For: Journal of Experimental Biology

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

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KEYWORDS: feeding behavior, dispersal, reproductive physiology, nutrient allocation, sand cricket

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 alternate 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 vs. reproduction trade-off was modified across a heterogeneous protein-carbohydrate nutritional landscape. Newly molted adult female 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) vs. flight muscle maintenance (flight muscle mass and somatic lipid stores). Feeding responses in both morphs were influenced more by total macronutrient concentration than 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 lipids 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.

Introduction

Since the 1980's, 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, 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 practice compensatory feeding (Karasov and Martínez del Rio, 2007; Behmer, 2009) or (3) variation in calories was often confounded with variation in the amount of specific nutrients (discussed in 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 underlays 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, 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*), 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 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 protein 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 to not only 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 to 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-to-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 vs. reproduction life-history trade-off over the first five days of adulthood. To do so, we gave LW(f) and SW females one of thirteen diets with different concentrations and ratios of protein and carbohydrate for five 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 the pilot study of 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) were 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 response to nutrient heterogeneity, but would also set the stage for an analysis of the biochemical mechanisms underlying this response.

Results

Body conditions 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) flight muscles, (2) ovaries, (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±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±1.3%) than LW(f) crickets (50.2 ±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±1.9%).

Results from experimental manipulation of food protein-carbohydrate content. Throughout the rest of the results we present findings from a five-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 five-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*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 five-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 text) 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 reduced model, which omitted all "morph" terms, we found a significant quadratic carbohydrate term (c^2 = -112.57 ± 44.59), significant linear protein term (p = -52.44 ± 21.99), significant intercept (369.62 ± 87.21), significant covariate (initial mass, 0.49 ± 0.13); the protein-by-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 [CB], 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-by-protein-by-carbohydrate interaction effect (partial F-test: $F_{6,157}$=2.27, P=0.039; Table 2, supplementary material, Tables S1 and S2). A significant negative quadratic carbohydrate term (supplementary 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 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 to either protein-biased or carbohydrate-biased diets. This produced a curved line on the intake array for these diets (Figs. 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 to low-concentration diets (160 mg total p+c consumed).

Physiological consequences of diets on mass gains, tissue gains, 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 five-day feeding period, a significant morph effect, and a significant protein-by-carbohydrate interaction, were observed for mass gain (Table 2, supplementary Tables S1 and 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 five 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-by-protein interaction (Table 2, supplementary Tables S1 and S2). For SW crickets, flight muscle mass was similar (on average 5.0±0.2 mg) across all the diets (Fig. 3B); however, compared to day zero SW crickets, flight muscle mass decreased by ~50% (Fig. 1A; model coefficients < 1, see supplementary Table S2). In contrast, flight muscle mass for LW(f) crickets increased on all treatments after five 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 Table S1).

Both cricket morphs had increased ovary mass by day five of adulthood (Fig. 1), but more importantly we found that ovary mass changed in a significant morph-by-protein-by-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 to LW(f) crickets (higher intercept estimate, supplementary Tables S1 and S2). Second, the range of ovary masses across the protein-carbohydrate nutritional landscape was much wider for SW crickets (21-86 mg) compared to 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 Tables S1 and 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 ~2X (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 to 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 Tables S1 and 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 $\pm$ 8 mg occurred on the very carbohydrate-biased diet (p8:c34). Second, analysis of ovary lipids revealed a significant morph-by-protein effect (Table 3, supplementary Tables S1 and S2). Ovary lipid amounts were consistently higher in SW crickets across all of the diets, and peaked strongly on 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 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 comprised 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 to 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-by-protein-by-carbohydrate manner (Table 3, supplementary Tables S1 and 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 Tables S1 and S2). Flight muscle protein content also shifted in a significant morph-by-protein-by carbohydrate fashion, in correspondence with the changes in flight muscle size (Table 3, supplementary Tables S1 and 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 lower macronutrient content (e.g. p8:c34), flight muscle contained at least 13 mg of protein. Meanwhile, SW crickets had only between ~3-4 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-by-carbohydrate effect (supplementary Table S3). The percentage of flight muscle consisting of protein occurred across a narrower range for LW(f) crickets (71-76%) compared to the SW morph (65-77%; supplementary Figure S1B). Protein percentages were lower on diets with a higher carbohydrate content (e.g. p8:c34).

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 vs. reproduction. The differences in intake and in the nutrient requirements for dispersal vs. reproduction translated into separate optima for each morph, and in 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-to-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 p:c 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 nutrient is in excess, while maximizing intake of the nutrient in surfeit (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 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 – e.g. 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, Clark et al., 2013). In contrast, for the SW morph, ovary masses were 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 to 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. (2008) and 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 due to 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 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. Since 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 how 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 (2001) found that on a dilute (25%) diet, the LW(f) morph had lower triglyceride levels compared to 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. Zera, R. Clark, S. Behmer, 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 constraints observed in the SW morph.

The differential morph responses across the nutritional landscape in terms of protein and carbohydrate intake, indicates 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 efforts to explicitly link diet protein-carbohydrate (nutrient) content to allocation have 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 balances of protein-to-carbohydrate, 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.

Methods

Crickets - "Insects and Experimental Chambers"

Female crickets came from large, outbred populations (greater than 200 breeders each generation) maintained at the University of Nebraska-Lincoln, that were artificially selected to produce either the flight-capable [LW(f)] or flightless (SW) morphs (see Zera and Larsen, 2001 and Zera, 2005 for details). 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 day five of adulthood (Zera et al., 1997). Past day five, 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 five LW flight muscle histolysed 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-by-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 five (χ^2=1.65, df=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

448 biochemical, endocrine, morphological and reproductive differences between LW(f) and SW
449 selected populations of any block are similar to differences between selected populations of the
450 other two blocks (Zera, 2005). Therefore, comparisons made between LW(f) and SW
451 populations of any one block should be representative of general differences between LW(f) and
452 SW selected populations.
453
454 Juvenile crickets were shipped from the University of Nebraska-Lincoln to Texas A&M
455 University, where they were raised to adulthood for experimental work. Groups of
456 approximately 50 individuals were reared in 17-L transparent plastic boxes kept in an incubator
457 with a 16h:8h light:dark cycle at a temperature of 28-29° C. Crickets were fed an *ad libitum*
458 "standard" diet of wheat germ, wheat bran, whole milk powder, and nutritional yeast (Zera and
459 Larsen, 2001), and were given water in two-oz plastic deli containers fitted with cotton wicks.
460 Boxes were checked two to three times a day for newly molted adults, which were weighed and
461 placed individually into small, plastic arenas (18.9 x 13.5 x 9.5 cm). In the arenas, crickets were
462 provided with preweighed, spill-resistant dishes of dry synthetic foods, which varied in their
463 protein-carbohydrate content (Raubenheimer and Simpson, 1990; see below). The plastic arenas
464 also housed an aluminum perch and distilled drinking water in a one-ounce plastic container with
465 a cotton wick.
466
467 *Diets*
468 A total of 13 experimental diets that varied in their protein (p) and digestible carbohydrate (c)
469 content were used (Table 1). These represented five p:c ratios, characterized relative to the
470 crickets' nutritional needs: (1) balanced [B], (2) carbohydrate-biased [CB], (3) protein-biased
471 [PB], (4) very carbohydrate-biased [VCB], and (5) very protein-biased [VPB]. For each p:c ratio
472 two or three total macronutrient levels (ranging from 21% to 63%) were studied. The first three
473 diets listed in Table 1 had 21% total macronutrient content: (a) 4% protein and 17%
474 carbohydrate [p4:c17; VCB], (b) p9:c12 [B], and (c) p14:c7 [VPB]. The next two diets had
475 31.5% total macronutrient content: (d) p9.75:c21:75 [CB] and (e) p17.25:c14.25 [PB]. The third
476 set contained 42% total macronutrient content: (f) p8:c34 [VCB], (g) p13:c29 [CB], (h) p18:c24
477 [B], (i) p23:c19 [PB], and (j) p28:c14 [VPB]. Two diets contained 52.5% macronutrients: (k)
478 p16.25:c36.25 [CB] and (l) p28.75:c23.75 [PB]. Finally, the thirteenth diet contained 63% total

macronutrient content: (m) p27:c36 [B]. 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. Undigestible bulk cellulose was substituted for protein and carbohydrate to adjust total macronutrient contents, 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 in Behmer et al. (2003). Protein and digestible carbohydrates have approximately equivalent caloric value, so diets with similar total macronutrient content (despite having different ratios of protein-to-carbohydrate) 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 five 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 three days, after which dry masses were measured. To estimate total somatic lipids, carcasses were homogenized with a mortar and pestle, and a subsample was weighed, placed into filter paper; soaked in three 24-hour changes of chloroform, dried for 24 hours, 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.

510

511 *Statistical Analysis*

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

525

526

Acknowledgements

We are grateful to M. Le Gall for help with diet preparation, to K. Alander and L. Sherer for help with cricket rearing, and to two anonymous reviewers for helpful comments on the manuscript.

Funding

This research was supported by the US National Science Foundation [grant number IOS-1121960 to S.T.B., grant number IOS-1122075 to A.J.Z.].

Author Contributions

S.T.B. and A.J.Z. developed the concepts and approach, R.M.C. performed experiments and data analysis, and S.T.B., A.J.Z. and R.M.C. prepared and edited the manuscript prior to submission.

References

Bass, T.M., Grandison, R.C., Wong, R., Martinez, P., Partridge, L., and Piper, M.D.W. (2007). Optimization of dietary restriction protocols in *Drosophila*. *J. Gerontol. A-Biol.* **62**(10), 1071-1081.

Behmer, S.T. (2009). Insect herbivore nutrient regulation. *Annu. Rev. Entomol.* **54**, 165-187.

Behmer, S., Raubenheimer, D., and Simpson, S. J. (2001). Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Anim. Behav.* **61**, 995–1005.

Behmer, S., Cox, E., Raubenheimer, D., and Simpson, S. J. (2003). Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Anim Behav.* **66**, 665–675.

Boggs, C. (1992). Resource allocation - Exploring connections between foraging and life history. *Funct. Ecol.* **6**(5), 508–518.

Boggs, C.L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Funct. Ecol.* **23**(1), 27-37.

Boggs, C., and Ross, C. (1993). The effect of adult food limitation on life history traits in Speyeria mormonia (Lepidoptera: Nymphalidae). *Ecology*, **74**(2), 433–441.

Capinera, J.L., Scott, R.D., and Walker, T.J. (2004). *Field Guide to Grasshoppers, Katydids, and Crickets of the United States*, 249 pp. Ithaca, NY: Cornell University Press.

Carvalho, G.B., Kapahi, P., and Benzer, S. (2005). Compensatory ingestion upon dietary restriction in *Drosophila melanogaster*. *Nat. Methods* **2**(11), 813-815.

Chambers, P.G., Simpson, S.G., and Raubenheimer, D. (1995). Behavioural mechanisms of nutrient balancing in *Locusta migratoria* nymphs. *Anim. Behav.* **50**, 1513-1523.

Clark, R. M., McConnell, A., Zera, A. J., and Behmer, S. T. (2013). Nutrient regulation strategies differ between cricket morphs that trade-off dispersal and reproduction. *Funct. Ecol.* **27**(5), 1126–1133.

Cook, S.C., Eubanks, M.D., Gold, R.E., and Behmer, S.T. (2010). Colony-level macronutrient regulation in ants: Mechanisms, hoarding and associated costs. *Anim. Behav.* **79**(2), 429-437.

Dadd, R. (1961). The nutritional requirements of locusts .4. Requirements for vitamins of the B-complex. *J. Insect Physiol.* **6**(1), 1–12.

Everitt, B.S., and Hothorn, T. (2010). *A Handbook of Statistical Analyses Using R, Second*

Edition, pp. 117-138. Boca Raton, FL: CRC Press.

Fanson, B.G., and Taylor, P.W. (2012). Protein:carbohydrate ratios explain life span patterns found in Queensland fruit fly on diets varying in yeast:sugar ratios. *Age* **34**(6), 1361-1368.

Fanson, B.G., Weldon, C.W., Perez-Staples, D., Simpson, S.J., and Taylor, P.W. (2009). Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocerca tryoni*). *Aging Cell* **8**(5), 514-523.

Fanson, B.G., Fanson, K.V., and Taylor, P.W. (2012). Cost of reproduction in the Queensland fruit fly: Y-model versus lethal protein hypothesis. *Proc. R. Soc. B* **279**, 4893-4900.

Furrer, R., Nychka, D., and Sain, S. (2012). fields: Tools for spatial data (Version 6.7). Retrieved from http://CRAN.R-project.org/package=fields

Grandison, R.C., Piper, M.D.W., and Partridge, L. (2009). Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature* **462**(7276), 1061-1064.

Harshman, L.G., and Zera, A.J. (2007). The cost of reproduction: The devil in the details. *Trends Ecol. Evol.* **22**(2), 80-86.

Karasov, W.H., and Martínez del Rio, C. (2007). *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*, 471 pp. Princeton: Princeton University Press.

Lee, K., Raubenheimer, D., and Simpson, S. J. (2004). The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiol. Entomol.* **29**(2), 108–117.

Lee, K., Simpson, S. J., Clissold, F., Brooks, R., Ballard, J., Taylor, P., Soran, N., and Raubenheimer, D. (2008). Lifespan and reproduction in Drosophila: New insights from nutritional geometry. *P. Natl. Acad. Sci. USA* **105**, 2498–2503.

Lenth, R. V. (2009). Response-Surface Methods in R, Using rsm. *J. Stat. Softw.* **32**(7), 1–17.

Loveridge, J. (1973). Age and the changes in water and fat content of adult laboratory-reared *Locusta migratoria* migratorioides. *Rhod. J. Agr. Res.* **11**, 131–143.

Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., and Brooks, R. C. (2008). Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr. Biol.* **18**(14), 1062–1066.

Mole, S., and Zera, A. (1993). Differential allocation of resources underlies the dispersal-reproduction trade-off in the wing-dimorphic cricket, Gryllus rubens. *Oecologia*, **93**(1),

121–127.

Piper, M.D.W., Blanc, E., Leitão-Gonçalves, R., Yang, M, He, X., Linford, N.J., Hoddinott, M.P., Hopfen, C., Soultoukis, G.A., Niemeyer, C., Kerr, F., Pletcher, S.D., Ribeiro, C., and Partridge, L. (2014). A holidic medium for *Drosophila melanogaster*. *Nat. Methods* **11**(1), 100-108.

Raubenheimer, D., and Simpson, S. J. (1990). The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behavior of larval *Locusta migratoria* and *Schistocerca gregaria* I: Short-term studies. *Physiol. Entomol.* **15**(2), 219–233.

Raubenheimer, D., and Simpson, S.J. (1993). The geometry of compensatory feeding in the locust. *Anim. Behav.* **45**(5), 953-964.

Raubenheimer, D., and Simpson, S.J. (1997). Integrative models of nutrient balancing: Application to insects and vertebrates. *Nutr. Res. Rev.* **10**, 151-179.

Robyt, J.F., and White, B.J. (1990). *Biochemical Techniques: Theory and Practice*, 407 pp. Monterey, California: Brooks-Cole Publishing Company.

Roeder, K.A., and Behmer, S.T. (2014). Lifetime consequences of food protein-carbohydrate content for an insect herbivore. *Funct. Ecol.* **28**, 1135-1143.

Simpson, S. J., and Abisgold, J. (1985). Compensation by locusts for changes in dietary nutrients - behavioral mechanisms. *Physiol. Entomol.* **10**(4), 443–452.

Simpson, S.J., and Raubenheimer, D. (1993). A multi-level analysis of feeding behaviour: The geometry of nutritional decisions. *Phil. Trans. R. Soc. B* **342**, 381-402.

Simpson, S.J., and Raubenheimer, D. (2007). Caloric restriction and aging revisited: The need for a geometric analysis of the nutritional bases of aging. *J. Gerontol. A-Biol.* **62**(7), 707-713.

Simpson, S.J., and Raubenheimer, D. (2012). *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. 239 pp. Princeton, N.J.: Princeton University Press.

Simpson, S.J., Raubenheimer, D., Behmer, S.T., Whitworth, A., and Wright, G.A. (2002). A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. *J. Exp. Biol.* **205**, 121-129.

Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T., and Raubenheimer, D. (2004). Optimal

foraging when regulating intake of multiple nutrients. *Anim. Behav.* **68**, 1299-1311.

Skorupa, D.A., Dervisefendic, A., Zwiener, J., and Pletcher, S.D. (2008). Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell* **7**, 478-490.

Slansky, F., and Wheeler, G. (1989). Compensatory increases in food consumption and utilization efficiencies by velvetbean caterpillars mitigate impact of diluted diets on growth. *Entomol. Exp. Appl.* **51**(2), 175–187.

Tatar, M. (2011). The plate half-full: Status of research on the mechanisms of dietary restriction in *Drosophila melanogaster*. *Exp. Gerontol.* **46**(5), 363-368.

van Milgen, J. (2002). Modeling biochemical aspects of energy metabolism in mammals. *J. Nutr.* **132**, 3195-3202.

van Noordwijk, A.J., and de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *Am. Nat.* **128**(1), 137-142.

Waldbauer, G.P., and Friedman, S. (1991). Self-selection of optimal diets by insects. *Annu. Rev. Entomol.* **36**, 43-63.

Yang, Y., and Joern, A. (1994). Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. *Physiol. Entomol.* **19**(1), 75–82.

Zera, A. J. (2005). Intermediary metabolism and life history trade-offs: Lipid metabolism in lines of the wing-polymorphic cricket, *Gryllus firmus*, selected for flight capability vs. early age reproduction. *Integr. Comp. Biol.* **45**, 511–524.

Zera, A. J., and Mole, S. (1994). The physiological costs of flight capability in wing-dimorphic crickets. *Res. Popul. Ecol.* **36**(2), 151–156.

Zera, A. J., and Denno, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annu. Rev. Entomol.* **42**(1), 207–230.

Zera, A.J., and Brink, T. (2000). Nutrient absorption and utilization by wing and flight muscle morphs of the cricket *Gryllus firmus*: Implications for the trade-off between flight capability and early reproduction. *J. Insect Physiol.* **46**(8), 1207-1218.

Zera, A.J., and Harshman, L.G. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* **32**(1), 95-126.

Zera, A., and Larsen, A. (2001). The metabolic basis of life history variation: Genetic and phenotypic differences in lipid reserves among life history morphs of the wing-

polymorphic cricket, *Gryllus firmus*. *J. Insect Physiol.* **47**(10), 1147–1160.

Zera, A. J., and Zhao, Z. (2006). Intermediary metabolism and life-history trade-offs: Differential metabolism of amino acids underlies the dispersal-reproduction trade-off in a wing-polymorphic cricket. *Am. Nat.* **167**(6), 889–900.

Zera, A., Mole, S., and Rokke, K. (1994). Lipid, carbohydrate and nitrogen content of long- and short-winged *Gryllus firmus*: Implications for the physiological cost of flight capability. *J. Insect Physiol.* **40**, 1037–1044.

Zera, A. J., Sall, J., and Grudzinski, K. (1997). Flight-muscle polymorphism in the cricket *Gryllus firmus*: Muscle characteristics and their influence on the evolution of flightlessness. *Physiol. Zool.* **70**(5), 519–529.

Zera, A.J., Potts, J., and Kobus, K. (1998). The physiology of life-history trade-offs: Experimental analysis of a hormonally induced life-history trade-off in *Gryllus assimilis*. *Am. Nat.* **152**(1), 7-23.

Zera, A.J., Zhao, Z., and Kaliseck, K. (2007). Hormones in the field: Evolutionary endocrinology of juvenile hormone and ecdysteroids in field populations of the wing-dimorphic cricket *Gryllus firmus*. *Physiol. Biochem. Zool.* **80**(6), 592-606.

Zhao, Z., and Zera, A.J. (2001). Enzymological and radiotracer studies of lipid metabolism in the flight-capable and flightless morphs of the wing-polymorphic cricket, *Gryllus firmus*. *J. Insect Physiol.* **47**(11), 1337-1347.

Zhao, Z., and Zera, A.J. (2006). Biochemical basis of specialization for dispersal vs. reproduction in a wing-polymorphic cricket: Morph-specific metabolism of amino acids. *J. Insect Physiol.* **52**(6), 646-658.

684 **Figures and Tables**

685

686 **Fig. 1. Mean (± s.e.m.) cricket body allocation patterns, during early adulthood, for**
687 **flightless (SW) and flight-capable [LW(f)] female crickets.** Panels (A) and (B) show total
688 mean (± s.e.m.) cricket mass, and the mass of flight muscle, ovaries, lipid vs. non-lipid carcass
689 fractions, and flight muscle and somatic protein at day zero. Asterisks indicate significant
690 differences (MANOVA with post-hoc t-tests, $P<0.05$, $N=17$ SW and 16 LW(f) crickets). Panels
691 (C) and (D) show how, after five days, allocation patterns shift and differ between morphs (see
692 subsequent figures and text for details and statistics). Day five data are averages from across all
693 13 dietary treatments and are from 79 SW and 91 LW crickets.

694

695 **Fig. 2. Mean cricket consumption patterns across diets with different protein-**
696 **carbohydrate content (each nutrient expressed as a percentage of dry mass).** Flight-capable
697 (long-winged; LW(f); n=91) and flight-incapable (short-winged; SW; n= 79) crickets were given
698 access to one of 13 diets (open circles) containing different amounts of protein and carbohydrate
699 for the first five days of adulthood. The total amount of food consumed (panel A) and
700 macronutrients ingested (panel B) are mapped as non-parametric thin-plate spline response
701 surfaces to allow detailed visualization of responses across the nutrient landscape. The dashed
702 line indicates the average self-selected ratio of protein to carbohydrate from a prior experiment
703 (Clark et al., 2013). Associated parametric statistics are given in Table 2. Macronutrient
704 ingestion is also presented as a bicoordinate plot of the mean (± s.e.m.) amounts of protein and
705 carbohydrate ingested for each diet (panel C). Dashed lines indicate food ratios, and the colored,
706 solid lines connect intake points across each of the five macronutrient concentrations offered.

707

708 **Fig 3. Mean cricket wet mass gains (A), flight muscle masses (B), and ovary masses (C), in**
709 **mg, as a function of diet protein and carbohydrate content**. For detailed information about
710 sample sizes and symbols, refer to the legend for Fig. 2. Associated parametric statistics are
711 given in Table 2.

712

713 **Fig 4. Mean cricket somatic lipid levels as a function of diet protein and carbohydrate**
714 **content**. Body lipid patterns (panel A) and ovary lipid patterns (panel B) are compared for flight-

715 incapable (short-winged; SW) and flight-capable [long-winged; LW(f)] adult crickets provided
716 for five days with one of 13 diets containing different total amounts of protein and carbohydrate
717 (open circles). For additional figure details, including sample sizes, refer to Fig. 2. Associated
718 statistics are given in Table 3.
719
720 **Fig 5. Mean cricket somatic (A) and flight muscle (B) protein content as a function of**
721 **dietary protein and carbohydrate content.** Protein levels are compared for flight-incapable
722 (short-winged; SW) and flight-capable [long-winged; LW(f)] adult crickets provided for five
723 days with one of 13 diets containing different total amounts of protein and carbohydrate (open
724 circles). For additional figure details, including sample sizes, refer to Fig. 2. Statistics are given
725 in Table 3.
726

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, 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 on each treatment are also given.

Diet protein:carbohydrate content		Total macronutrients (% dry mass)	LW(f)	SW
(a) p4:c17	very carbohydrate-biased	21	9	6
(b) p9:c12	balanced		7	6
(c) p14:c7	very protein-biased		6	6
(d) p9.75:c21.75	carbohydrate-biased	31.5	7	6
(e) p17.25:c14.25	protein-biased		8	6
(f) p8:c34	very carbohydrate-biased	42	9	6
(g) p13:c29	carbohydrate-biased		5	6
(h) p18:c24	balanced		5	6
(i) p23:c19	protein-biased		9	6
(j) p28:c14	very protein-biased		5	7
(k) p16.25:c36.25	carbohydrate-biased	52.5	5	6
(l) p28.75:c2375	protein-biased		9	6
(m) p27:c36	balanced	63	7	6
Total			**91**	**79**

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

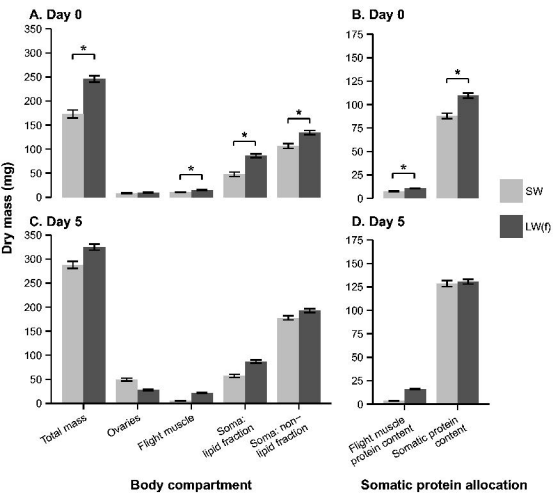
Model terms	Amount of food consumed	Macro-nutrient intake	Mass gain	Flight muscle mass	Ovary mass
Full model	**$F_{6,163}$=5.9 *P*<0.001**	**$F_{12,157}$=16.0 *P*<0.001**	**$F_{12,157}$=31.6 *P*<0.001**	**$F_{12,156}$=161 *P*<0.001**	**$F_{12,156}$=12.9 *P*<0.001**
Intercept	**$F_{1,163}$=17.8 *P*<0.001**	**$F_{1,157}$=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*Carb-ohydrate		**$F_{1,157}$=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 6 "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**

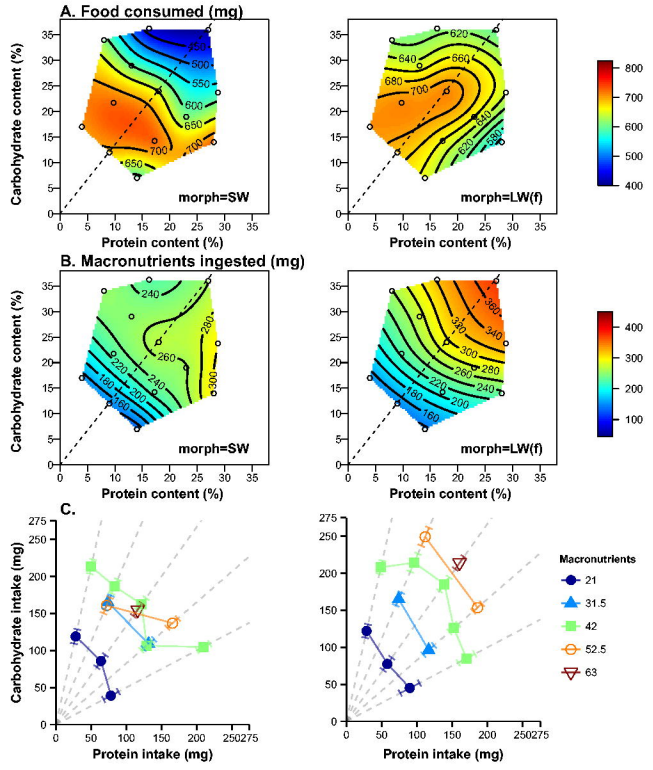
Initial cricket mass was included in models as a covariate, and protein and carbohydrate model terms were standardized to a scale from -1 to 1. Model term significance was assessed with partial F-tests. Bold indicates significance at the alpha=0.05 level and italicized terms are marginally significant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

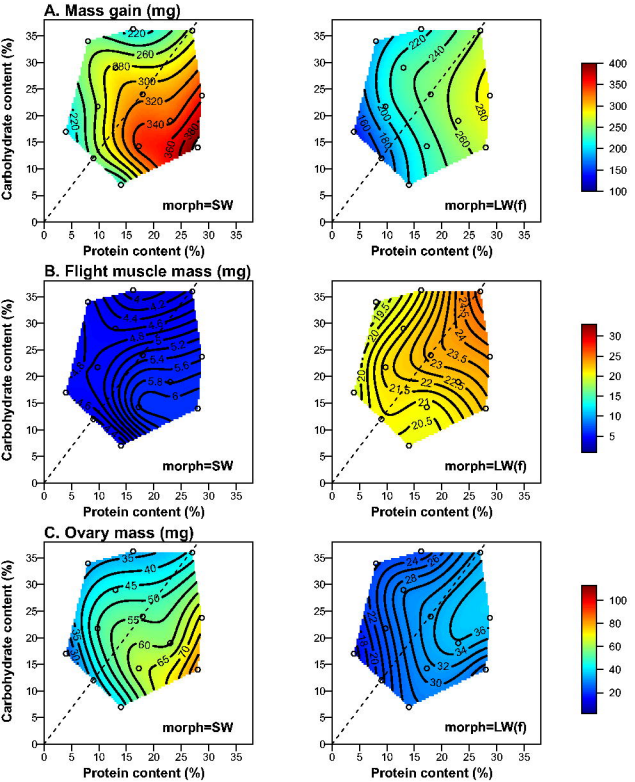
740 **Table 3.** Statistical results for response surface models testing the effects of protein and
741 carbohydrate concentration and morph type [LW(f) vs. SW] on day five body composition.

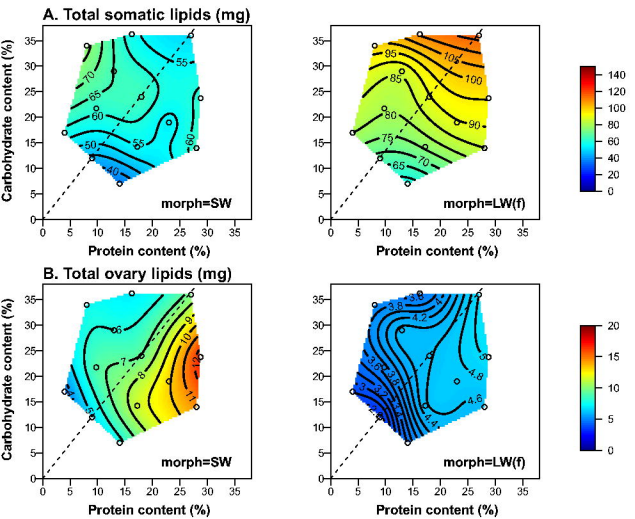
Model terms	Somatic + ovary lipids	Somatic lipids	Ovary lipids	Somatic protein	Flight muscle protein
Full model	**$F_{6,141}$=23.4 *P*<0.001**	**$F_{12,142}$=12.8 *P*<0.001**	**$F_{12,146}$=8.47 *P*<0.001**	**$F_{12,151}$=34.7 *P*<0.001**	**$F_{12,155}$=168 *P*<0.001**
Intercept	**$F_{1,141}$=11.0 *P*=0.001**	**$F_{1,157}$=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*Carb-ohydrate		$F_{1,142}$=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 6 "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**

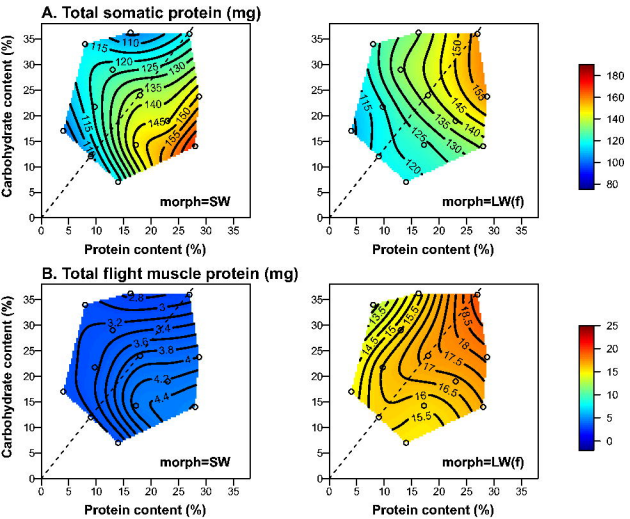
742 Initial cricket mass was included in models as a covariate, and protein and carbohydrate model
743 terms were standardized to a scale from -1 to 1. Model term significance was assessed with
744 partial F-tests. Bold indicates significance at the alpha=0.05 level and italicized terms are
745 marginally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


A. Day 0
B. Day 0
C. Day 5
D. Day 5
Dry mass (mg)
SW
LW(f)
Total mass
Ovaries
Flight muscle
Soma: lipid fraction
Soma: non-lipid fraction
Flight muscle protein content
Somatic protein content
Body compartment
Somatic protein allocation


A. Food consumed (mg)
Carbohydrate content (%)
Protein content (%)
morph=SW
morph=LW(f)
B. Macronutrients ingested (mg)
C.
Carbohydrate intake (mg)
Protein intake (mg)
Macronutrients
21
31.5
42
52.5
63


A. Mass gain (mg)
B. Flight muscle mass (mg)
C. Ovary mass (mg)
Carbohydrate content (%)
Protein content (%)
morph=SW
morph=LW(f)
0
5
10
15
20
25
30
35
100
150
200
250
300
350
400


A. Total somatic lipids (mg)
Carbohydrate content (%)
Protein content (%)
morph=SW
morph=LW(f)
B. Total ovary lipids (mg)
Carbohydrate content (%)
Protein content (%)
morph=SW
morph=LW(f)
0 5 10 15 20 25 30 35
140
120
100
80
60
40
20
0


A. Total somatic protein (mg)
Carbohydrate content (%)
Protein content (%)
morph=SW
morph=LW(f)
B. Total flight muscle protein (mg)
Carbohydrate content (%)
Protein content (%)
morph=SW
morph=LW(f)