



SYMPOSIUM

Effects of Protein and Carbohydrate on an Insect Herbivore: The Vista from a Fitness Landscape

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Synopsis Protein and carbohydrates are important nutrients driving the growth of herbivores; however, their content in plants is highly variable. Multiple studies have explored their effect on herbivores, but only one other study (using a caterpillar) has provided a comprehensive overview that includes a simultaneous evaluation of their ratios and concentrations. In the present work, we ran two experiments using nymphs of the generalist grasshopper *Melanoplus differentialis*. Grasshoppers and caterpillars differ in a number of important ways, which might affect their feeding and physiological responses to foods with variable content of protein and carbohydrates. First, in a choice experiment, we measured performance and related this to the self-selected intake of nutrients. No differences were found for duration of development across treatments, but gain in mass was lower on a diet of low macronutrient concentration. Consumption of protein was always tightly regulated, but intake of carbohydrate was significantly reduced when consuming diluted food. In the second experiment, insects were constrained to one of nine diets and we plotted performance and consumption using a fitness-landscape approach that mimics the natural variation of nutrients in plants. We found significant effects of protein and carbohydrate content on gain in mass and in duration of development. The concentration of macronutrients in the food had more pronounced effects than did the protein-to-carbohydrate ratio. The protein–carbohydrate content also significantly affected the intake of food and energy (calories), production of frass, and digestive efficiency. On foods with low macronutrient concentration consumption was high, but digestive efficiency was low. Our results suggest that insects will favor protein-biased foods when the total macronutrient content of available foods is low, and that in the short-term compensatory feeding responses can overcome nutritional deficits and/or imbalances. However, over the long term, insect herbivores might pay substantial costs when eating foods that are nutritionally suboptimal.

Introduction

With the exceptions of nutrients provided by endosymbionts (Karasov and Douglas 2013), all animals acquire the multiple nutrients needed for growth, maintenance, and reproduction by eating. Proteins and carbohydrates are particularly important dietary nutrients (Behmer 2009; Simpson and Raubenheimer 2012), and although they have identical caloric value, from an animal’s perspective they are functionally very different. Dietary proteins provide the amino acids needed to build new tissues, enzymes, and proteins, whereas carbohydrates are commonly used as the key energy source needed to fuel this biosynthesis. Amino acids can, through gluconeogenesis, act as a substrate to generate glucose, but generally this

only happens when dietary digestible carbohydrates are low (Thompson 2000; Thompson et al. 2002).

All foods vary in their protein and carbohydrate content, but generally variation of these two nutrients in plants is much greater than in animals (Schoonhoven et al. 2005; Clissold et al. 2006, 2009; Behmer and Joern 2012). For plants, variation in proteins and carbohydrates exists at a number of levels, including between species (Yeoh et al. 1992), within species (Sattelmacher et al. 1994), and within an individual plant (Mattson 1980) depending on the type of tissue (i.e., leaves, flowers, seeds, and stems), and its age (i.e., young versus old leaves). Additionally, a plant’s protein and carbohydrate content can vary in response to environmental factors,

including the amount of light it receives, the chemical composition of the soil, and inputs of water (Holt and Hilst 1969; Hendrix and Huber 1986; Chapin et al. 1987; Felton 1996; Walter et al. 2012). The concentration of nutrients in plants is also generally low, compared with animal tissue (Bernays and Chapman 1994; Karasov and Martínez del Río 2007). Thus, the challenge for herbivores is to find and eat foods that contain the best blend of proteins and carbohydrates, and at a high concentration.

Plants with nutritionally optimal vegetative tissue are rare, but herbivores can still meet their requirements for protein and carbohydrate using a suite of pre-ingestive and post-ingestive mechanisms (Behmer 2009). For example, they can regulate their intake of protein and carbohydrate by mixing their diet, either by switching between plants or plant tissues (Singer et al. 2002; Villalba et al. 2002; Wright et al. 2003; Villalba and Provenza 2005; Clements et al. 2009; Felton et al. 2009). When the opportunity to mix their diet is limited, or constrained (e.g., predators), insect herbivores can adjust feeding responses both to the balance and the concentration of nutrients in their food (Slansky and Wheeler 1992; Raubenheimer and Simpson 1993; Yang and Joern 1994a; Lee et al. 2004; Berner and Blanckenhorn 2006; Fanson et al. 2012). However, under natural conditions, these mechanisms can sometimes be restricted. For instance, balancing the intake of protein and of carbohydrate by alternating host plants might not be possible because of the threat of predation (Beckerman et al. 1997; Schmitz and Suttle 2001; Danner and Joern 2003; Hawlena and Schmitz 2010). Likewise, because the probability of attack by predators is higher when herbivores are actively feeding (Bernays 1997; Bowers 1993), compensatory feeding on foods that have low concentrations of nutrients can increase the risk of predation because insects are forced to feed for a longer period of time.

A fitness-landscape approach offers the best way to fully understand how the protein and carbohydrate content of food, both in terms of absolute amounts and ratios, affects an animal, including herbivores. This approach was first employed by Simpson et al. (2004), by feeding a generalist caterpillar (*Spodoptera littoralis*) a range of diets that varied in two ways. First, diets differed in their protein:carbohydrate (P:C) ratio. Second, they differed in their concentrations of protein and carbohydrates (for each P:C ratio, total macronutrient content varied from low to high). The collection of diets used in this study delimited a nutritional space, and for each diet in this nutritional space, a measure of animal

performance was calculated (a combination of survival, duration of development, and gain in mass). This variable was then visualized as a response surface (plot of a fitness landscape). This study revealed, in protein-carbohydrate nutritional space, how fitness costs increase in a continuous and smooth fashion, as distance from the self-selected intake of protein and carbohydrate increases.

This article also highlighted how fitness-landscape plots can be valuable in terms of understanding an organism's ecological niche. With a few exceptions, caterpillars typically live in protein-rich habitats, and demonstrate protein-biased intakes (Behmer 2009). In contrast, many rangeland insects (e.g., grasshoppers) experience nutritional habitats that are more carbohydrate-biased (Behmer and Joern 2012; Lenhart et al. 2014), and they tend to self-select diets that are balanced, or slightly carbohydrate-biased (Behmer and Joern 2008; Behmer 2009). Grasshoppers, in contrast to caterpillars, also generally tend to be much more mobile, which provides them greater opportunity to practice selective feeding. Finally, caterpillars and grasshoppers differ in one other important way—they have dramatically different alimentary canals (Dow 1987). Caterpillars basically lack a foregut, have a large midgut (which is the primary site of absorption), and a relatively short hindgut. This design favors rapid throughput over storage, which is beneficial considering how rapidly caterpillars grow (three orders of magnitude within 2 weeks). In contrast, grasshoppers invest equally in the foregut, midgut, and hindgut; this design allows for short-term storage, and longer residence time, which can lead to greater absorption efficiency of nutrients from ingested food (Yang and Joern 1994a, 1994b). Given these important differences, caterpillars and grasshoppers may employ different regulatory and growth rules in response to variable inputs of protein and carbohydrate. The extent to which these differences might occur is best investigated using a fitness-landscape approach.

In this article, we conducted two experiments that examined how the content of protein and carbohydrate in food affects performance, composition of the body, feeding behavior, and digestion in the grasshopper *Melanoplus differentialis*. This species occurs throughout much of the United States where it is recorded as feeding on a wide range of herbaceous and successional plants (Capinera et al. 2004). We first performed a choice experiment, in which final instar nymphs were given three different paired foods that were nutritionally complementary to one another; for each pairing, we measured the consumption of protein and carbohydrate, gain in

mass, and duration of development. In our second experiment, grasshoppers were constrained to one of nine diets, encompassing a range of concentrations and ratios of protein and carbohydrate that mimicked what might typically be found in plants eaten by this species. For each diet, we recorded a suite of physiological variables (duration of development, gain in dry mass, and lipid content), plus measures of consumption (intake of food, total macronutrients, protein, and carbohydrate), egestion (production of feces), and the efficiency of digestion. We analyzed these data using response-surface methods, and visualized them as fitness landscapes plotted over the protein–carbohydrate nutrient space defined by our experimental diets. This approach allowed us to disentangle the relative effect of the concentrations and ratios of protein and carbohydrate both on feeding behavior and on physiology. We discuss our results in light of earlier published work, and its implications for future research.

Materials and methods

Insects and experimental chambers

Insects used in this study came from a culture fed a diet of greenhouse-grown seedling wheat and wheat germ, and maintained in the Department of Entomology, Texas A&M University, College Station, TX, USA; the colony was initiated in 2008 from a local population. They were maintained under standard laboratory conditions with a 14 h:10 h L:D photoperiod, and under radiant heat at a temperature of 32–35°C during the light phase (supplied by 60W full-spectrum incandescent bulbs), and at 24–26°C during the dark phase.

Nymphs of both sexes were removed from the culture within 4–6 h after ecdysis to the sixth stadium (day 0), weighed (to the nearest 0.1 mg), and then transferred individually into clear plastic chambers (19 cm long × 14 cm wide × 9 cm high). Each chamber contained either two food dishes (experiment 1), or one food dish (experiment 2), an expanded aluminum roost for perching, and a source of water (a 30-mL Solo Cup, fitted with a lid that had been modified to allow the use of a cotton wick for drinking); the food dishes were modified Petri dishes designed to minimize spillage (Raubenheimer and Simpson 1990). Insects were maintained throughout the experiment under a 14 h:10 h L:D regime in an incubator. In the choice experiment, temperatures varied between 32°C (light cycle) and 26°C (dark cycle). In the no-choice experiment, the temperatures were maintained at 28°C both for the light and the dark cycles.

Synthetic foods

The chemically defined foods used in this study were based on those used by Behmer et al. (2003). In total, nine foods differing in their absolute amounts and ratios of protein (p) and digestible carbohydrate (c) were prepared: (1) p7:c7 (this diet contained 7% protein and 7% digestible carbohydrate, expressed on a dry mass basis), (2) p7:c14, (3) p14:c7, (4) p7:c35, (5) p14:c28, (6) p21:c21, (7) p28:c14, (8) p35:c7, and (9) p35:c35. These diets, shown in Fig. 1, span a range of concentrations and ratios of protein and carbohydrate that *M. differentialis* nymphs can encounter in the field (see Behmer and Joern 2012). The protein component of all foods was a 3:1:1 mixture of casein, peptone, and albumen, whereas the digestible carbohydrate (henceforth carbohydrate) component was a 1:1 mixture of sucrose and dextrin. All foods contained similar amounts of Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%), and vitamin mix (0.2%) (Dadd 1961a, 1961b). The remainder of the diet was cellulose, a non-nutritive bulking agent.

Experimental treatments

Two separate experiments were performed. The first, a choice-experiment, had three treatments (each containing two food dishes): (1) p7:c35 paired with p35:c7, (2) p7:c35 paired with p28:c14, and (3) p7:c14 paired with p14:c7. The total macronutrient content of each food, in the first two food pairings, was 42%; for the third food pairing, the total macronutrient content of each food was 21%. Previous work has shown that *M. differentialis* nymphs actively regulate their intake of protein and carbohydrate when macronutrient concentration is high (e.g., food pairings 1 and 2) (see Behmer and Joern 2008). The third pairing allowed us to test the extent to which *M. differentialis* nymphs regulate their intake of protein and carbohydrate when macronutrient concentration is low. For each treatment, we measured: (1) amount of food consumed from each dish, (2) intake of protein and carbohydrate (from both food dishes, combined), (3) gain in dry mass, (4) duration of development, and (5) lipid content of the body.

The second experiment was a study that included all nine treatments listed above, but without offering a choice. We measured the same variables as outlined in experiment 1, as well as fecal production. This allowed us to quantify the efficiency at which our grasshoppers converted digested food (total consumption—feces) into body mass. This measurement, the efficiency of conversion of digested food

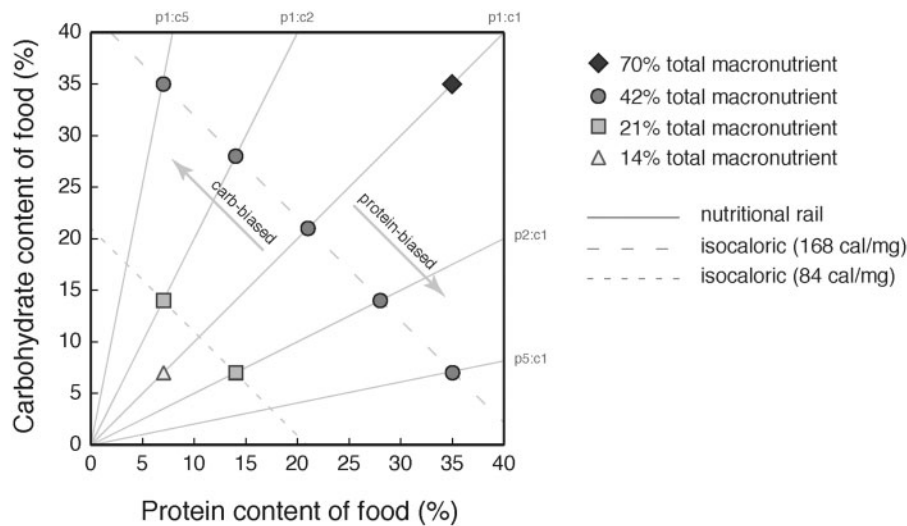


Fig. 1 The protein (p) and digestible carbohydrate (c) content of the nine artificial diets used in this study. The diets represent five different P:C ratios, with each shown as a “nutritional rail” (the thin gray lines projecting from the origin). The three diets on the middle nutritional rail have equal amounts of protein and carbohydrate (p1:c1), but differ in their total macronutrient concentration (14%, 42%, and 70%). Three diets occur to the left of the p1:c1 rail; two have twice as much carbohydrate as protein (p1:c2; total macronutrient content of 21% and 42%), whereas the third has five times as much carbohydrate as protein (p1:c5; total macronutrient content of 42%). The three remaining foods, left of the p1:c1 rail, are protein-biased; two have twice as much protein as carbohydrate (p2:c1), whereas the third has five times as much protein as carbohydrate (p5:c1; total macronutrient content of 42%). Finally, two diet groups are isocaloric to one another. The diets in the first group (squares on the short-dashed line) each contain 84 cal/mg. The diets in the second group (circles on the long-dashed line) each contain 168 cal/mg.

(ECD) is calculated as follows:

$$\text{ECD} = \left[\frac{\text{gain in dry mass (mg)}}{(\text{total consumption of food (mg)} - \text{frass (mg)})} \right]$$

Experimental protocol

In both experiments, similar protocols were followed. First, food was scooped into individual dishes, left to equilibrate under stable ambient room humidity for 24 h, and then weighed to the nearest 0.1 mg. On the first day of the sixth-stadium (day 0), newly molted nymphs were placed, individually, into the experimental chambers and allowed to feed for 72 h; at the end of this time, food dishes were then replaced with fresh, pre-weighed dishes of the same food, every 3 days, until nymphs molted to the adult stage. All removed food dishes were left to equilibrate to room humidity for 24 h before being weighed (to the nearest 0.1 mg). Upon molting, grasshoppers were weighed (to the nearest 0.1 mg) and then placed into a freezer and stored. At this time, we also collected and measured (to the nearest 0.1 mg) total fecal production. Shortly following the conclusion of the experiment, grasshoppers were removed from the freezer, dried in a desiccating oven at 40°C until they reached a constant mass, and

weighed to the nearest 0.1 mg. We then extracted lipids from each individual grasshopper, using a series of chloroform washes (see Behmer et al. 2001); lipid content was calculated by subtracting the dry mass post-chloroform wash from the dry mass pre-chloroform wash. To calculate the gain in dry mass, we estimated the initial dry mass with a regression equation that was derived from the dry mass of 20 newly molted sixth instar grasshoppers from the laboratory colony.

Statistical analyses and presentation of data

In the choice experiment, the amount of protein and carbohydrate eaten was compared using multiple analysis of covariance (MANCOVA) (with food pairing treatment as the main effect in the model), using Pillai’s test statistic; starting mass was used as a covariate to correct for differences in size among individuals (females are typically larger than males). When significant effects were observed, *post hoc* comparisons were performed using contrasts, following the techniques employed by Scheiner (1993). Intake of protein and carbohydrate was also compared, independently, using univariate tests (analysis of variance). Gain in dry mass was analyzed using analysis of covariance (ANCOVA), with initial starting mass as a covariate to account for differences

Table 1 Results of MANCOVA (P:C intake) and univariate ANCOVAs for the intake of protein and carbohydrate by grasshoppers from the choice experiment

Variable	Source	df	F-ratio	P-value
P + C intake	Treatment	4	3.29	0.018
	Sex	2	2.20	0.196
	Start mass	2	1.74	0.133
Individual contrasts associated with P + C intake				
	p7:c35 w/p35:c7 compared with p7:c35 w/p28:c14	2	0.61	0.550
	p7:c35 w/p28:c14 compared with p7:c14 w/p14:c7	2	7.69	0.003
	p7:c35 w/p35:c7 compared with p7:c14 w/p14:c7	2	4.09	0.030
Protein intake	Treatment	2	0.59	0.564
	Sex	1	4.58	0.042
	Start mass	1	3.58	0.070
Carbohydrate intake	Treatment	2	8.58	0.002
	Sex	1	0.29	0.592
	Start mass	1	0.54	0.471
Individual contrasts associated with carbohydrate intake				
	p7:c35 w/p35:c7 compared with p7:c35 w/p28:c14	1	1.02	0.322
	p7:c35 w/p28:c14 compared with p7:c14 w/p14:c7	1	15.95	<0.001
	p7:c35 w/p35:c7 compared with p7:c14 w/p14:c7	1	8.49	0.008

Notes: When treatment effects were significant, individual contrasts were used to identify where differences occurred. Bold indicates significant effect ($P < 0.05$).

in body size. Duration of development was compared using failure-time analysis.

In the no-choice experiments, we used response-surface methodology (Lee et al. 2008; Maklakov et al. 2008) to determine how the protein and carbohydrate content of food affected various responses by the insects. The broad objective was to find the values of the terms that produced a maximum or minimum across the protein–carbohydrate landscape generated by our nine experimental diets. As in the no-choice experiment, starting mass was used as a covariate to adjust for differences in size between the sexes. For each measured variable, data were mapped over the protein–carbohydrate nutrient landscape (see Fig. 1), and presented as fitness surfaces. Because these surfaces are best visualized with nonparametric techniques that do not constrain the shape of the surface (Blows and Brooks 2003), thin-plate splines were used within the fields package of R (version R 2.11.1), following the approach developed by Simpson et al. (2004).

Results

Choice experiment

Nutrient intake

Grasshoppers' ratio of intake of protein relative to carbohydrate (P:C) differed significantly between the three pairing of food (Table 1). As seen in Fig. 2a,

P:C intake was similar for treatments in which the total macronutrient content of food in each dish was 42% (pairings 1 and 2), but P:C intake on the treatment with foods having only 21% total macronutrient content (pairing 3) differed significantly compared with the pairings with 42% total macronutrient content (Table 1). An ANCOVA test showed that this was a function of differences in carbohydrate intake (Table 1); on average, grasshoppers given foods with the lower total macronutrient content ate about a third less total carbohydrate (Fig. 2a). In contrast, protein intake was regulated in a similar fashion between the three pairings of food (Table 1 and Fig. 2a). Patterns of food consumption revealed how grasshoppers achieved their P:C intake (Fig. 2b). Grasshoppers on the treatment pairing p7:c35 with p35:c7 ate significantly more from the dish containing food with p7:c35 (t -test: $t_9 = 4.94$, $P < 0.001$); for the other two treatments (p7:c14 w/p14:c7 and p7:c35 w/p28:c14), consumption from the two food dishes was not statistically different (t -test: $t_9 = 0.87$, $P = 0.407$ and t -test: $t_9 = 1.07$, $P = 0.310$, respectively). Total food consumption differed between the treatments, but not between males and females (Table 2). Food consumption was highest for the treatment in which both food dishes had 21% macronutrient content, it was lower for the two treatments in which total macronutrient content was 42% (Fig. 2b).

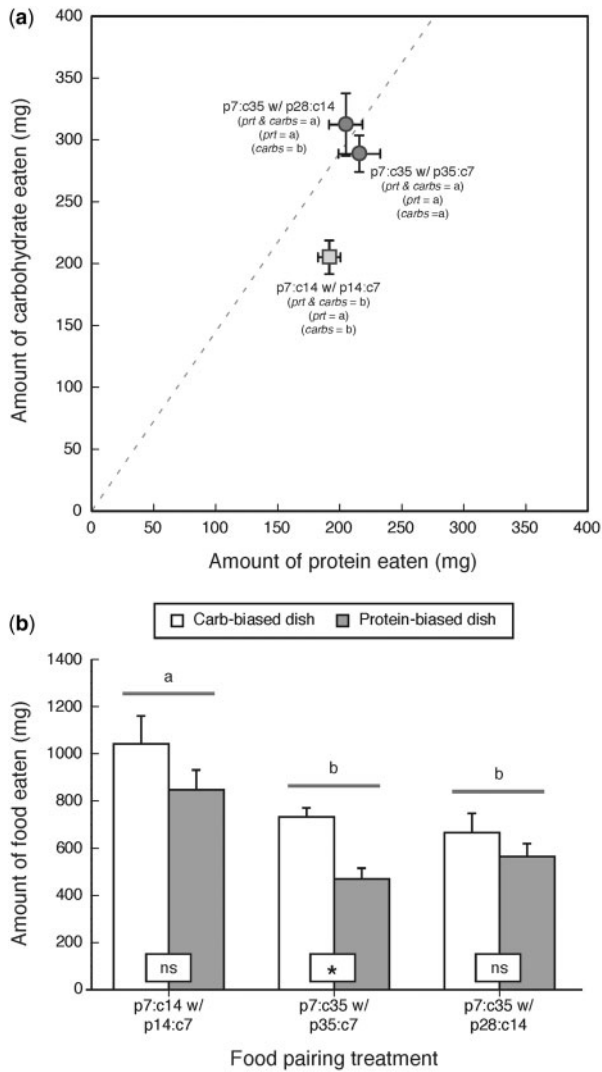


Fig. 2 Feeding behavior in the choice experiment. (a) Protein and carbohydrate consumption. The round symbols show treatments in which both foods had 42% total macronutrient content; the square symbol shows the treatment in which both foods had 21% total macronutrient content. Different letters adjacent to different treatments indicate statistically significant differences ($P < 0.05$) for a MANCOVA (for protein (prt) and carbohydrate (carb)) and univariate ANCOVAs (prt and carb). The dashed-line shows the mean P:C ratio of the two treatments with high concentration of nutrients. (b) The amounts eaten from each food dish in each treatment. Different letters above the bars indicate a significant difference in total consumption ($P < 0.05$). An asterisk indicates that significantly more food was eaten from one of the two food dishes ($P < 0.05$); ns = not significant ($P > 0.05$).

Gain in dry mass and duration of development

Females, on average, were heavier than males, and grasshoppers that had food dishes with 42% total macronutrient content were on average 30% heavier than grasshoppers from the treatment in which total macronutrient content was 21% (Table 3 and Fig. 3a). Females took longer to develop than

Table 2 Results of ANCOVA for consumption and gain in dry mass by grasshoppers from the choice experiment

Variable	Source	df	F-ratio	P-value
Total food eaten	Treatment	2	44.20	<0.001
	Sex	1	0.03	0.857
	Start mass	1	12.64	0.002
Gain in dry mas	Treatment	2	5.25	0.013
	Sex	1	5.54	0.027
	Start mass	1	0.31	0.580

Notes: Treatment refers to the food-pairing combination; initial mass was used as a covariate to adjust for differences in size among insects. Bold indicates significant effect ($P < 0.05$).

Table 3 The full parametric model for the linear and quadratic effects of protein and carbohydrate on measures of gain in mass, duration of development, and final lipid mass

Model terms	Duration of development	Gain in dry mass	Final lipid mass
Full model	$F = 21.63$ $P < 0.001$	$F = 9.82$ $P < 0.001$	$F = 7.68$ $P < 0.001$
Protein	$\chi^2 = 3.05$ $P = 0.081$ (-0.002)	$F = 2.09$ $P = 0.153$ (0.442)	$F = 0.17$ $P = 0.685$ (0.095)
Carbohydrate	$\chi^2 = 1.55$ $P = 0.213$ (-0.002)	$F = 21.52$ $P < 0.001$ (1.308)	$F = 12.58$ $P = 0.001$ (0.762)
Protein ²	$\chi^2 = 0.71$ $P = 0.399$ (-0.000)	$F = 0.20$ $P = 0.665$ (-0.018)	$F = 0.98$ $P = 0.327$ (0.033)
Carbohydrate ²	$\chi^2 = 3.25$ $P = 0.072$ (-0.000)	$F = 0.50$ $P = 0.482$ (-0.029)	$F = 4.61$ $P = 0.036$ (-0.014)
Protein-by-Carbohydrate	$\chi^2 = 3.56$ $P = 0.059$ (0.000)	$F = 0.21$ $P = 0.651$ (-0.009)	$F = 0.85$ $P = 0.359$ (-0.069)
Sex	$\chi^2 = 15.76$ $P < 0.001$ (0.072)	$F = 4.32$ $P = 0.042$ (6.576)	$F = 0.006$ $P = 0.938$ (0.191)
Start mass	$\chi^2 = 6.33$ $P = 0.012$ (-0.000)	$F = 10.98$ $P = 0.002$ (0.158)	$F = 16.59$ $P < 0.001$ (0.157)

Notes: Bold indicates significant effect ($P < 0.05$).

did males (failure-time analysis: $df = 1$, $\chi^2 = 15.17$, $P < 0.001$; Fig. 3b), but developmental time was not affected by composition of the food (failure-time analysis: $df = 2$, $\chi^2 = 4.43$, $P = 0.109$).

No-choice experiment

Measures of performance across the nutrient landscape
The full parametric models from response-surface analysis for duration of development, gain in mass,

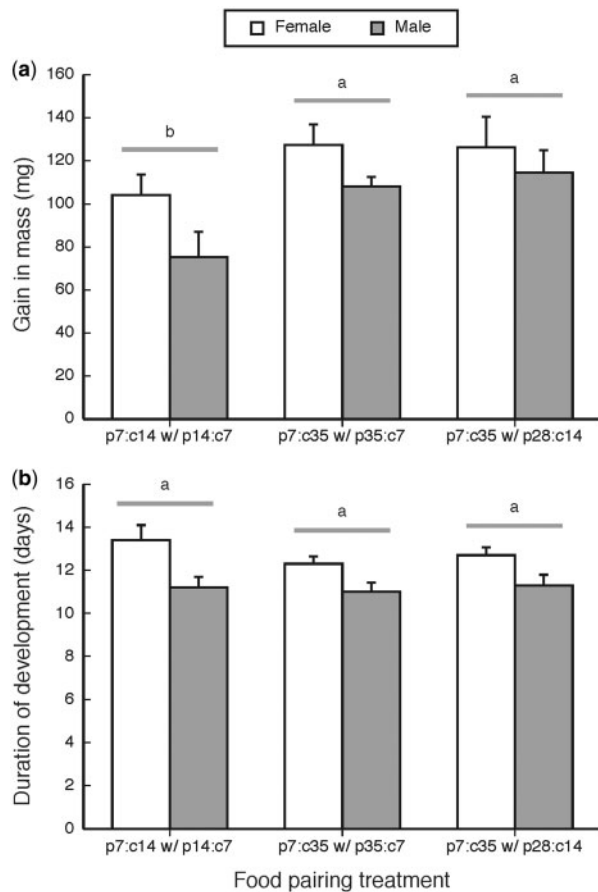


Fig. 3 Grasshoppers' performance in the choice experiment. (a) Mean gain in dry mass (\pm SEM) for females and males. (b) Mean duration of development (\pm SEM) for females and males. Different letters indicate statistically significant differences between treatments ($P < 0.05$).

and lipid content are reported in Table 3, and the associated response surfaces are shown in Fig. 4. There were no significant effects of protein or carbohydrate on the duration of development, although the protein-by-carbohydrate interaction term was nearly significant ($P = 0.059$), with grasshoppers on diets having low levels of macronutrients taking slightly longer to develop compared with those on other diets (Fig. 4a). On all diets, males developed significantly faster than did females, and developmental time was positively associated with initial mass (larger insects developed faster). Gain in mass increased significantly, and in a linear fashion, as the content of carbohydrate in the food increased (Fig. 4b), but it was not affected by the protein content of the food. Females gained more mass than did males, and gains in mass were higher in insects with high starting mass. Finally, levels of lipid were significantly affected by the carbohydrate content of food, but the coefficients showed that the linear effects (0.245)

were stronger than the quadratic effects (-0.014). Insects that were larger at the start of the experiment had higher lipid levels at the end of the experiment.

Measures of feeding and digestion across the nutrient landscapes

The full parametric models from response-surface analysis for various measures of consumption and digestion are reported in Table 4, and the associated response surfaces are shown in Fig. 5. There was a strong protein-by-carbohydrate interaction effect on total intake of food, with a peak occurring when the content of protein and carbohydrate was lowest (Fig. 5a). However, as the protein and carbohydrate content of the food increased, total intake of food gradually decreased evenly in all directions, away from this peak. When the intake of total macronutrients ($P + C$) was analyzed, significant linear and quadratic carbohydrate responses were observed (Fig. 5b), but the coefficients indicated a stronger linear response (2.840 versus -0.249). We also separately examined the intake of protein and carbohydrate. The intake of protein showed both significant linear and quadratic responses as the protein content of the diets increased, but the linear trend was much stronger (6.452 versus -0.197). The intake of protein also decreased in a strong linear fashion as the carbohydrate content of the food decreased. As a result, the intake of protein was greatest on protein-biased diets with high total macronutrient content (Fig. 5c). With respect to the intake of carbohydrates, significant linear and quadratic responses to the protein and carbohydrate content of the diets were observed, but the coefficients showed much stronger linear responses for both classes of nutrients (Table 4); the intake of carbohydrate was highest on carbohydrate-biased diets that had high total macronutrient content (Fig. 4d). For each of these four categories (intake of food, intake of total macronutrient, intake of protein, and intake of carbohydrate), values were always higher for females, and for grasshoppers that had higher initial mass (Table 4).

Our final two analyses examined how grasshoppers processed and digested the food they ate. First, the amount of feces produced, like the intake of food, showed a significant protein-by-carbohydrate interaction, with peak production occurring for grasshoppers eating foods with a low protein and carbohydrate content (Fig. 5e). Second, the efficiency at which grasshoppers converted digested food to body mass (ECD) showed significant linear carbohydrate and protein effects (Table 4); ECD gradually increased as both the carbohydrate and the protein content of the food increased (Fig. 5f), and was

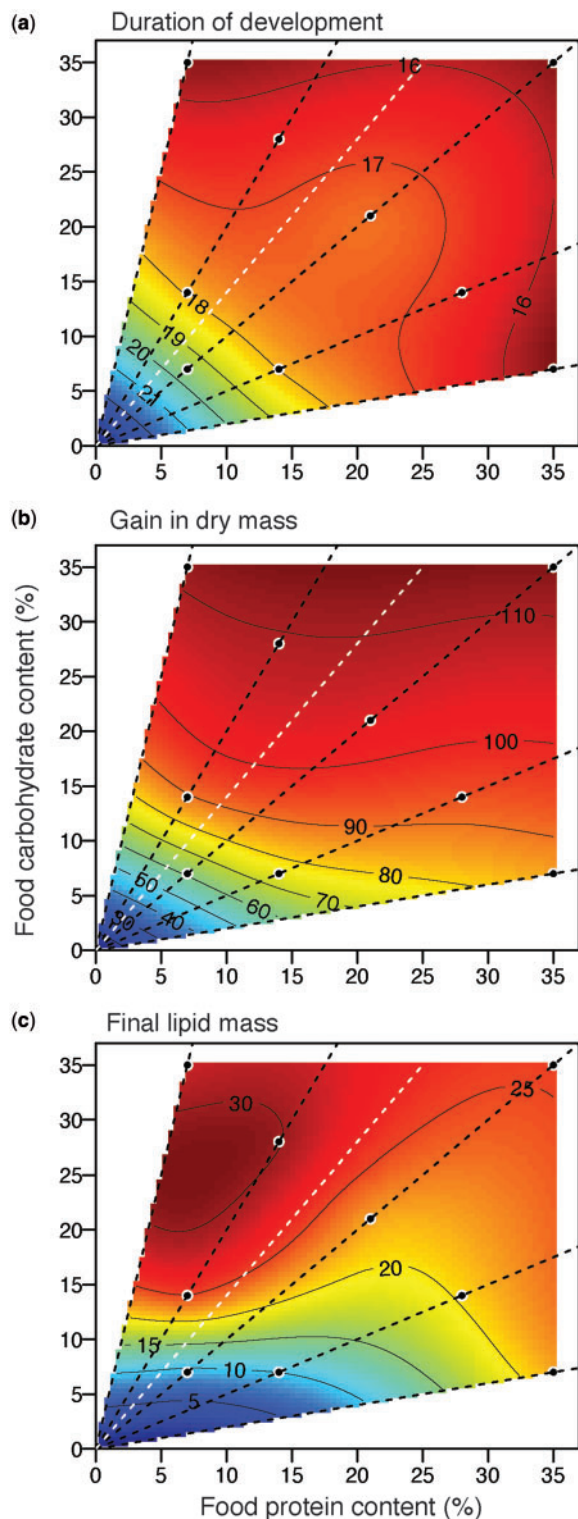


Fig. 4 Grasshoppers' performance, represented as fitness-landscape plots, on the nine diets in the no-choice experiment. (a) Duration of development, (b) gain in dry mass, and (c) final lipid mass (mg). The five P:C ratios tested are shown as black-dashed lines (nutritional rails) projecting from the origin; the self-selected P:C ratio from the first experiment is indicated by the white-dashed line.

highest for grasshoppers given the food with the greatest macronutrient content (p35:c35).

Discussion

We now know much about how food P:C ratio affects insect herbivores (Raubenheimer and Simpson 1993, 1999, 2003; Simpson and Raubenheimer 2000; Simpson et al. 2002; Lee et al. 2003), but in most of these studies P:C ratios have been examined at a single total macronutrient concentration (42%). For most insect herbivores, especially grasshoppers, host plants rarely contain total macronutrient concentrations at this level (Behmer and Joern 2012; Lenhart et al. 2014); a more typical situation is that total macronutrient content is lower (between 15% and 35%, and not all of this is available [Clissold 2007; Clissold et al. 2009]). In the current article, we show how the concentration of protein and carbohydrate in food modifies feeding behavior when grasshoppers are allowed to self-select their diets. However, on data on performance from both the choice and no-choice experiments reveal that at least in the short term (a single immature developmental stage), grasshoppers can employ effective compensatory responses across a range of nutritional conditions. Collectively, these results enhance our understanding of how an insect herbivore's physiological design and behavior are adapted to their particular ecological niche.

When given the opportunity, most herbivorous insects actively regulate their intake of protein and carbohydrate by selectively eating foods that contain different blends of these nutrients (Behmer 2009). However, our data, combined with data from caterpillars presented with variable foods over their final stage of development (Lee et al. 2002), suggested that when insect herbivores encounter foods that have low macronutrient concentration, they will favor foods that are protein-biased (when they are available). For both caterpillars and grasshoppers, this behavior will keep them from reaching their preferred intake of protein and carbohydrate. Interestingly, though, this behavior has different outcomes for grasshoppers and caterpillars. In the case of grasshoppers, prioritizing the intake of protein over carbohydrate negatively impacted gain in mass, but not the duration of development. In contrast, for caterpillars, the size of pupae was similar regardless of the intake of protein and carbohydrate, whereas caterpillars on treatments in which both foods had high macronutrient content developed fastest (Lee et al. 2002). Caterpillars are designed to grow bigger and faster than grasshoppers, so

Table 4 The full parametric model for the linear and quadratic effects of protein and carbohydrate on consumption

Model terms	Intake of food	Intake of macronutrients	Intake of protein	Intake of carbohydrate	Production of feces	Efficiency of digestion
Full model	$F = 114.10$ $P < 0.001$	$F = 23.52$ $P < 0.001$	$F = 99.14$ $P < 0.001$	$F = 123.13$ $P < 0.001$	$F = 111.00$ $P < 0.001$	$F = 6.44$ $P < 0.001$
Protein	$F = 308.87$ $P < 0.001$ (-42.310)	$F = 0.60$ $P = 0.442$ (0.494)	$F = 301.32$ $P < 0.001$ (6.452)	$F = 224.20$ $P < 0.001$ (-5.957)	$F = 266.89$ $P < 0.001$ (-34.225)	$F = 19.10$ $P < 0.001$ (0.004)
Carbohydrate	$F = 214.45$ $P < 0.001$ (-32.765)	$F = 22.92$ $P < 0.001$ (2.840)	$F = 164.62$ $P < 0.001$ (-4.432)	$F = 386.94$ $P < 0.001$ (7.274)	$F = 292.49$ $P < 0.001$ (-33.657)	$F = 9.04$ $P = 0.004$ (0.003)
Protein ²	$F = 15.05$ $P = 0.003$ (1.26)	$F = 0.09$ $P = 0.763$ (0.026)	$F = 15.60$ $P < 0.001$ (-0.197)	$F = 17.43$ $P < 0.001$ (0.223)	$F = 5.65$ $P = 0.028$ (0.683)	$F = 1.42$ $P = 0.238$ (-0.000)
Carbohydrate ²	$F = 0.86$ $P = 0.358$ (-0.296)	$F = 8.58$ $P = 0.005$ (-0.249)	$F = 3.46$ $P = 0.068$ (0.092)	$F = 41.46$ $P = 0.001$ (-0.340)	$F = 1.88$ $P = 0.175$ (0.395)	$F = 1.83$ $P = 0.182$ (0.000)
Protein-by-Carbohydrate	$F = 82.16$ $P < 0.001$ (1.404)	$F = 0.03$ $P = 0.862$ (-0.007)	$F = 0.01$ $P = 0.944$ (-0.002)	$F = 0.05$ $P = 0.832$ (-0.005)	$F = 77.21$ $P < 0.001$ (1.197)	$F = 3.34$ $P = 0.073$ (-0.000)
Sex	$F = 32.22$ $P < 0.001$ (140.087)	$F = 47.10$ $P < 0.001$ (44.934)	$F = 33.97$ $P < 0.001$ (22.209)	$F = 31.04$ $P < 0.001$ (22.725)	$F = 19.65$ $P < 0.001$ (99.141)	$F = 2.39$ $P = 0.127$ (-0.017)
Start mass	$F = 10.08$ $P = 0.002$ (1.189)	$F = 13.60$ $P = 0.001$ (0.366)	$F = 5.85$ $P = 0.019$ (0.140)	$F = 13.39$ $P = 0.001$ (0.226)	$F = 4.56$ $P = 0.037$ (0.743)	$F = 1.66$ $P = 0.202$ (0.000)

Notes: Bold indicates significant effect ($P < 0.05$).

grasshoppers may trade-off developing faster over growing bigger given that actively regulating the intake both of protein and of carbohydrate, by eating greater total amounts of food, could increase the risk of predation (Bernays and Minkenbergh 1997; Lima 1998). Additionally, compensatory feeding, in response to nutrient dilution can also be physiologically costly (Martin and Vanthof 1988; Slansky and Wheeler 1989; Slansky 1993).

Our first experiment also helps interpret responses in the no-choice experiment because it provides a reference point indicating *M. differentialis*' preferred P:C ratio (shown as the white dashed line on the fitness-landscape plots in Figs. 4 and 5). This reference point is important because for insect herbivores, self-selected ratios are known to be functionally optimal (Simpson and Raubenheimer 1993; Lee et al. 2002; Simpson et al. 2004; Behmer and Joern 2008; Roeder and Behmer 2014). Duration of development and gain in mass are two important performance variables for insect herbivores (often associated with fitness), and results from our response-surface analyses demonstrated that duration of development was a function of an interaction between the protein content and the carbohydrate content of food, whereas gain in mass was a function of carbohydrate content. Protein is considered the most limiting

nutrient for insect herbivores (Bernays and Chapman 1994; Schoonhoven et al. 2005), but our results clearly demonstrate a key role for digestible carbohydrates, as it directly impacted mass. However, high carbohydrate intake is not necessarily better, because it leads to excessive levels of lipids, especially when the protein concentration in food is low (Fig. 4d). The importance of keeping lipid levels low has been demonstrated in caterpillars reared on carbohydrate-biased foods for multiple generations—in only eight generations they evolved the ability to eat excess carbohydrate without laying it down as fat (Warbrick-Smith et al. 2006). Deposition of lipids also has important physiological costs—theoretical estimates suggest that the conversion of hexoses to storage fat can take up to 20–25% of the energy content of the food supplied (Westerterp 1994).

Interestingly, and in contrast with our results, the caterpillars used by Simpson et al. (2004) showed a clear fitness peak, centered on the self-selected intake of protein and carbohydrate. A number of factors might explain why a clear peak centered on the self-selected intake of protein and carbohydrate was not observed for our grasshoppers. First, our data show that the grasshoppers in our study practice strong compensatory feeding, especially when given foods that have low macronutrient concentration (see Fig.

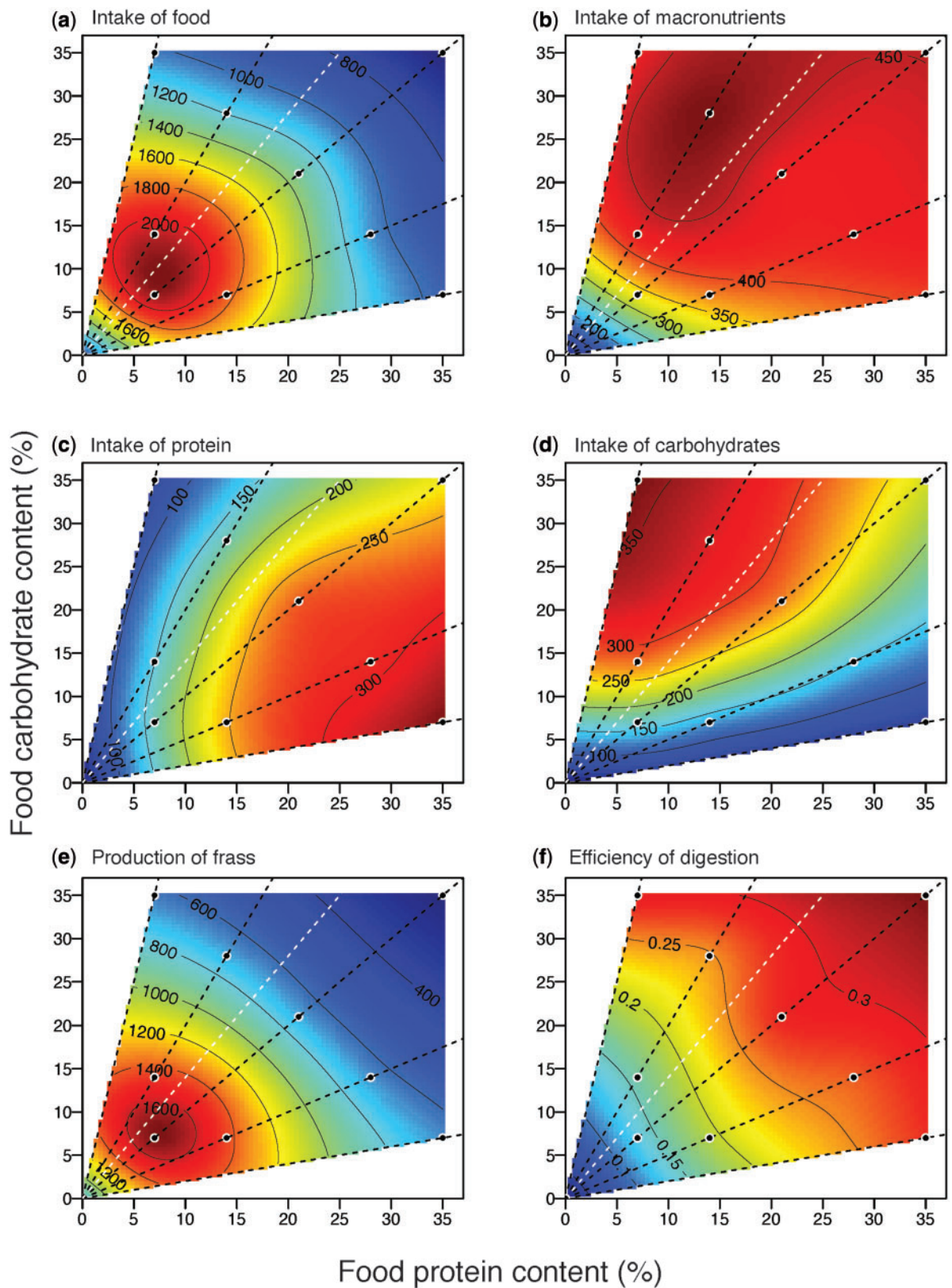


Fig. 5 Grasshoppers' feeding and digestive responses, represented as fitness-landscape plots, on the nine diets in the no-choice experiment. (a) Intake of food, (b) intake of macronutrients, (c) intake of protein, (d) intake of carbohydrate, (e) production of feces, and (f) efficiency of digestion. The five P:C ratios tested are shown as black-dashed lines (nutritional rails) projecting from the origin; the self-selected P:C ratio from the first experiment is indicated by the white-dashed line.

5a). Dietary compensation in insect herbivores is well documented (Slansky and Wheeler 1989, 1991; Raubenheimer and Simpson 1993; Zanotto et al. 1993; Chambers et al. 1995; Clissold et al. 2010), but in our study we demonstrated the extent to which this occurs across a broad nutritional landscape. Second, when grasshoppers were eating foods that were less concentrated (e.g., p7:c14 and p14:c7) their selected intake of protein and carbohydrate was more balanced than when they were offered foods with high macronutrient content (e.g., p7:c35 and p35:c7). Third, because grasshoppers eat in discrete feeding bouts (Simpson 1995), and have alimentary canals designed to hold and process foods with high efficiency across a number of different conditions (Dow 1987; Clissold et al. 2010; Coggan et al. 2011), they may be utilizing ingested nutrients more efficiently than caterpillars. However, two other factors might also partially explain why performance differences between the treatments were so minimal for different protein: carbohydrate ratios. First, Lee and Roh (2010) showed, using caterpillars, that the magnitude of nutritional effects can be dampened as temperature is reduced. When Behmer and Joern (2008) examined *M. differentialis* on a similar suite of diets those used in the current study (they had the total macronutrient content of 42%), but at a slightly higher temperature, growth rates were observed (especially on the most carbohydrate-biased diets). Second, Parsons and Joern (2014) showed a latitudinal effect on growth rates in the grasshopper *Melanoplus femurrubrum*. In this case, southern populations had slower growth rates than did northern populations. In the current study, grasshoppers were derived from a central Texas population, whereas those in the earlier study (Behmer and Joern 2008) originated from western Nebraska.

Feeding is a fundamental process, important to all animals, and although insect herbivores have a suite of mechanisms available to overcome nutritionally suboptimal food (either as a function of low concentration or imbalanced macronutrient content relative to species-specific requirements), they show better performance when they have access to foods containing protein and digestible carbohydrate in the right ratio, and at high concentrations. Our study, like most studies to date, examined only a single developmental stage. Collectively, these studies suggest that in the short-term insect herbivores can adapt to differences in food protein and carbohydrate, for example by growing faster, ratios when examined across a single developmental stage, but they also suggest that differences in insect herbivores' digestive physiology, specifically alimentary canal design, might impact compensatory responses and

physiological outcomes in unique ways (e.g., like grasshoppers growing faster when they have limited access to food). Recently, the effects of the content of protein and carbohydrate in food were examined over a longer time frame—from hatching to pupation to eclosion, and finally through to reproduction (Roeder and Behmer 2014). That study revealed that larval performance (e.g., pupal mass, duration of development, and survival) did not differ dramatically between seven diets that varied broadly in their P:C ratios (ranging from p1:c3 up to p3:c1). Instead, the effects only began to show during pupal development, including strong differences between males and females. When data from this study were used in a simple model of macronutrient dietary effects at the population level, the importance of eating foods that match the preferred P:C ratio was revealed, including how even small deviations from this preferred intake can have pronounced effects. We encourage researchers to further our understanding of nutritional ecology and physiology, in all types of organisms, by combining a fitness-landscape approach, across an organism's lifetime, as it will best capture the full effects of both food macronutrient concentrations and ratios.

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References

- Beckerman AP, Uriarte M, Schmitz OJ. 1997. Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proc Natl Acad Sci USA* 94:10735–8.
- Behmer ST. 2009. Insect herbivore nutrient regulation. *Annu Rev Entomol* 54:165–87.
- Behmer ST, Joern A. 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proc Natl Acad Sci USA* 105:1977–82.
- Behmer ST, Joern A. 2012. Insect herbivore outbreaks viewed through a physiological framework: insights from Orthoptera. In: Barbosa P, Letourneau DK, Agrawal AA,

- editors. Insect outbreaks revisited. Oxford (UK): Blackwell Publishing Ltd. p. 1–29.
- Behmer ST, Cox E, Raubenheimer D, Simpson SJ. 2003. Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Anim Behav* 66:665–75.
- Behmer ST, Raubenheimer D, Simpson SJ. 2001. Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Anim Behav* 61:995–1005.
- Bernays EA. 1997. Feeding by lepidopteran larvae is dangerous. *Ecol Entomol* 22:121–3.
- Bernays EA, Chapman RF. 1994. Host-plant selection by phytophagous insects. New York (NY): Chapman and Hall.
- Bernays EA, Minkenberg OPJM. 1997. Insect herbivores: different reasons for being a generalist. *Ecology* 78:1157–69.
- Berner D, Blanckenhorn WU. 2006. Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. *J Anim Ecol* 75:130–9.
- Blows MW, Brooks R. 2003. Measuring nonlinear selection. *Am Nat* 162:815–20.
- Bowers MD. 1993. Aposematic caterpillars: lifestyles of the unpalatable and wringingly colored. In: Stamp N, Casey T, editors. Ecological and evolutionary constraints on caterpillars and the implications for pest management. New York (NY): Chapman and Hall. p. 331–71.
- Capinera JL, Scott RD, Walker TJ. 2004. Field guide to grasshoppers, katydids, and crickets of the United States. Ithaca (NY): Cornell University Press.
- Chambers PG, Simpson SJ, Raubenheimer D. 1995. Behavioural mechanisms of nutrient balancing in *Locusta migratoria* nymphs. *Anim Behav* 50:1513–23.
- Chapin FS, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. *Bioscience* 37:49–57.
- Clissold FJ. 2007. The biomechanics of chewing and plant fracture: mechanisms and implications. *Adv Insect Physiol* 34:317–72.
- Clissold FJ, Sanson GD, Read J. 2006. The paradoxical effects of nutrient ratios and supply rates on an outbreaking insect herbivore, the Australian plague locust. *J Anim Ecol* 75:1000–13.
- Clissold FJ, Sanson GD, Read J, Simpson SJ. 2009. Gross vs. net income: how plant toughness affects performance of an insect herbivore. *Ecology* 90:3393–405.
- Clissold FJ, Tedder BJ, Conigrave AD, Simpson SJ. 2010. The gastrointestinal tract as a nutrient-balancing organ. *Proc R Soc B Biol Sci* 277:1751–9.
- Clements KD, Raubenheimer D, Choat JH. 2009. Nutritional ecology of marine herbivorous fishes: ten years on. *Funct Ecol* 23:79–92.
- Coggan N, Clissold FJ, Simpson SJ. 2011. Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. *Proc R Soc B Biol Sci* 278:2745–52.
- Dadd RH. 1961a. The nutritional requirements of locusts—5. Observations on essential fatty acids, chlorophyll, nutritional salt mixtures, and the protein or amino acid components of synthetic diets. *J Insect Physiol* 6:126–45.
- Dadd RH. 1961b. The nutritional requirements of locusts. IV. Requirements for vitamins of the B-Complex. *J Insect Physiol* 6:1–12.
- Danner BJ, Joern A. 2003. Stage-specific behavioral responses of *Ageneotettix deorum* (Orthoptera: Acrididae) in the presence of lycosid spider predators. *J Insect Behav* 16:453–64.
- Dow JAT. 1987. Insect midgut function. *Adv Insect Physiol* 19:187–328.
- Fanson BG, Yap S, Taylor PW. 2012. Geometry of compensatory feeding and water consumption in *Drosophila melanogaster*. *J Exp Biol* 215:766–73.
- Felton GW. 1996. Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Arch Insect Biochem Physiol* 32:107–30.
- Felton AM, Felton A, Raubenheimer D, Simpson SJ, Foley WJ, Wood JT, Wallis IR, Lindenmayer DB. 2009. Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behav Ecol* 20:685–90.
- Hawlena D, Schmitz OJ. 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *Am Nat* 176:537–56.
- Hendrix DL, Huber SC. 1986. Diurnal fluctuations in cotton leaf carbon export, carbohydrate content, and sucrose synthesizing enzymes. *Plant Physiol* 81:584–6.
- Holt DA, Hilst AR. 1969. Daily variation in carbohydrate content of selected forage crops. *Agron J* 61:239–42.
- Karasov BA, Douglas AE. 2013. Comparative digestive physiology. *Comp Physiol* 3:741–83.
- Karasov WH, Martínez del Río C. 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton (NJ): Princeton University Press.
- Lee KP, Roh C. 2010. Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. *Entomol Exp Appl* 136:151–63.
- Lee KP, Behmer ST, Simpson SJ, Raubenheimer D. 2002. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). *J Insect Physiol* 48:655–65.
- Lee KP, Raubenheimer D, Behmer ST, Simpson SJ. 2003. A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker). *J Insect Physiol* 49:1161–71.
- Lee KP, Raubenheimer D, Simpson SJ. 2004. The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiol Entomol* 29:108–17.
- Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO, Taylor PW, Soran N, Raubenheimer D. 2008. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc Natl Acad Sci USA* 105:2498–503.
- Lenhart PA, Eubanks MD, Behmer ST. 2014. Water stress in grasslands: dynamic responses of plants and insect herbivores. *Oikos J* published online (doi:10.1111/oik.01370).
- Lima SL. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Stress Behav* 27:215–90.
- Maklakov AA, Simpson SJ, Zajitschek F, Hall MD, Dessmann J, Clissold F, Raubenheimer D, Bonduriansky R, Brooks RC. 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr Biol* 18:1062–6.
- Martin MM, Vanthof HM. 1988. The cause of reduced growth of *Manduca sexta* larvae on a low-water

- diet: increased metabolic processing costs or nutrient limitation? *J Insect Physiol* 34:515–25.
- Mattson WJ. 1980. Herbivory in relation to plant nitrogen content. *Annu Rev Ecol Syst* 11:119–61.
- Parsons SMA, Joern A. 2014. Life history traits associated with body size covary along a latitudinal gradient in a generalist grasshopper. *Oecologia* 174:379–91.
- Raubenheimer D, Simpson SJ. 1990. The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behavior of larval *Locusta migratoria* (L) and *Schistocerca gregaria* (Forsk.) 1. Short-term studies. *Physiol Entomol* 15:219–33.
- Raubenheimer D, Simpson SJ. 1993. The geometry of compensatory feeding in the locust. *Anim Behav* 45:953–64.
- Raubenheimer D, Simpson SJ. 1999. Integrating nutrition: a geometrical approach. *Entomol Exp Appl* 91:67–82.
- Raubenheimer D, Simpson SJ. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *J Exp Biol* 206:1669–81.
- Roeder KA, Behmer ST. 2014. Lifetime consequences of food protein–carbohydrate content for an insect herbivore. *Funct Ecol* 28:1135–43.
- Sattelmacher B, Horst WJ, Becker HC. 1994. Factors that contribute to genetic-variation for nutrient efficiency of crop plants. *Zeitschrift für Pflanzenernährung und Bodenkunde* 157:215–24.
- Scheiner SM. 1993. MANOVA: multiple response variables and multispecies interactions. In: Scheiner SM, Gurevitch J, editors. *The design and analysis of ecological experiments*. New York (NY): Chapman and Hall. p. 94–112.
- Schmitz OJ, Suttle KB. 2001. Effects of top predator species on direct and indirect interactions in a food web. *Ecology* 82:2072–81.
- Schoonhoven LM, van Loon JJA, Dicke M. 2005. *Insect–plant biology*. Oxford: Oxford University Press.
- Simpson SJ. 1995. Regulation of a meal: chewing insects. In: Chapman RF, De Boer JG, editors. *Regulatory mechanisms in insect feeding*. New York (NY): Chapman and Hall. p. 137–56.
- Simpson SJ, Raubenheimer D. 1993. A multilevel analysis of feeding-behavior: the geometry of nutritional decisions. *Phil Trans R Soc Lond Ser B Biol Sci* 342:381–402.
- Simpson SJ, Raubenheimer D. 2000. The hungry locust. *Adv Study Behav* 29:1–44.
- Simpson SJ, Raubenheimer D. 2012. *The nature of nutrition: a unifying framework from animal adaptation to human obesity*. Princeton (NJ): Princeton University Press.
- Simpson SJ, Raubenheimer D, Behmer ST, Whitworth A, Wright GA. 2002. A comparison of nutritional regulation in solitary- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. *J Exp Biol* 205:121–9.
- Simpson SJ, Sibly RM, Lee KP, Behmer ST, Raubenheimer D. 2004. Optimal foraging when regulating intake of multiple nutrients. *Anim Behav* 68:1299–311.
- Singer MS, Bernays EA, Carriere Y. 2002. The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. *Anim Behav* 64:629–43.
- Slansky F. 1993. Nutritional ecology: the fundamental quest for nutrients. In: Stamp NE, Casey TM, editors. *Caterpillars: ecological and evolutionary constraints on foraging*. London: Chapman and Hall. p. 29–91.
- Slansky F, Wheeler GS. 1989. Compensatory increases in food consumption and utilization efficiencies by velvetbean caterpillars mitigate impact of diluted diets on growth. *Entomol Exp Appl* 51:175–87.
- Slansky F, Wheeler GS. 1991. Food consumption and utilization responses to dietary dilution with cellulose and water by velvetbean caterpillars, *Anticarsia gemmatalis*. *Physiol Entomol* 16:99–116.
- Slansky F, Wheeler GS. 1992. Caterpillars compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. *Entomol Exp Appl* 65:171–86.
- Thompson SN. 2000. Pyruvate cycling and implications for regulation of gluconeogenesis in the insect, *Manduca sexta* L. *Biochem Biophys Res Commun* 274:787–93.
- Thompson SN, Redak RA, Borchardt DB. 2002. The glucogenic response of a parasitized insect *Manduca sexta* L. is partially mediated by differential nutrient intake. *Biochim Biophys Acta* 1571:138–50.
- Villalba JJ, Provenza FD. 2005. Foraging in chemically diverse environments: energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. *J Chem Ecol* 31:123–38.
- Villalba JJ, Provenza FD, Bryant JP. 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? *Oikos* 97:282–92.
- Walter J, Hein R, Auge H, Beierkuhnlein C, Löffler S, Reifenrath K, Schädler M, Weber M, Jentsch A. 2012. How do extreme drought and plant community composition affect host plant metabolites and herbivore performance? *Arthropod Plant Interact* 6:15–25.
- Warbrick-Smith J, Behmer ST, Lee KP, Raubenheimer D, Simpson SJ. 2006. Evolving resistance to obesity in an insect. *Proc Natl Acad Sci USA* 103:14045–9.
- Westerterp KR. 1993. Food quotient, respiratory quotient, and energy balance. *Am J Clin Nutr* 57:759S–64S.
- Wright GA, Simpson SJ, Raubenheimer D, Stevenson PC. 2003. The feeding behavior of the weevil, *Exophthalmus jekelianus*, with respect to the nutrients and allelochemicals in host plant leaves. *Oikos* 100:172–84.
- Yang YL, Joern A. 1994a. Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. *Physiol Entomol* 19:75–82.
- Yang YL, Joern A. 1994b. Influence of diet quality, developmental stage, and temperature on food residence time in the grasshopper *Melanoplus differentialis*. *Physiol Zool* 67:598–616.
- Yeoh H-H, Wee Y-C, Watson L. 1992. Leaf protein contents and amino acid patterns of dicotyledonous plants. *Biochem Syst Ecol* 20:657–63.
- Zanotto FP, Simpson SJ, Raubenheimer D. 1993. The regulation of growth by locusts through postingestive compensation for variation in the levels of dietary protein and carbohydrate. *Physiol Entomol* 18:425–34.