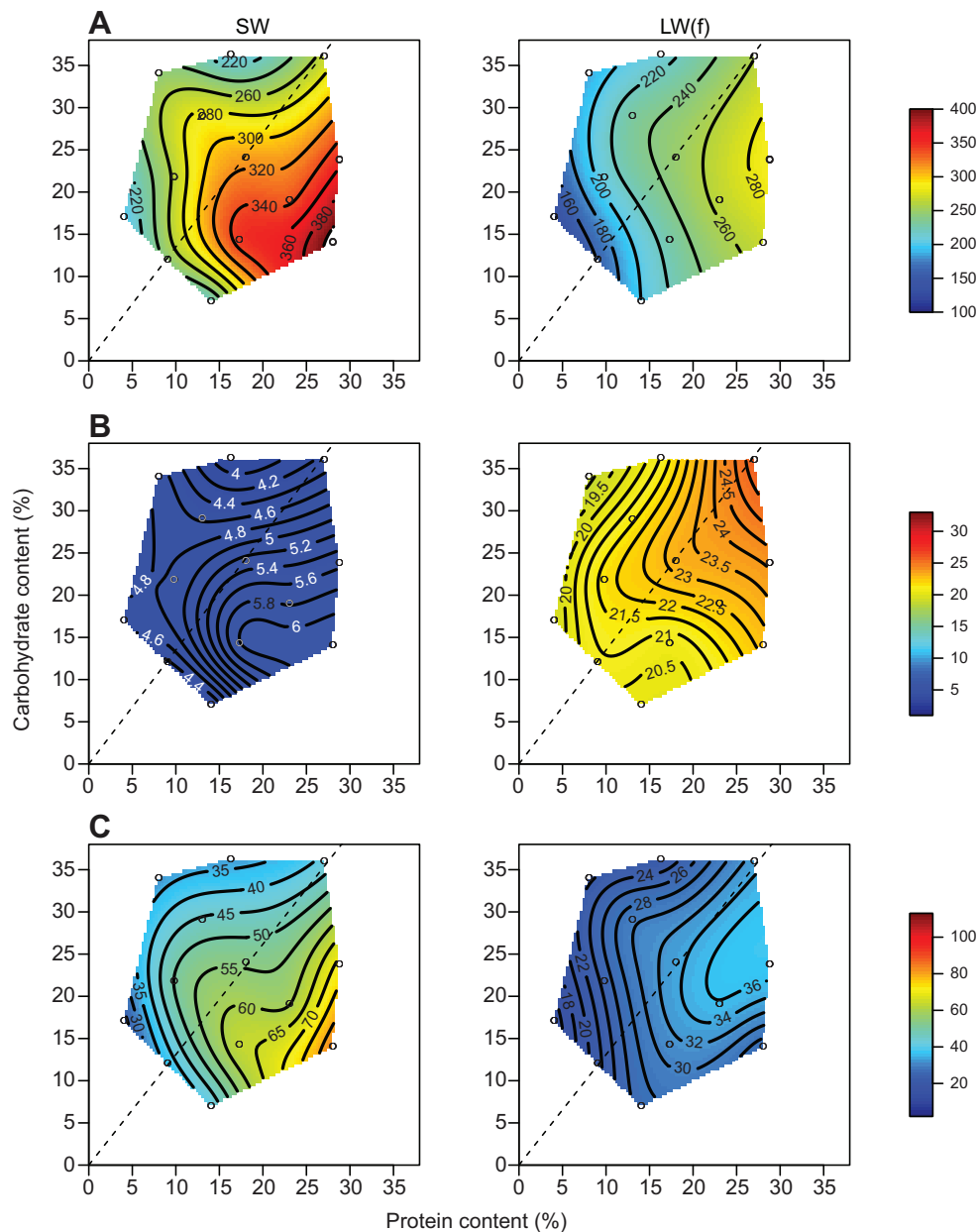


Fig. 3. Tissue mass responses to diets that differ in protein and carbohydrate content. Mean cricket wet mass gain (A), flight muscle mass (B) and ovary mass (C) (all in mg). For detailed information about sample sizes and symbols, refer to the legend for Fig. 2. Associated parametric statistics are given in Table 2.

diet, as well as diet p27:c36, but only reached a maximum of 5 ± 1 mg, up from a minimum of just 2 ± 1 mg on diet p9:c12. Viewed on a percentage basis, analysis of the proportion of the ovaries that was composed of lipid indicated no difference between the morphs, but a significant quadratic protein effect, due to a low percentage of ovary lipids for diets in the center of the nutrient landscape (13.2% lipids) compared with the fringes (~15–19% lipids; see supplementary material Fig. S1, Table S3).

Finally, we analyzed the protein content of the soma (carcass minus flight muscle and ovaries) and flight muscles. Total somatic protein content changed in a significant morph \times protein \times carbohydrate manner (Table 3; supplementary material Tables S1, S2). For LW(f) crickets, the highest somatic protein amounts (~157–158 mg) occurred in individuals on balanced, high-concentration diets (e.g. p27:c36 and p28.75:c23.75), whereas SW crickets had the highest somatic protein amounts (peaking at 174 ± 1 mg) on a high-nutrient, very protein-biased diet (e.g. p28:c14; Fig. 5). Correspondingly, the quadratic protein and carbohydrate model terms were significant for the SW

morph, whereas they were non-significant for the LW(f) morph (supplementary material Tables S1, S2). Flight muscle protein content also shifted in a significant morph \times protein \times carbohydrate fashion, in correspondence with the changes in flight muscle size (Table 3; supplementary material Tables S1, S2). Flight muscle protein was unilaterally higher in the LW(f) morph across all the treatments. It peaked in LW(f) crickets at 20 ± 7 mg on diet p27:c36, but even on diets with a lower macronutrient content (e.g. p8:c34), flight muscle contained at least 13 mg of protein. Meanwhile, SW crickets had only between ~3 and 4 mg of protein in flight muscle across the nutrient landscape. When flight muscle protein was analyzed as a percentage of total flight muscle composition, the morph difference remained, in the form of a significant morph \times carbohydrate effect (supplementary material Table S3). The percentage of flight muscle consisting of protein occurred across a narrower range for LW(f) crickets (71–76%) compared with the SW morph (65–77%; supplementary material Fig. S1B). Protein percentages were lower on diets with a higher carbohydrate content (e.g. p8:c34).

Table 3. Statistical results for response surface models testing the effects of protein and carbohydrate concentration and morph type [LW(f) versus SW] on day 5 body composition

Model terms	Somatic + ovary lipids	Somatic lipids	Ovary lipids	Somatic protein	Flight muscle protein
Full model	$F_{6,141}=23.4$ $P<0.001$	$F_{12,142}=12.8$ $P<0.001$	$F_{12,146}=8.47$ $P<0.001$	$F_{12,151}=34.7$ $P<0.001$	$F_{12,155}=168$ $P<0.001$
Intercept	$F_{1,141}=11.0$ $P=0.001$	$F_{1,157}=18.8$ $P<0.001$	$F_{1,157}=43.6$ $P<0.001$	$F_{1,156}=34.1$ $P<0.001$	$F_{1,156}=18.3$ $P<0.001$
Initial cricket mass (covariate)	$F_{1,141}=103.2$ $P<0.001$	$F_{1,157}=9.29$ $P=0.003$	$F_{1,157}=213.2$ $P<0.001$	$F_{1,156}=111.2$ $P<0.001$	$F_{1,156}=2.9$ $P=0.09$
Morph		$F_{1,142}=4.00$ $P=0.047$	$F_{1,146}=13.3$ $P<0.001$	$F_{1,151}=6.46$ $P=0.01$	$F_{1,155}=351$ $P<0.001$
Protein	$F_{1,141}=0.10$ $P=0.76$	$F_{1,142}=1.40$ $P=0.24$	$F_{1,146}=32.1$ $P<0.001$	$F_{1,151}=72.3$ $P<0.001$	$F_{1,155}=0.86$ $P=0.35$
Carbohydrate	$F_{1,141}=32.5$ $P<0.001$	$F_{1,142}=9.6$ $P=0.002$	$F_{1,146}=0.39$ $P=0.53$	$F_{1,151}=6.58$ $P=0.01$	$F_{1,155}=0.002$ $P=0.97$
Protein ²	$F_{1,141}=0.024$ $P=0.88$	$F_{1,142}=0.22$ $P=0.64$	$F_{1,146}=0.08$ $P=0.77$	$F_{1,151}=0.90$ $P=0.34$	$F_{1,155}=0.77$ $P=0.38$
Carbohydrate ²	$F_{1,141}=1.83$ $P=0.18$	$F_{1,142}=63$ $P=0.43$	$F_{1,146}=3.00$ $P=0.085$	$F_{1,151}=6.84$ $P=0.01$	$F_{1,155}=0.23$ $P=0.63$
Protein×carbohydrate	$F_{1,141}=0.004$ $P=0.95$	$F_{1,142}=0.31$ $P=0.58$	$F_{1,146}=1.28$ $P=0.26$	$F_{1,151}=6.15$ $P=0.01$	$F_{1,155}=1.29$ $P=0.26$
Morph×protein		$F_{1,142}=1.6$ $P=0.22$	$F_{1,146}=8.64$ $P=0.004$	$F_{1,151}=2.31$ $P=0.13$	$F_{1,155}=8.54$ $P=0.004$
Morph×carbohydrate		$F_{1,142}=0.95$ $P=0.33$	$F_{1,146}=0.51$ $P=0.48$	$F_{1,151}=10.8$ $P=0.001$	$F_{1,155}=0.087$ $P=0.77$
Morph×protein ²		$F_{1,142}=0.05$ $P=0.82$	$F_{1,146}=0.56$ $P=0.46$	$F_{1,151}=0.009$ $P=0.93$	$F_{1,155}=0.14$ $P=0.71$
Morph×carbohydrate ²		$F_{1,142}<0.001$ $P>0.99$	$F_{1,146}=0.57$ $P=0.45$	$F_{1,151}=1.08$ $P=0.30$	$F_{1,155}=1.56$ $P=0.21$
Morph×protein×carbohydrate		$F_{1,142}=0.22$ $P=0.64$	$F_{1,146}=0.61$ $P=0.43$	$F_{1,151}=7.63$ $P=0.006$	$F_{1,155}=4.22$ $P=0.04$
Model adjusted R^2	0.48	0.48	0.36	0.71	0.92
Morph differences: partial F -test between models with/without six 'Morph' terms	$F_{6,135}=1.13$ $P=0.35$	$F_{6,142}=2.44$ $P=0.028$	$F_{6,146}=10.2$ $P<0.001$	$F_{6,151}=7.0$ $P<0.001$	$F_{6,155}=205$ $P<0.001$

Initial cricket mass was included in models as a covariate, and protein and carbohydrate model terms were standardized to a scale from -1 to 1 . Model term significance was assessed with partial F -tests. Bold indicates significance at the $\alpha=0.05$ level and italicized terms are marginally significant ($\alpha<0.10$).

DISCUSSION

The expression and evolution of life-history trade-offs is hypothesized to be linked to the forms of nutrient limitation that an organism experiences (Boggs, 1992; Boggs and Ross, 1993; Zera and Harshman, 2001; Fanson et al., 2012). Here, we have explicitly tested how the ratio and amounts of food protein and carbohydrate affect consumption and allocation patterns in association with a key life-history trade-off between dispersal and reproduction. In line with our predictions, *G. firmus* crickets showed morph-specific intake responses to food protein–carbohydrate content and balance, which in turn influenced mass gain and allocation to organs and the corresponding metabolites used for dispersal versus reproduction. The differences in intake and in the nutrient requirements for dispersal versus reproduction translated into separate optima for each morph and variation in the magnitude of the flight–dispersal trade-off across the nutrient landscape. Our results show, for the first time, how food protein and carbohydrate ratio and content are coupled to allocation on an organ-specific level, affecting the expression of this well-characterized life-history trade-off.

A food's nutrient content is a primary driver of animal feeding behavior, so the analysis of an animal's macronutrient intake patterns across a nutritional landscape is a critical step in understanding how nutrition influences subsequent aspects of performance (Waldbauer and Friedman, 1991; Chambers et al., 1995; Simpson et al., 2004; Behmer, 2009; Simpson and Raubenheimer, 2012). Without knowing what is consumed, and how much, it is difficult to elucidate how nutrients are allocated, and the

nature of constraints affecting allocation. The finding that each morph adjusted macronutrient intake in different ways confirms earlier work that showed, over five isocaloric diets, that the morphs employ different 'consumption rules' (Clark et al., 2013). Because the current study explored nutrient intake patterns over a broad protein–carbohydrate nutritional landscape that included changes in both protein–carbohydrate ratio and total macronutrient concentration, the full nature of the morphs' consumption rules is now revealed. The intake response of the SW crickets – similar total macronutrient intake across all except the most nutrient-poor diets, even those with widely different protein–carbohydrate ratios – suggests SW crickets have an intrinsic upper limit or ceiling to total nutrient intake, as has been previously observed in generalist caterpillars (Simpson et al., 2004; Lee et al., 2004). The presence of a ceiling suggests there might be a cost for SW crickets overeating total amounts of protein and carbohydrate, perhaps due to limits on the morph's ability to increase its overall rate of nutrient processing above a threshold level. The LW(f) intake pattern, in contrast, indicates a lack of tight regulation in response to total diet macronutrient content, particularly on diets that were not strongly imbalanced with respect to their protein–carbohydrate ratio. This suggests that LW(f) crickets will maximize food intake when they have access to foods that are nutrient rich and have a relatively balanced protein–carbohydrate ratio. However, LW(f) crickets did show sensitivity to protein–carbohydrate balance across the five isocaloric diets, reinforcing our prior finding that LW(f) crickets employ a consumption strategy to minimize intake of whichever

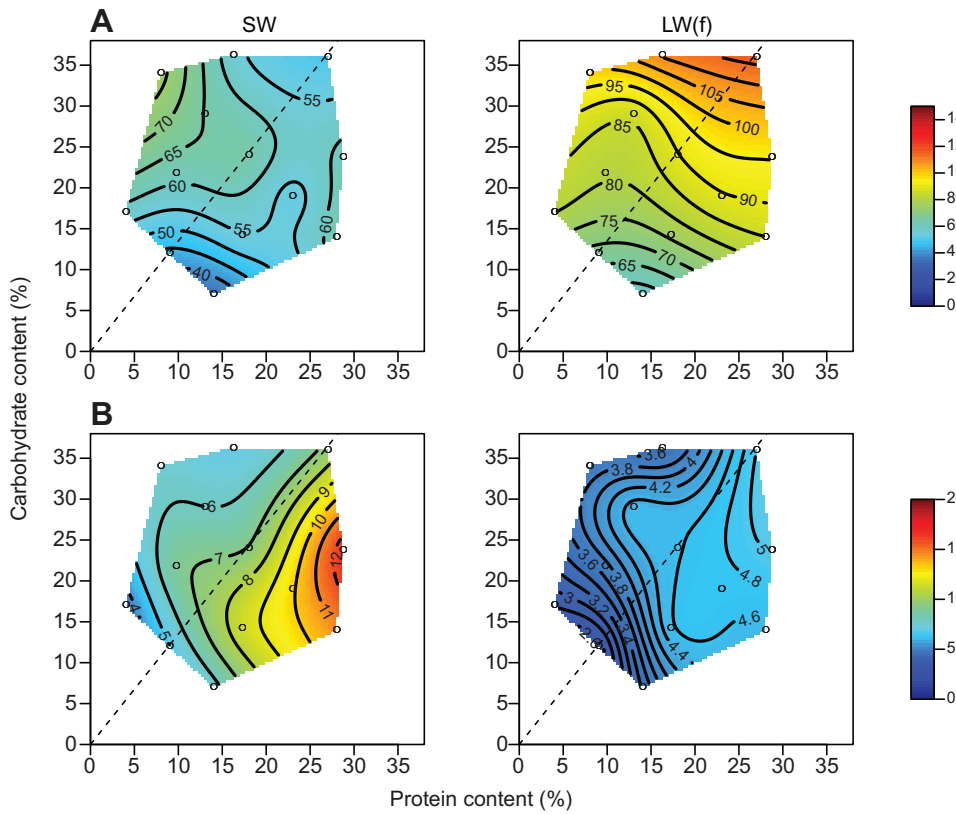


Fig. 4. Mean cricket lipid levels as a function of diet protein and carbohydrate content. Body lipid patterns (A) and ovary lipid patterns (B) (both in mg) are compared for SW and LW(f) adult crickets provided for 5 days with one of 13 diets containing different total amounts of protein and carbohydrate (open circles). For additional figure details, including sample sizes, refer to Fig. 2. Associated statistics are given in Table 3.

nutrient is in excess, while maximizing intake of the nutrient in deficit (Clark et al., 2013).

The contrasting effects of protein–carbohydrate ratio and concentration on macronutrient intake in the two morphs had direct

implications for how the morphs allocated resources to tissues and metabolite pools under different nutritional contexts, particularly because proteins and carbohydrates are only partially interchangeable (Simpson et al., 2004). For instance, while

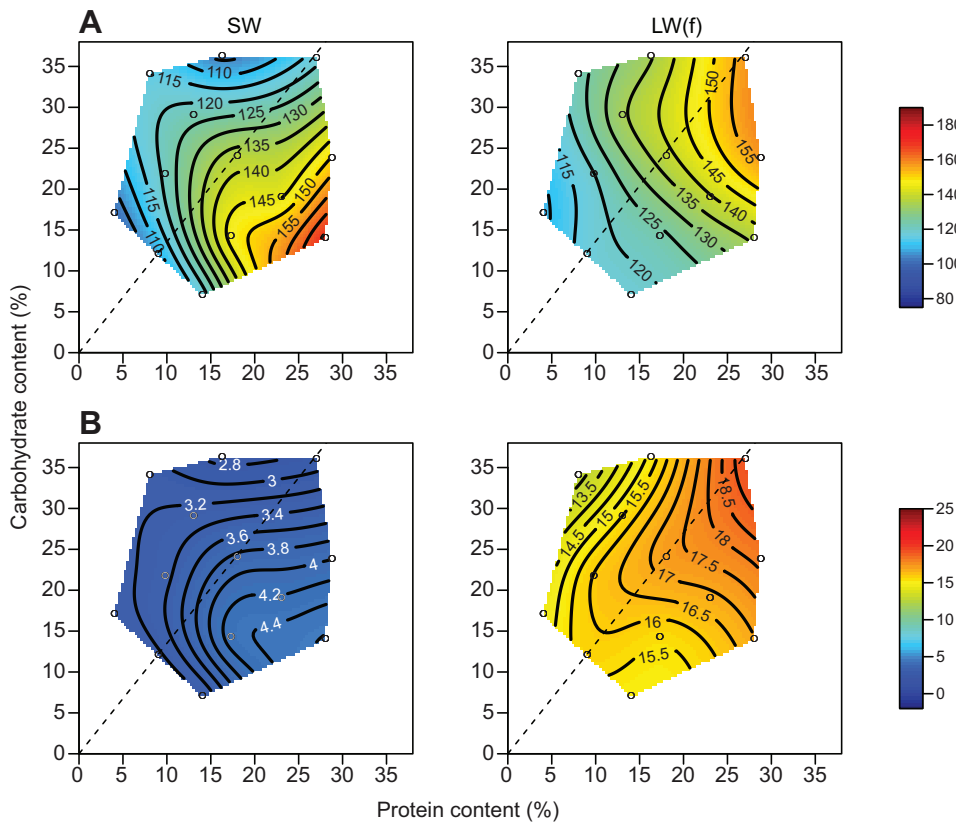


Fig. 5. Cricket tissue protein amounts relative to diet composition. Mean cricket somatic (A) and flight muscle (B) protein content (both in mg) as a function of dietary protein and carbohydrate content. Protein levels are compared for SW and LW(f) adult crickets provided for 5 days with one of 13 diets containing different total amounts of protein and carbohydrate (open circles). For additional figure details, including sample sizes, refer to Fig. 2. Statistics are given in Table 3.

gluconeogenesis provides a pathway for the generation of glucose from amino acids, this process is metabolically expensive and inefficient (van Milgen, 2002; Karasov and Martínez del Rio, 2007), and normally only happens under extreme conditions (e.g. starvation). In contrast, carbohydrates can never substitute for amino acids required in the assembly of structural, storage or enzymatic proteins. Indeed, changes in diet nutrient concentration and ratio had morph-specific effects on the end products of metabolic and allocation trade-offs within each morph – for example, on organ masses and nutrient stores in the soma, flight muscles and ovaries (Zhao and Zera, 2006).

Interestingly, the diet treatments that correspond to optima for each life-history strategy differed between morphs, and are distinct from, but related to, their previously identified self-selected nutrient targets (Clark et al., 2013). Optimal lipid acquisition in the LW(f) morph occurred on the balanced, concentrated diet p27:c36, which corresponds clearly and directly with their self-selected intake ratio (p1:c1.62; dashed line used to center the nutrient landscape in Fig. 4) (Clark et al., 2013). In contrast, for the SW morph, ovary mass was greatest on the very protein-biased diet p28:c14, which deviates from the SW self-selected ratio (p1:c1.30) (Clark et al., 2013), which was protein-biased compared with that of the LW(f) morph but not nearly so extreme as the 2:1 ratio of diet p28:c14. The deviation for SW crickets suggests that factors other than reproductive demands may influence their nutrient intake during early adulthood. Lee et al. (Lee et al., 2008) and Maklakov et al. (Maklakov et al., 2008) also found distinct and different nutritional optima for the life-history traits of survival and reproduction in *Drosophila* fruit flies and *Teleogryllus* field crickets. In both cases, self-selected protein–carbohydrate ratios occurred intermediate between the optima for survival and reproduction.

Largely as a result of their strategy of ‘compensatory’ feeding in response to changes in total nutrient concentration, the mass and ovary gains in SW females showed a strong, positive response to food protein content; the SW morph gained the most mass and developed the largest ovaries on the most protein-biased diet. This means that the mechanisms used by SW females to preferentially divert protein towards egg production are enhanced in settings where protein is abundantly available. SW somatic lipid content showed the opposite pattern, and was sensitive to food carbohydrate content, peaking on carbohydrate-biased foods with high-nutrient concentrations. SW females do not utilize somatic lipid stores for flight in the same fashion as their LW(f) counterparts, meaning that the SW morph may process and store excess carbohydrates as lipids either as a buffer against environmental variation in energy availability or as an intermediate step before allocation to egg production.

Consistent with findings from a prior series of simple diet dilution experiments (Zera and Brink, 2000; Zera and Larsen, 2001), we found that LW(f) females preferentially retained flight muscle and somatic lipid stores at the expense of ovary development and overall mass gain, across the entire nutritional landscape. That is, the flight–fecundity trade-off is highly canalized. As LW(f) crickets retained flight muscle and high-lipid stores, once crickets are committed to the dispersal strategy, the maintenance of flight capability is inflexible and prioritized across nutritional environments. However, the means to this end may depend on the specific dietary context, especially given that LW(f) nutrient intake depended upon food protein–carbohydrate ratio and concentration. On a ‘standard’ laboratory diet, increased lipid synthesis by the LW(f) morph was shown to result from the utilization of a greater

proportion of fatty acid for glyceride biosynthesis over oxidation (Zera, 2005), as well as preferential metabolism of amino acids for use in fatty acid production and storage (Zera and Zhao, 2006; Zhao and Zera, 2006). In a simple diet dilution experiment, Zera and Larsen (Zera and Larsen, 2001) found that on a dilute (25%) diet, the LW(f) morph had lower triglyceride levels compared with early adulthood, but still managed to retain higher levels than the SW morph, most likely via decreased lipid utilization. Together, these lead to increases in somatic triglyceride storage relative to SW crickets. All of these mechanisms are likely to have been involved in generating the higher somatic lipid levels observed here, and are currently under investigation. Indeed, we have found that triglyceride biosynthesis is strongly elevated in the LW(f) compared with the SW morph across the entire nutritional landscape (A.J.Z., R.M.C. and S.T.B., unpublished data). In the present experiment, enhanced lipid biosynthesis most likely came at the cost of lower overall mass gain by LW(f) crickets across all diets, despite the general positive effect of food protein content on mass gain in both morphs. The diversion of nutrients to lipid synthesis might also be what allowed LW(f) crickets to consume greater total amounts of macronutrients when provided with more nutrient-dense foods, preventing the form of nutrient constraint observed in the SW morph.

The differential morph responses across the nutritional landscape in terms of protein and carbohydrate intake indicate that morph differences in protein and carbohydrate acquisition need to be explicitly taken into account in biochemical studies of internal resource allocation underlying the flight capability–fecundity trade-off. This will result in more refined nutritionally explicit models of internal allocation with respect to protein and carbohydrate inputs and their effects on specific metabolic processes and life-history traits (Boggs, 2009; Fanson et al., 2012). To date, much of the effort to explicitly link diet protein–carbohydrate (nutrient) content to allocation has focused on a putative nutrition-mediated trade-off between lifespan and reproduction, as detailed for *Drosophila*, Queensland fruit flies and crickets (Lee et al., 2008; Maklakov et al., 2008; Skorupa et al., 2008; Fanson and Taylor, 2012; Piper et al., 2014). In these cases, optimal lifespan and maximal reproduction occur at different balance of proteins to carbohydrates, indicating that animals are forced to compromise their intake strategy to reach a point between two different optima. It is critical to identify how specific nutrient allocation mechanisms generate such purported trade-offs, as in at least one case, what appeared superficially to be a lifespan–reproduction trade-off mediated by protein–carbohydrate balance can be more directly explained as a protein or amino acid dosage effect that can be decoupled from reproduction (Grandison et al., 2009; Fanson et al., 2012). Part of the remaining challenge for studies of allocation will therefore be to characterize the causal mechanisms connecting differential nutrient intake to differential allocation in the context of clearly defined life-history trade-offs, such as how diet protein–carbohydrate content is linked to hormonal shifts and changes in the flow of metabolites through specific pathways of intermediary metabolism (Harshman and Zera, 2007; Karasov and Martínez del Rio, 2007). This should effectively link a rich research tradition in nutritional biology to an equally rich body of work on organismal growth and production historically based on bioenergetics.

MATERIALS AND METHODS

Crickets and experimental chambers

Female crickets came from large, outbred populations (greater than 200 breeders in each generation), maintained at the University of Nebraska-

Lincoln, that were artificially selected to produce either the flight-capable [LW(f)] or flightless (SW) morph (see Zera and Larsen, 2001; Zera, 2005). Nearly all (>95%) SW adults have vestigial flight muscles and are flightless. LW(f) individuals emerge with large flight muscles, which most (>85%) retain through to day 5 of adulthood (Zera et al., 1997). Past day 5, flight muscle histolysis, coupled with enhanced ovarian growth, occurs with increasing frequency in LW(f) individuals, converting them to the flightless [LW(h)] morph. All LW crickets used in the present experiment were dissected to confirm flight muscle status, and 28 LW(h) crickets were excluded from the sample sizes and analyses reported below because they represent a physiologically indistinct intermediate phenotype (Zera et al., 2007). We tested for the effects of the diet treatments on the probability that day 5 LW flight muscle histolyzed by constructing a generalized linear model with a binomial link function, flight muscle condition as the dependent variable (pink and flight capable or white and histolyzed), and linear protein and carbohydrate terms and the protein×carbohydrate interaction term as predictors. This model was tested by comparison against a null model (intercept only), using a likelihood ratio test with the chi-square statistic (Everitt and Hothorn, 2010). There were no differences in the incidence of LW(h) individuals across the diet treatments at day 5 ($\chi^2=1.65$, d.f.=3, $P=0.65$).

The present experiments compared one LW(f)-selected and one SW-selected population from one of three blocks (block 2) of a larger artificial selection experiment. Each block of the selection experiment represents an independent artificial selection trial involving one pair of LW(f) and SW selected populations. Previous studies have shown, without exception, that the biochemical, endocrine, morphological and reproductive differences between LW(f) and SW selected populations of any block are similar to differences between selected populations of the other two blocks (Zera, 2005). Therefore, comparisons made between LW(f) and SW populations of any one block should be representative of general differences between LW(f) and SW selected populations.

Juvenile crickets were shipped from the University of Nebraska-Lincoln to Texas A&M University, where they were raised to adulthood for experimental work. Groups of ~50 individuals were reared in 171 transparent plastic boxes kept in an incubator with a 16 h:8 h light:dark cycle at a temperature of 28–29°C. Crickets were fed an *ad libitum* 'standard' diet of wheatgerm, wheatbran, whole milk powder and nutritional yeast (Zera and Larsen, 2001), and were given water in 2 ounce (~57 ml) plastic deli containers fitted with cotton wicks. Boxes were checked two to three times a day for newly molted adults, which were weighed and placed individually into small, plastic arenas (18.9×13.5×9.5 cm). In the arenas, crickets were provided with pre-weighed, spill-resistant dishes of dry synthetic food, which varied in protein–carbohydrate content (Raubenheimer and Simpson, 1990) (see below). The plastic arenas also housed an aluminium perch and distilled drinking water in a 1 ounce (~28 ml) plastic container with a cotton wick.

Diets

A total of 13 experimental diets that varied in their protein (p) and digestible carbohydrate (c) content were used (Table 1). These represented five p:c ratios, characterized relative to the crickets' nutritional needs: (1) balanced, (2) carbohydrate biased, (3) protein biased, (4) very carbohydrate biased and (5) very protein biased. For each p:c ratio, two or three total macronutrient levels (ranging from 21% to 63%) were studied. The first three diets listed in Table 1 had 21% total macronutrient content: (a) 4% protein and 17% carbohydrate [p4:c17], (b) p9:c12 and (c) p14:c7. The next two diets had 31.5% total macronutrient content: (d) p9.75:c21:75 and (e) p17.25:c14.25. The third set contained 42% total macronutrient content: (f) p8:c34, (g) p13:c29, (h) p18:c24, (i) p23:c19 and (j) p28:c14. Two diets contained 52.5% macronutrients: (k) p16.25:c36.25 and (l) p28.75:c23.75. Finally, the thirteenth diet contained 63% total macronutrient content: (m) p27:c36. The protein portion of the diet was a 3:1:1 mixture of casein, peptone and albumin; the digestible carbohydrate portion was a 1:1 mixture of sucrose and starch. Indigestible bulk cellulose was substituted for protein and carbohydrate to adjust total macronutrient content, while other diet ingredients were kept consistent between diets (e.g. vitamins, cholesterol and fatty acids). The synthetic diets used here were based on synthetic diets

originally created and modified for grasshoppers (Dadd, 1961; Simpson and Abisgold, 1985; Behmer et al., 2001); they were prepared as described elsewhere (Behmer et al., 2003). Protein and digestible carbohydrates have approximately equivalent caloric value, so diets with similar total macronutrient content (despite having different protein–carbohydrate ratios) are calorically equivalent.

Feeding experiment

To study the consequences of food intake under predefined nutritional conditions, newly emerged adult females were weighed and then allowed to feed *ad libitum* for 5 days on one of the 13 foods described above (sample sizes given in Table 1). Five days later, food dishes were removed and re-weighed, and crickets were weighed to determine their final wet mass. The crickets were frozen for dissection and measurement of flight muscles, ovaries and body tissue composition. Ovaries and flight muscles (including both dorsoventral and dorsal–longitudinal muscles) were dissected from cricket carcasses and dried along with the carcasses at 70°C for at least 3 days, after which dry mass was measured. To estimate total somatic lipids, carcasses were homogenized with a mortar and pestle, and a subsample was weighed, enclosed in filter paper, soaked in three 24 h changes of chloroform, dried for 24 h and re-weighed (Loveridge, 1973). Somatic nitrogen content was measured in a second carcass subsample and in intact, dried flight muscle via combustion analysis with an Elementar CN vario Max (Elementar, Germany). Nitrogen measurements were converted to protein by multiplying by 6.25 (Robyt and White, 1990).

To assess changes in body condition and allocation from the beginning of adulthood, a second set of newly emerged day zero adult females was also collected as 'reference crickets'; these individuals were weighed following emergence, and then immediately frozen for the same dissection and measurement procedures described above.

Statistical analysis

All statistical analyses were performed in R (version 2.15.3), and values reported in the text are means ± s.e.m. Diet effects were assessed via general linear models of response surfaces using the package 'rsm' to standardize the protein and carbohydrate treatment axes (Lenth, 2009). The cricket's initial mass was used as a covariate to control for size differences. Response surface models included linear and quadratic terms for diet protein and carbohydrate content, as well as a protein×carbohydrate interaction term. To test for differences between cricket morphs, 'morph' and 'morph interaction' terms were added to a given model, and this model was compared against the original reduced model with a partial F-test. Where the two models were statistically significantly different, we interpreted this to indicate significant overall morph differences. Non-parametric response surface figures were generated with the thin-plate splines function (Tps) from the 'fields' package (Furrer et al., 2012), as these surfaces provide a more detailed visualization of the cricket data as compared with graphing the best-fitting response surface regression models.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.T.B. and A.J.Z. developed the concepts, approach, and experimental design, R.M.C. performed experiments and data analysis, and S.T.B., A.J.Z. and R.M.C. drafted, edited, and revised the manuscript.

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Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.112888/-DC1>

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