

Nutrient regulation strategies differ between cricket morphs that trade-off dispersal and reproduction

Rebecca M. Clark¹, Ashley McConnell^{1,2}, Anthony J. Zera³ and Spencer T. Behmer^{*,1}

¹Department of Entomology, Texas A&M University, College Station, Texas, 77843 USA; ²Department of Biological Sciences, University of Maryland-Baltimore County, Baltimore, Maryland, 21250 USA; and ³School of Biological Sciences, University of Nebraska, Lincoln, Nebraska, 68588 USA

Summary

1. Nutrient regulation should covary with life history, but actual demonstrations of this connection are rare.

2. Here, we use a wing-polymorphic cricket, *Gryllus firmus*, that trades off dispersal and reproduction; the long-winged morph with functional flight muscles [LW(f)] is adapted for dispersal at the expense of egg production, while the short-winged (SW) morph is adapted for egg production at the expense of flight. We explore the extent to which these two morphs differentially regulate macronutrient intake to best match their life-history strategy.

3. In a 'choice' experiment, we offered female crickets of each morph [LW(f) and SW] two nutritionally complementary foods varying in protein and digestible carbohydrate content. In a second 'no-choice' experiment, we confined crickets to one of five foods, each with a different protein/carbohydrate ratio. In both experiments, and for both morphs, we measured food intake, mass gain and lipid concentration.

4. In the 'choice' experiments, LW(f) females selected a more carbohydrate-biased diet than SW females. The two morphs gained similar total mass, but the LW morph had higher lipid concentration.

5. In the no-choice experiment, the two morphs practised different nutrient 'consumption rules'. SW females ate similar total nutrient amounts (protein plus carbohydrate) across diets, while LW(f) females decreased intake as the protein/carbohydrate ratio of the available food became increasingly imbalanced. Overall mass gain was marginally higher in the SW morph and lowest for both morphs on the diets that were extremely carbohydrate biased. LW(f) and SW females had similar lipid concentrations across the diets, even though LW(f) crickets ate less carbohydrate on the two carbohydrate-biased diets. Our data suggest that for LW(f) females, there are costs of overeating nutrients in excess of requirements, but they are efficient at utilizing ingested nutrients.

6. Our results shed new light on how the nutritional environment interacts with the direct trade-off between dispersal and reproduction occurring in adult *G. firmus* crickets. Dispersal is linked to heightened diet selectivity and an emphasis on nutrients promoting flight fuel (lipid) storage over protein acquisition for egg laying, such that nutritional regulation complements the metabolic mechanisms that generate this trade-off.

Key-words: carbohydrate, cricket, life history, nutrition, protein, trade-off

Introduction

Theoretical and experimental studies have shown that nutrient input can profoundly affect life-history traits and trade-offs (Boggs & Ross 1993; Zera & Harshman 2001; Lee *et al.* 2008; Boggs 2009). However, we know surpris-

ingly little about how variation in nutrient quality affects life-history strategies. Given that organisms can experience tremendous variation in the nutritional quality of their food, understanding organismal responses to different nutritional environments is critical for gaining insights into how life-history traits evolve (Partridge & Harvey 1985; Barnes & Partridge 2003; Boggs 2009). This information is also important in the context of understanding how

*Correspondence author. E-mail: s-behmer@tamu.edu

organisms respond to ongoing, widespread, anthropogenically driven shifts in resource availability (Parmesan 2006; Rosenzweig *et al.* 2007).

A classical life-history trade-off, useful for beginning to address this question, involves the relative investment in dispersal (flight) ability vs. fecundity, as is observed in wing-polymorphic insect species (Harrison 1980; Zera & Denno 1997; Zera 2009). The wing-polymorphic cricket, *Gryllus firmus*, has been extensively studied in this context (reviewed in Zera & Denno 1997; Zera 2009; Zera & Harshman 2009, 2011). This species has two main adult morphs that differentially trade-off early age fecundity with flight capability. Females of the flight-capable [LW(f)] morph exhibit fully developed wings and flight muscles and produce a large amount of lipid flight fuel, but delay egg production. By contrast, obligately flightless, short-winged (SW) females produce only vestigial wings and flight muscles and a low quantity of triglyceride, but exhibit a fourfold increase in egg production during the first 1–2 weeks of adulthood, compared with the LW(f) morph (Zera 2004, 2005, 2009). To date, virtually all physiological work on this trade-off in this cricket has focused on genetic and hormonal aspects of the differential allocation of internal resources in long-winged (LW) and SW morphs in artificially selected populations reared on diets with fixed nutrient ratios (Zera 2005, 2009).

However, crickets are natural omnivores and feed on both plant and animal material in the field (Capinera, Scott & Walker 2004); thus, their diet can vary greatly in nutrient content, especially the amounts and ratios of protein and digestible carbohydrates. Both protein and carbohydrates affect insect and animal performance in distinct ways (Behmer 2009; Simpson & Raubenheimer 2012). Currently, though, we have a limited grasp of what constitutes optimal protein–carbohydrate intake for adult crickets (e.g. Maklakov *et al.* 2008) and know nothing about how the trade-off between flight and early reproduction in LW(f) and SW morphs might influence protein–carbohydrate regulatory strategies (*sensu* Raubenheimer & Simpson 1999). For instance, do the two morphs possess different ‘intake targets’, that is, do they prefer to consume different amounts of protein and/or carbohydrate? Secondly, when confronted with foods with imbalanced, and thus sub-optimal, protein/carbohydrate ratios, do the morphs practise different ‘consumption rules/compromises’, making different decisions about how much to eat? One might expect, *a priori*, that life-history adaptation is tied to differential consumption of key nutrients (Boggs & Ross 1993; Boggs 1997a,b). For example, a phenotype that emphasizes egg production should prefer foods that are proteinaceous, while a phenotype that emphasizes flight and delayed reproduction should favour a more energy- or carbohydrate-rich diet from which lipid flight fuel can be biosynthesized (Wheeler 1996).

In this study, we use the experimental approach of the geometric framework (Simpson & Raubenheimer 1993, 1995, 2012; Raubenheimer & Simpson 1999) to explore the

extent to which nutrient regulation strategies differ between crickets exhibiting trade-offs between dispersal and reproduction. We first identify the protein–carbohydrate intake points for the two morphs. The different metabolic requirements for flight vs. reproduction lead us to predict that SW crickets will select a more protein-biased diet relative to LW individuals, given the importance of protein towards reproductive output. Next, we examined consumption patterns across a range of foods with different protein and carbohydrate ratios, to test whether morphs use different regulatory strategies to cope with imbalanced diets, as might be expected if there are morph-specific differences in costs of over- or underconsumption of nutrients (Lee *et al.* 2008). Potential costs of overconsumption are much greater for flight-capable compared with flightless individuals, as overconsumption represents added load during flight, so LW crickets should employ a more selective intake strategy as compared with SW crickets.

Materials and methods

INSECTS AND EXPERIMENTAL CHAMBERS

Female crickets were raised in large outbred populations (>200 breeders each generation) that were artificially selected to produce either the flight-capable [LW(f)] or flightless SW morphs; the methods used to create the laboratory populations have been previously described (Zera & Larsen 2001; Zera 2005). Nearly all (>95%) SW individuals have vestigial flight muscles throughout adulthood and are flightless. All LW(f) individuals emerge with large flight muscles, which are retained in most (>85%) individuals through day 5 of adulthood (Zera, Sall & Grudzinski 1997). After this time, flight muscle histolysis (coupled with enhanced ovarian growth) occurs with increasing frequency in LW(f) individuals, converting them into the flightless [LW(h)] morph. In this study, we compared LW(f) females from one LW(f)-selected population and SW females from another, SW-selected population; these populations both come from one of three blocks (block 2) of a larger artificial selection experiment (Zera & Larsen 2001; Zera 2005). Each block of that experiment constitutes an independent artificial selection trial involving one pair of LW(f)- and SW-selected populations. In previous studies, without exception, biochemical, endocrine, morphological and reproductive differences between LW(f)- and SW-selected populations of any one block are very similar to differences between the LW(f)- and SW-selected populations of the other two blocks (e.g. see Zera 2005). Thus, comparisons made between LW(f) and SW populations of any one block are expected to be representative of general differences between LW(f)- and SW-selected populations of *G. firmus*. It is worth noting here that the purpose of this study was to investigate phenotypic differences between the LW(f) and SW morphs. A more comprehensive genetic analysis would require comparisons of population means of LW(f) and SW populations from replicate blocks.

Cricket colonies were maintained at the University of Nebraska–Lincoln, but juveniles were shipped to Texas A&M University, where they were raised to adulthood for experimental work. Crickets were reared in groups of *c.* 50 individuals in 10-gallon aquaria kept in an incubator with a 16 h:8 h light/dark cycle at a temperature of 28 °C. During rearing, crickets were fed an *ad libitum* ‘standard’ diet composed of wheat germ, wheat bran, milk powder and nutritional yeast (Zera & Larsen 2001) and

were provided with water in a 50-mL plastic tube plugged with cotton. Aquaria were checked every 3 h over the course of the day for newly moulted adults, which were transferred individually to small, plastic arenas (18.9 × 13.5 × 9.5 cm) where they were given preweighed, spill-resistant dishes of dry synthetic foods which varied in their protein–carbohydrate content (Raubenheimer & Simpson 1990; see below). Crickets also had free access to an aluminium perch and distilled drinking water in a plastic container with a cotton wick.

EXPERIMENTAL DIETS

Experimental diets that varied in their protein (p) and digestible carbohydrate (c) content were prepared based on synthetic diets originally made and modified for grasshoppers (Dadd 1961; Simpson & Abisgold 1985; Behmer, Raubenheimer & Simpson 2001). Diets were prepared as described in Behmer, Simpson & Raubenheimer (2002). In total, there were five diets: (i) 7% protein and 35% carbohydrate (p7 : c35), (ii), p14 : c28, (iii) p21 : c21, (iv) p28 : c14 and (v) p35 : c7; these diets had the same total macronutrient content (42%) and identical amounts of other dietary ingredients (e.g. cellulose, vitamins, cholesterol, fatty acids).

EXPERIMENTAL PROTOCOL

Choice experiments

To test whether crickets actively regulated nutrient intake, newly emerged adult female crickets of each morph [LW(f) and SW] were weighed and then given one of two preweighed food pairing treatments: (i) p7 : c35 with p35 : 7 and (ii) p7 : c35 with p28 : c14. Alone these foods are nutritionally suboptimal, but together they are nutritionally complementary. Two food pairings were used to confirm that crickets were selectively feeding to reach a target intake and not simply feeding equally from both dishes. Nine crickets of each morph were used for the first treatment, and 10 crickets of each morph were used in the second treatment. Data from five LW crickets were excluded from analysis due to loss of sample material during the experiment, resulting in final sample sizes of 9 SW and 8 LW(f) crickets for the first treatment, and 10 SW and 6 LW(f) crickets for the second treatment.

After 3 days, the initial pair of feeding dishes was removed and weighed; a pair of fresh preweighed food dishes (containing the same two food types) was placed into arenas, and crickets were allowed to feed for an additional 3 days. At the end of day 6, the second set of dishes was removed and weighed, and the crickets were weighed to determine their final mass. The crickets were then frozen, dried to constant mass and reweighed. Flight muscle type was not checked in the LW(f) morph because, as mentioned previously, nearly all (>85%) LW individuals have pink functional muscles [i.e. are the LW(f) morph] at this age; in addition, even those few LW(h) individuals produced during the experiment existed as the LW(f) morph during most of the study (Zera, Sall & Grudzinski 1997; Zera & Cisper 2001). To estimate total lipid content, lipids were extracted from the dried crickets with chloroform, as described by Lovern (1973); lipid content was calculated within individuals as the difference between a cricket's dry mass and its mass after lipid extraction (lean mass). Two crickets were excluded from lipid analysis due to sample loss during lipid measurement (one LW from treatment (i), one SW from treatment (ii)).

No-choice experiments

In no-choice experiments, newly emerged adult female crickets were weighed and then allowed to feed *ad libitum* on one of the

five previously described foods (p7 : c35, p14 : c28, p21 : c21, p28 : c14, p35 : c7). Sample sizes per treatment were initially 10, 10, 8, 9 and 9 SW crickets, respectively, and 9, 8, 8, 10 and 6 LW(f) crickets, but 13 crickets were excluded from analysis due to the following: failure to eat the offered food, net mass loss or loss of sample material. This resulted in final sample sizes of 9, 8, 9, 6 and 6 SW crickets, and 6, 8, 6, 7 and 9 LW(f) crickets. As with the choice experiments, crickets were presented with an initial dish of preweighed food for 3 days, which was replaced with a second, fresh dish of food for the following 3 days (days 3 through 6). Final cricket masses (wet, dry and lean mass, plus lipid content) were measured as previously described. Lipid data also had to be excluded for two additional SW crickets and three additional LW(f) crickets.

DATA ANALYSIS

Statistics were calculated and figures generated using the software program R (version 2.14.0; R Development Core Team 2010). Many of the measured characteristics are likely to be influenced by an individual's initial mass (Horton & Redak 1993; Raubenheimer & Simpson 1992). Therefore, where appropriate, comparisons were made and reported using ANCOVA (analysis of covariance) or MANCOVA (multivariate analysis of covariance) using a cricket's initial adult mass (wet mass) as the covariate.

To calculate dry mass gains, a set of 10 newly moulted adult LW and 10 SW crickets was collected, weighed, frozen, dried to constant mass at 70 °C and reweighed. Initial dry mass for each morph was regressed against initial wet mass to calculate wet-to-dry conversion coefficients (for LW: $0.352 * M_i$, $r^2 = 0.99$; for SW: $0.335 * M_i$, $r^2 = 0.99$). Dry mass gains were calculated as the initial wet masses of LW(f) and SW crickets multiplied by conversion coefficients; this value was subtracted from final cricket dry masses.

Results

CHOICE EXPERIMENTS

To determine whether morphs actively regulated protein–carbohydrate intake, we allowed crickets to choose between pairs of nutritionally suboptimal foods offered in two treatments (p7 : c35 paired with p35 : c7, and p7 : c35 paired with p28 : c14). For a given morph, crickets consumed similar amounts of protein and carbohydrate across both pairing treatments (MANCOVA for protein and carbohydrate consumption within each morph: $F_{LW} = 1.65$, d.f. = 2,11, $P = 0.235$; $F_{SW} = 0.04$, d.f. = 2,15, $P = 0.963$). This allowed us to combine consumption rates across the food pairing treatments and compare patterns between morphs (data shown in Fig. 1). Analysis of the combined data revealed that SW and LW morphs self-selected statistically different protein–carbohydrate intake points (MANCOVA: $F_{morph} = 4.20$, d.f. = 2,30, $P = 0.045$; $F_{mass} = 0.45$, d.f. = 1,29, $P = 0.506$). *Post hoc* univariate analyses showed that SW crickets ate more protein than LW crickets (ANCOVA: $F_{protein} = 6.32$, d.f. = 1,31, $P = 0.017$), but similar total amounts of carbohydrate (ANCOVA: $F_{carbohydrate} = 0.45$, d.f. = 1,31, $P = 0.506$). Differences in protein–carbohydrate intake occurred even though newly moulted adults of both morphs had similar starting mass (*t*-test: $t_{mass} = 0.69$, d.f. = 28, $P = 0.494$) and

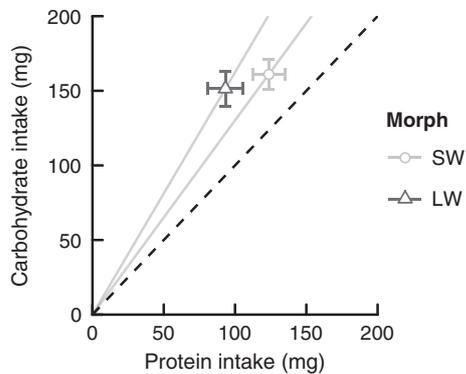


Fig. 1. Self-selected protein and carbohydrate intake points (means \pm SEM) for short-winged (SW) and long-winged (LW) crickets ($n = 18$ and 14 , respectively). Protein intake, but not carbohydrate intake, differed between morphs (see text for statistics). The dashed line indicates the trajectory for balanced intake (equal amounts of protein and carbohydrate; a 1 : 1 ratio).

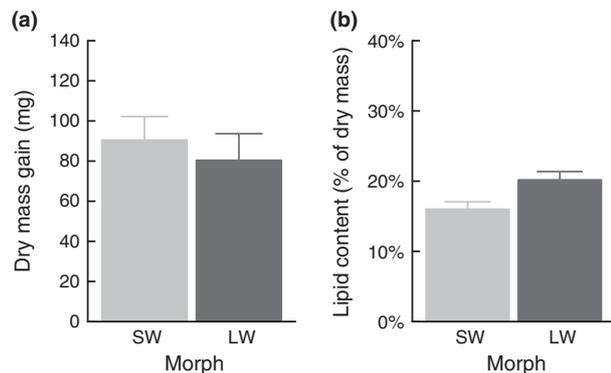


Fig. 2. Cricket performance on self-selected diets. Panel (a) shows dry mass gains (means \pm SEM) for short-winged (SW, $n = 18$) vs. long-winged (LW, $n = 14$) morphs allowed to self-selected diets from two complementary, but nutritionally imbalanced foods. Panel (b) shows cricket lipid content, as a percentage of dry mass.

consumed similar total quantities of food over the duration of the experiment (ANCOVA: $F_{\text{morph}} = 2.37$, d.f. = 1,29, $P = 0.135$; see Fig. S1, Supporting information).

Despite differences in protein and carbohydrate intake points, both morphs showed similar dry mass gains (ANCOVA: $F = 0.449$, d.f. = 1,29, $P = 0.508$; Fig. 2). However, the nature of dry mass gains differed qualitatively between morphs (Fig. 2). Specifically, LW crickets had higher final lipid content as compared to SW crickets (ANCOVA with morph and carbohydrates eaten as factors and initial cricket mass as the covariate: $F_{\text{morph}} = 7.18$; d.f. = 1,28; $P = 0.012$; $F_{\text{carbohydrates}} = 2.78$, d.f. = 1,28, $P = 0.107$).

NO-CHOICE EXPERIMENTS

In this set of experiments, we explored how LW and SW crickets regulate macronutrient intake when they cannot self-select their protein-carbohydrate intake. Here, each morph received a single food with a fixed protein/carbo-

hydrate ratio (p7 : c35, p14 : c28, p21 : c21, p28 : c14, or p35 : c7), and for each morph on each food, we measured consumption, protein-carbohydrate intake, mass gain and lipid content. We observed significant differences in consumption between morphs and across diets (ANCOVA with morph and diet as factors, initial cricket mass as covariate: $F_{\text{morph}} = 8.55$, d.f. = 1,59, $P = 0.005$; $F_{\text{diet}} = 3.91$, d.f. = 4,59, $P = 0.007$), but the morph-by-diet interaction was not significant ($F = 0.66$, d.f. = 4,59, $P = 0.62$). Consumption for the SW crickets, averaged across all diets, was 632 ± 27 mg of food (mean \pm SE) versus 518 ± 30 mg for LW crickets (see Fig. S2, Supporting information). Averaged across the morphs, consumption was highest on the food containing equal amounts of protein and carbohydrate (p21 : c21), lowest on the two most carbohydrate-biased foods and intermediate on the two protein-biased foods.

To better understand how our two focal macronutrients affected food consumption responses, we plotted mean protein and carbohydrate intake for each morph for each diet (Fig. 3a). We then used these data points to fit separate intake arrays for each morph, which enabled us to characterize nutrient regulation strategies where two nutrients were being regulated simultaneously (Raubenheimer & Simpson 1999; Behmer 2009). The array for the SW morph approximated a linear fit, while that for the LW morphs approximated a quadratic fit (Fig. 3a). We also generated protein (p) + carbohydrate (c) 'error plots' (Fig. 3b), which reveal the extent to which each morph overingests p and c relative to the intake target (for more details, see Raubenheimer & Simpson 1999). For SW crickets, there was no difference in p + c consumption between the different diets (ANCOVA: $F_{\text{diet}} = 1.67$, d.f. = 4,34, $P = 0.270$). In contrast, the LW cricket p + c consumption pattern differed between diets (ANCOVA: $F_{\text{diet}} = 3.45$, d.f. = 4,28, $P = 0.018$), showing a significant negative quadratic fit (Quadratic contrast: $F = 5.11$, d.f. = 2,30, $P = 0.032$).

Cricket dry mass gain was significantly affected by food protein/carbohydrate ratio (ANCOVA: $F_{\text{diet}} = 10.80$, d.f. = 4,59, $P < 0.001$; Fig. 4). It was highest on the equal-ratio and protein-biased diets, lowest on the most carbohydrate-biased diet (p7 : c35) and intermediate on the slightly carbohydrate-biased diet (p14 : c28). There was no significant morph-by-diet interaction with respect to mass gain (ANCOVA: $F_{\text{morph} \times \text{diet}} = 0.31$, d.f. = 4,59, $P = 0.870$). The SW morphs, on average, gained more mass compared with the LW morphs, but this difference was only marginally significant (ANCOVA: $F_{\text{morph}} = 3.97$, d.f. = 1,59, $P = 0.051$). LW and SW crickets confined to single diets did not differ in final lipid content (ANCOVA: $F_{\text{morph}} = 0.160$, d.f. = 1,54, $P = 0.691$; Fig. 5), but lipid content varied with diet composition (ANCOVA: $F_{\text{diet}} = 11.05$, d.f. = 4,54, $P < 0.001$). There was no significant morph-by-diet interaction (ANCOVA: $F_{\text{morph} \times \text{diet}} = 0.54$, d.f. = 4,54, $P = 0.704$). Lipid content was highest on the two carbohydrate-biased foods and lowest on the equal-ratio and protein-biased foods (Fig. 5).

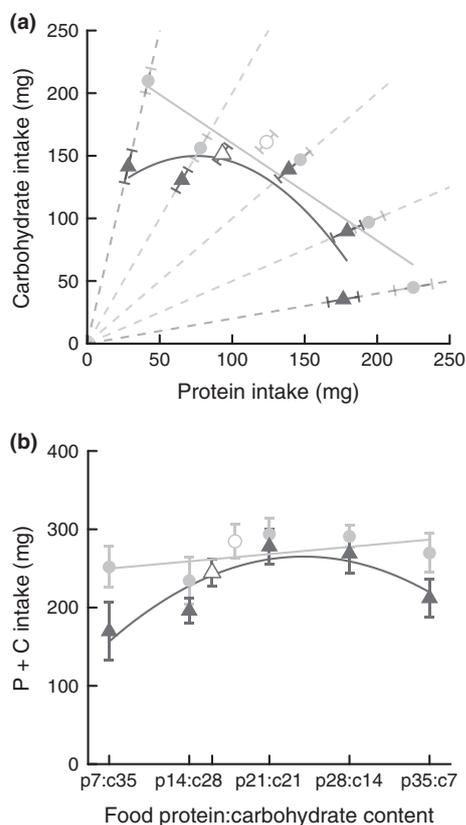


Fig. 3. Protein and carbohydrate intake for long-winged (LW; triangles) and short-winged (SW; circles) crickets restricted to one of five diets. Panel (a) shows protein and carbohydrate intake (means), for SW and LW crickets, as a bi-coordinate plot. Dashed lines indicate the protein-to-carbohydrate ratios of the five foods, which are, from left to right, p7 : c35 [7% protein and 35% digestible carbohydrate (as a percentage of dry mass)], p14 : c28, p21 : c21, p28 : c14 and p35 : c7. Open symbols indicate intake points, for reference. Panel (b) shows total protein and carbohydrate (P + C) consumption by LW and SW crickets as a function of each diet's protein content, with intake points indicated as open symbols, for reference. For both plots, the linear fits represent best-fit models for the SW morph's functional feeding rule, while the curved fits represent best-fit models for the LW morph (see text for more detail).

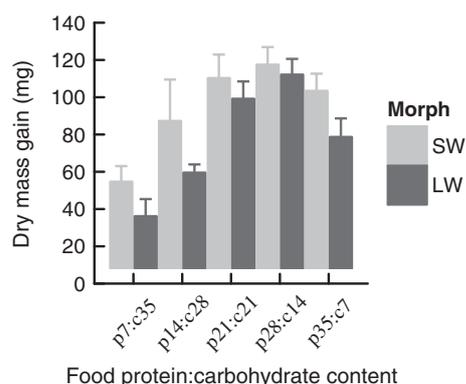


Fig. 4. Dry mass gains (means \pm SEM) across diets for short-winged (SW) compared with long-winged (LW) crickets which fed on diets with different protein and carbohydrate content (as a percentage of dry mass).

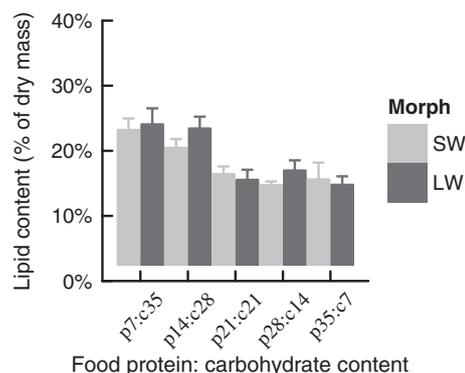


Fig. 5. Cricket lipid content (as a percentage of mass) in short-winged (SW) and long-winged (LW) crickets feeding on five diets that varied in their protein and carbohydrate content (as a percentage of dry mass).

Discussion

The extent and mechanisms by which variation in nutrient input contributes to variation in life-history traits and trade-offs have been a central, longstanding subject of research. However, many key aspects of this topic remain poorly understood, which constitutes a major roadblock to attaining a deep understanding of the mechanistic basis of life-history evolution (Boggs 1992, 2009; Zera & Harshman 2001, 2009; Simpson *et al.* 2002; Lee *et al.* 2008). In particular, we know little about the influence of nutrient heterogeneity on life-history evolution. In the current study, we show, for the first time, how life-history trade-offs can differentially affect nutrient regulation strategies. Specifically, we found that adult *G. firmus* females that trade-off early reproduction (SW) for dispersal capability (LW) practise different protein-carbohydrate regulation strategies, both when allowed to self-select their protein-carbohydrate intake and when confined to a range of foods with different protein/carbohydrate ratios.

When SW and LW(f) morphs were free to self-select their protein-carbohydrate intake, LW crickets selected a more carbohydrate-biased diet relative to SW crickets. Morph behaviour therefore differed in a manner that one might expect, given the different physiological requirements of flight and reproduction, and the different physiological functions of protein and carbohydrates. Flight is energetically intensive, requiring the acquisition and maintenance of flight muscle and production of flight fuel (primarily triglycerides), at the cost of allocation to other tissues (Zhao & Zera 2002; Zera 2005). Meanwhile, increased early reproductive output is associated with the diversion of nutritional resources, especially protein, towards ovarian and egg development and away from somatic function (Zera & Zhao 2006). While both amino acids and carbohydrates can be metabolized to contribute towards lipid synthesis (Zera & Larsen 2001; Zhao & Zera 2002; Zera 2005; Zera & Zhao 2006), protein synthesis and allocation, especially for oogenesis, are dependent on dietary protein availability (Wheeler 1996). The early ramping

up of reproductive output could therefore lead to increased demand for protein for the reproductive (SW) morph, reflected in higher protein intake when crickets could self-select. At the same time, consumption of excess protein relative to physiological demands can be harmful (Lee *et al.* 2008; Cease *et al.* 2012), especially for organisms preparing for dispersal, where overall storage capacity may be limited (Zera & Denno 1997). Consistent with this, Zera & Zhao (2006) showed that amino acid oxidation was higher in LW(f) compared with the SW morph, which would promote the elimination of excess ingested protein. Therefore, high costs of excess protein consumption could contribute towards relatively lower protein intake observed for the dispersing LW(f) morph.

In natural settings, various constraints (e.g. food scarcity, competition and predation) can prevent organisms from reaching their preferred nutrient intake target, making it important to characterize organisms' behavioural and physiological responses to nutritionally imbalanced foods (Raubenheimer & Simpson 1999). As with intake targets, consumption rules on nutritionally imbalanced foods might reflect an organism's life-history strategy, and our no-choice experiments demonstrate that LW(f) and SW crickets practise different consumption rules as the protein-carbohydrate content of their food becomes increasingly imbalanced. For LW(f) crickets, the observed quadratic relationship between food protein-carbohydrate content and total protein plus carbohydrate intake (see Fig. 3b) reflects a consumption strategy that minimizes the intake of the nonlimiting nutrient (i.e. protein on protein-biased diets and carbohydrate on carbohydrate-biased diets). In contrast, the combined protein plus carbohydrate intake for SW crickets on imbalanced diets was similar to that observed for crickets allowed to self-select. This reflects a consumption strategy that prioritizes intake of the limiting nutrient when confined to a nutritionally imbalanced food.

Nutrient regulatory strategies are known to vary depending on the evolutionary history, ecological context and physiological condition of the organism in question (Behmer 2009; Simpson & Raubenheimer 2012). For example, the desert locust (*Schistocerca gregaria*) is a generalist herbivore that is phenotypically plastic, existing in either a solitary or gregarious form. Solitary and gregarious locusts, unlike the LW(f) and SW crickets, exhibit similar protein-carbohydrate intake targets (Simpson *et al.* 2002). However, gregarious locusts, like SW crickets, maximize total nutrient intake when confined to nutritionally imbalanced foods, eating similar total amounts of protein plus carbohydrate across a range of diets with different protein/carbohydrate ratios (Simpson *et al.* 2002). In contrast, both solitary locusts and LW crickets minimize nutritional errors by not overeating nutrients that occur in excess. This raises an interesting question: Why should gregarious locusts and SW crickets practise a nutrient maximization strategy, while solitary locusts and LW(f) crickets minimize nutritional errors?

The most likely explanation is linked to population density. Gregarious locusts, like SW crickets, are often found as part of a large group (Pener & Simpson 2009). High-density living occurs in gregarious locusts because they are attracted to one another. *Gryllus* are also attracted to each other, and, unlike many other wing-polymorphic insects such as aphids, high density strongly promotes the induction of the SW morph in *Gryllus*, which cannot disperse by flight (Zera & Tiebel 1988; Zera & Denno 1997). The SW morph is also associated with high cricket density in the field (C. Mitra, pers. comm.). In both cases, high density leads to increased competition for nutritionally optimal food. Thus, both gregarious locusts and SW crickets should behave as opportunists, eating what is encountered. The consequences of being choosy for an animal living as part of a large group – namely, missing a meal, compounded by having a conspecific eat it – are too great, especially when food is limiting. On the other hand, when food is less limiting, choosiness with respect to nutrient intake should be practised. Indeed, this is the case for solitary locusts, which live in isolation from other locusts (Pener & Simpson 2009) and LW(f) crickets that can disperse away from places where competition for local resources is high. Being selective with respect to nutrient intake is also adaptive if there are costs associated with overconsuming nutrients that occur in excess of requirements (Simpson *et al.* 2004; Raubenheimer, Lee & Simpson 2005; Warbrick-Smith *et al.* 2009). Currently, our understanding of feeding behaviour of LW(f) and SW crickets in the field is limited – further field studies of *G. firmus* feeding ecology should help reveal how patterns of overall food availability, motility and habitat persistence interact to create different nutrient regulatory responses for wing-polymorphic crickets (Zera & Denno 1997).

Differential lipid production is a key component of the dispersal-reproduction life-history trade-off in *G. firmus* (Zera & Larsen 2001; Zera 2005). Our lipid data suggest that SW crickets are well suited to handling/processing excess nutrients, while LW(f) crickets are particularly efficient at utilizing the nutrients that are ingested. For instance, even though LW(f) and SW crickets ate similar total amounts of carbohydrate in the choice experiments, LW(f) crickets generated significantly more lipid, measured as per cent body fat. Previous radiotracer studies have documented that synthesis of new fatty acid is higher, and oxidation of fatty acid is lower, in the LW(f) morph compared with the SW morph of *G. firmus* (Zera 2005; Zera & Zhao 2006). In the no-choice experiments, on the two most carbohydrate-biased treatments (and especially on the p7 : c35 treatment), LW(f) crickets ingested *smaller* amounts of carbohydrate relative to SW crickets, yet both morphs had identical lipid levels (measured as per cent body fat) on these two diets. Together, these findings indicate that lipid acquisition and allocation, a key component of the life-history trade-off in *G. firmus*, are dependent on nutrient availability and quality in the environment, and

on the crickets' consequent nutrient consumption patterns. Results obtained in the present study set the stage for additional investigations. In particular, it would be useful to relate these findings to SW and LW individuals collected from the same habitats to determine whether dietary decisions have similar consequences for growth and lipid acquisition patterns in natural settings. It is also important to ultimately compare additional LW(f)- and SW-selected lines to determine the extent to which the phenotypic differences identified in the present study are due to genetic differences between the morphs. Such studies are currently in progress.

Conclusions

The availability and quality of food can have profound influences on organismal life history, especially in the context of allocation to reproduction vs. dispersal (reviewed in Zera & Harshman 2001). The feeding studies reported here reveal, for the first time, such an influence; morphs of *G. firmus* self-select diets have different nutritional composition (with respect to their protein/carbohydrate ratio) and employ different consumption rules when encountering nutritionally imbalanced foods. This demonstrates a clear link between the expression of life-history trade-offs and the nutritional environment.

Acknowledgements

Many thanks to K. Roder and S. Cook for technical assistance with the experiments. This research was supported by an NSF grant (IOS-1121960) awarded to STB and an NSF grant (IOS-1122075) awarded to AJZ. AM was partially supported by an NSF grant (REU BIO #0755264) awarded to Kevin M. Heinz and Texas AgriLife Research.

Data accessibility

Cricket measurements: DRYAD entry doi: 10.5061/dryad.rt950

References

- Barnes, A.I. & Partridge, L. (2003) Costing reproduction. *Animal Behaviour*, **66**, 199–204.
- Behmer, S.T. (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology*, **54**, 165–187.
- Behmer, S.T., Raubenheimer, D. & Simpson, S.J. (2001) Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Animal Behaviour*, **61**, 995–1005.
- Behmer, S.T., Simpson, S. & Raubenheimer, D. (2002) Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology*, **83**, 2489–2501.
- Boggs, C.L. (1992) Resource allocation: exploring connections between foraging and life history. *Functional Ecology*, **6**, 508–518.
- Boggs, C.L. (1997a) Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology*, **78**, 192–202.
- Boggs, C.L. (1997b) Reproductive allocation from reserves and income in butterfly species with differing adult diets. *Ecology*, **78**, 181–191.
- Boggs, C.L. (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, **23**, 27–37.
- Boggs, C. & Ross, C. (1993) The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). *Ecology*, **74**, 433–441.
- Capinera, J., Scott, R. & Walker, T. (2004) *Field Guide to Grasshopper, Katydid and Crickets of the United States and Canada*. Cornell University Press, Ithaca, NY.
- Cease, A.J., Elser, J.J., Ford, C.F., Hao, S., Kang, L. & Harrison, J.F. (2012) Heavy livestock grazing promotes locust outbreaks by lowering plant nitrogen content. *Science*, **335**, 467–469.
- Dadd, R. (1961) The nutritional requirements of locusts. 4. Requirements for vitamins of the B-complex. *Journal of Insect Physiology*, **6**, 1–12.
- Harrison, R. (1980) Dispersal polymorphisms in insects. *Annual Review of Ecology and Systematics*, **11**, 95–118.
- Horton, D. & Redak, R. (1993) Further comments on analysis of covariance in insect dietary studies. *Entomologia Experimentalis et Applicata*, **69**, 163–275.
- Lee, K.P., Simpson, S., Clissold, F., Brooks, R., Ballard, J., Taylor, P., Soran, N. & Raubenheimer, D. (2008) Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 2498–2503.
- Loverage, J. (1973) Age and the changes in water and fat content of adult laboratory-reared *Locusta migratoria migratorioides*. *Rhodesian Journal of Agricultural Research*, **11**, 131–143.
- Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessmann, J., Clissold, F., Raubenheimer, D., et al. (2008) Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Current Biology*, **18**, 1062–1066.
- Parnesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637–669.
- Partridge, L. & Harvey, P.H. (1985) Evolutionary biology: costs of reproduction. *Nature*, **316**, 20.
- Pener, M.P. & Simpson, S.J. (2009) Locust phase polyphenism: an update. *Advances in Insect Physiology*, **36**, 1–272.
- R Development Core Team (2010) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org>.
- Raubenheimer, D., Lee, K.P. & Simpson, S.J. (2005) Does Bertrand's rule apply to macronutrients? *Proceedings of the Royal Society of London, Series B*, **272**, 2429–2434.
- Raubenheimer, D. & 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. *Physiological Entomology*, **15**, 219–233.
- Raubenheimer, D. & Simpson, S. (1992) Analysis of covariance - an alternative to nutritional indexes. *Entomologia Experimentalis et Applicata*, **62**, 221–231.
- Raubenheimer, D. & Simpson, S. (1999) Integrating nutrition: a geometrical approach. *Entomologia Experimentalis et Applicata*, **91**, 67–82.
- Raubenheimer, D. & Simpson, S. (2003) Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology*, **206**, 1669–1681.
- Rosenzweig, C., Casassa, G., Karoly, D., Imeson, A., Liu, C., Menzel, A. & Rawlins, S., et al. (2007) Assessment of observed changes and responses in natural and managed systems. *Climate Change 2007: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds M. Parry, O. Canzani, J. Palutikof, P. van der Linden & C. Hanson), pp. 79–131. Cambridge University Press, Cambridge.
- Simpson, S. & Abisgold, J. (1985) Compensation by locusts for changes in dietary nutrients – behavioral mechanisms. *Physiological Entomology*, **10**, 443–452.
- Simpson, S. & Raubenheimer, D. (1993) A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **342**, 381–402.
- Simpson, S.J. & Raubenheimer, D. (1995) The geometric analysis of feeding and nutrition: a user's guide. *Journal of Insect Physiology*, **41**, 545–553.
- Simpson, S.J. & Raubenheimer, D. (2012) *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton University Press, Princeton, NJ.
- Simpson, S., Raubenheimer, D., Behmer, S., Whitworth, A. & Wright, G. (2002) A comparison of nutritional regulation in solitary- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. *Journal of Experimental Biology*, **205**, 121–129.
- Simpson, S., Sibly, R., Lee, K.P., Behmer, S. & Raubenheimer, D. (2004) Optimal foraging when regulating intake of multiple nutrients. *Animal Behaviour*, **68**, 1299–1311.

- Warbrick-Smith, J., Raubenheimer, D., Simpson, S.J. & Behmer, S.T. (2009) Three hundred and fifty generations of extreme food specialization: testing predictions of nutritional ecology. *Entomologia Experimentalis et Applicata*, **132**, 65–75.
- Wheeler, D. (1996) The role of nourishment in oogenesis. *Annual Review of Entomology*, **41**, 407–431.
- Zera, A.J. (2004) The endocrine regulation of wing polymorphism in insects: state of the art, recent surprises, and future directions. *Integrative and Comparative Biology*, **43**, 607–616.
- 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. *Integrative and Comparative Biology*, **45**, 511–524.
- Zera, A.J. (2009) Wing polymorphism in *Gryllus* (Orthoptera: Gryllidae): proximate endocrine, energetic and biochemical bases underlying morph specializations for flight vs. reproduction. *Phenotypic Plasticity in Insects: Mechanisms and Consequences* (eds T.N. Ananthakrishnan & D. Whitman), pp. 609–653. Science Publishers, Enfield, NH.
- Zera, A.J. & Cisper, G. (2001) Genetic and diurnal variation in the juvenile hormone titer in a wing-polymorphic cricket: implications for the evolution of life histories and dispersal. *Physiological and Biochemical Zoology*, **74**, 293–306.
- Zera, A.J. & Denno, R.F. (1997) Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, **42**, 207–230.
- Zera, A.J. & Harshman, L.G. (2001) The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics*, **32**, 95–126.
- Zera, A.J. & Harshman, L.G. (2009) Laboratory selection studies of life history physiology in insects. *Experimental Evolution: Concepts, Methods, and Applications* (eds T. Garland Jr & M.R. Rose), pp. 217–262. University of California Press, Berkeley, CA.
- Zera, A.J. & Harshman, L.G. (2011) Intermediary metabolism and the biochemical-molecular basis of life history variation and trade-offs in two insect models. *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-offs* (eds T. Flatt & A. Heyland), pp. 311–328. Oxford University Press, New York.
- Zera, A.J. & 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*. *Journal of Insect Physiology*, **47**, 1147–1160.
- Zera, A.J., Sall, J. & Grudzinski, K. (1997) Flight-muscle polymorphism in the cricket *Gryllus firmus*: muscle characteristics and their influence on the evolution of flightlessness. *Physiological Zoology*, **70**, 519–529.
- Zera, A.J. & Tiebel, K.C. (1988) Brachypterizing effect of group rearing, juvenile hormone-III and methoprene in the wing dimorphic cricket, *Gryllus rubens*. *Journal of Insect Physiology*, **34**, 489–498.
- Zera, A.J. & 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. *The American Naturalist*, **167**, 889–900.
- Zhao, Z. & Zera, A.J. (2002) Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 16829–16834.

Received 20 December 2012; accepted 14 March 2013

Handling Editor: Kwang Pum Lee

Supporting Information

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

Fig. S1. Mass of food consumed by morphs in ‘choice’ experiment.

Fig. S2. Mass of food consumed across diets in ‘no-choice’ experiment.