

# Lifetime consequences of food protein-carbohydrate content for an insect herbivore

Karl A. Roeder<sup>1,2</sup> and Spencer T. Behmer<sup>\*,1</sup>

<sup>1</sup>Department of Entomology, Texas A&M University, College Station, TX 77843, USA; and <sup>2</sup>Department of Biology, University of Oklahoma, Norman, OK 73019, USA

## Summary

1. Food protein–carbohydrate content has significant consequences for animal survival, growth and reproduction. Rarely, though, is its effect examined over an animal's entire lifetime.
2. In this study, we reared newly hatched caterpillars [*Heliothis virescens* Fabricius (Lepidoptera: Noctuidae)] on diets containing different protein/carbohydrate (p/c) ratios. We recorded larval survival, time to pupation, pupal mass, eclosion success, time to eclosion and pupal body lipid content. Additionally, for each treatment, we mated eclosed males and females and measured egg production and egg viability.
3. Larval performance (survival to pupation and time to pupation) was similar across all except the two most extreme treatments. In contrast, pupal performance (mass, eclosion success and time to eclosion) was best on diets that were balanced or slightly protein-biased. However, eclosion success differed between sexes. For males, it was best on diets with balanced p/c ratios, while female eclosion was strong across all but the most carbohydrate-biased diet. Pupal body lipid content in both males and females increased as the food p/c ratio decreased.
4. Egg production was best on diets with balanced or slightly protein-biased p/c ratios.
5. We also estimated the effect of food p/c ratio at the population level, using the data generated in this study. Population size was largest on diets with a balanced p/c ratio and declined steadily and strongly as the food p/c ratio became increasingly more imbalanced.
6. Our findings show, for the first time, the effect of food p/c content over an insect herbivore's entire life. Our data indicate that there is a narrow range of p/c ratios that maximize lifetime performance, and for *H. virescens*, this range coincides with its self-selected p/c ratio.

**Key-words:** foraging behaviour, nutritional ecology, nutritional physiology, physiological ecology, plant/herbivore interactions

## Introduction

The fundamental reason all animals eat is to acquire nutrients that are necessary for survival, growth and reproduction. Protein and carbohydrates, which provide necessary amino acids and energy, respectively, are two important macronutrients that influence animal survival, growth and reproduction (Karasov & Martínez del Río 2007; Simpson & Raubenheimer 2012), but for most animals there is a species-specific protein/carbohydrate blend that results in optimal performance (Raubenheimer & Simpson 2003; Lee, Behmer & Simpson 2006; Behmer & Joern 2008; Behmer 2009; Simpson & Raubenheimer 2012). Finding foods that contain protein and carbohydrates in optimal amounts or ratios is an important challenge for many

animals (Chown & Nicolson 2004; Karasov & Martínez del Río 2007; Price *et al.* 2011; Simpson & Raubenheimer 2012). This is especially true for herbivores because plants can be highly variable with respect to their protein and digestible carbohydrate content (Bernays & Chapman 1994; Schoonhoven, van Loon & Dicke 2005; Clissold, Sanson & Read 2006; Clissold *et al.* 2009; Behmer & Joern 2012).

Herbivores can, however, overcome some of the variation in food protein/carbohydrate content by practicing selective feeding, either by eating a range of different plants, or by feeding on different vegetative tissues within a plant (Chambers *et al.* 1996; Singer, Bernays & Carriere 2002; Villalba, Provenza & Bryant 2002; Wright *et al.* 2003; Villalba & Provenza 2005; Clements, Raubenheimer & Choat 2009; Felton *et al.* 2009). However, what are the consequences to an herbivore when it is restricted to foods

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

with suboptimal protein/carbohydrate ratios? In some situations, food may be limiting (e.g. by drought, over-grazing), or the quality of available food may be low (Joern, Provin & Behmer 2012). Alternatively, where high-quality food is available, it may not be accessible because of the threat of predation (Beckerman, Uriarte & Schmitz 1997; Schmitz & Suttle 2001; Danner & Joern 2003; Hawlena & Schmitz 2010).

The effects of food protein/carbohydrate content on herbivores have been investigated in a range of species, but the most detailed work comes from studies using grasshoppers and caterpillars (reviewed by Behmer 2009). However, in the majority of these studies, food protein/carbohydrate effects have only been investigated in the final immature developmental stage (e.g. Telang *et al.* 2001, 2003; Lee *et al.* 2002; Behmer *et al.* 2003; Raubenheimer & Simpson 2003; Lee, Behmer & Simpson 2006; Fielding & Defoliart 2007). A potential limitation with this approach is that the foods used to rear insects prior to the start of the experiment were likely of high quality and thus reported differences in performance (e.g. survival, growth rate) associated with food protein/carbohydrate content might be dampened because test insects likely had nutrient reserves to draw upon during the experimental phase of the study (Lee, Kwon & Roh 2012). It is also the case that experiments using caterpillars are usually terminated shortly after caterpillars have pupated. The pupal stage is a very metabolically active period, and if nutrient reserves are exhausted before metamorphosis is completed, eclosion may not proceed; failure to take caterpillars through to eclosion may mask important food nutrient shortcomings. Finally, outside of two studies on grasshoppers that examined egg production but not viability (Joern & Behmer 1997, 1998), no experiments to date have explored the full effect of food protein/carbohydrate content on insect herbivore reproductive output.

The aim of the current study was to explore, for the first time in an insect herbivore, the lifetime consequences (including egg production and viability) associated with feeding on foods that have different protein/carbohydrate content. This was carried out using a capital-breeding insect herbivore, the generalist caterpillar, *Heliothis virescens*. An advantage of using these caterpillars is that all nutrients allocated towards growth and reproduction are acquired during larval development (Jönsson 1997). We reared newly hatched neonate caterpillars, over their entire larval lifetime, on foods with different protein/carbohydrate (p/c) ratios and measured the consequences of food p/c ratio on larval, pupal and adult reproductive performance. We discuss our findings in relation to previous studies that explored the short-term effects of food macronutrient content on insect herbivores, and use our combined data to make inferences at the population level. Our results suggest that there is a narrow food protein/carbohydrate range that is functionally optimal (which corresponds to the self-selected protein/carbohydrate ratio for late stadium caterpillars) and that subtle shifts in host-

plant protein/carbohydrate content can have profound impacts for insect herbivores.

## Materials and methods

### EXPERIMENTAL INSECTS

Caterpillar eggs were obtained from a *Heliothis virescens* culture at North Carolina State University. These eggs came from moths that had been reared on a corn-soy-milk (CSM)-based diet modified by the methods described in the study of Burton (1970). All experimental neonates hatched approximately at the same time, and within a few hours of hatching, they were transferred, using a fine-tipped paint brush, to 2 oz. Solo cup that contained a block of experimental food. A lid was placed on each individual cup, and all cups were transferred to an insect growth chamber (Model I-66VL; Percival Scientific, Perry, IA, USA) set at  $29 \pm 1$  °C with a 12:12 h L : D photoregime.

### EXPERIMENTAL DIETS

A total of seven CSM-based diets were used for this experiment. They all had similar combined total protein (p) and digestible carbohydrate (c) amounts (42% by dry mass) but differed in their p/c ratio: (1) p10.5:c31.5 (10.5% protein and 31.5% carbohydrate), (2) p14:c28, (3) p17.5:c24.5, (4) p21:c21, (5) p24.5:c17.5, (6) p28:c14 and (7) p31.5:c10.5. These protein/carbohydrate ratios represent values found in plants that would likely be eaten by *H. virescens*, although vegetative tissues with the two most extreme p/c ratios should be rare (Behmer & Joern 2012). The inclusion of a basal amount of CSM to the diet (20% of the total dry mass of the experimental food, which contributed 3.68% protein and 10.0% carbohydrate to each treatment) was necessary because initial pilot studies demonstrated that a pure synthetic diet [as used for grasshoppers (see Behmer, Raubenheimer & Simpson 2001)] did not support development of caterpillars from hatch to eclosion. The remaining 80% of the experimental diet was synthetic [originally based on a recipe for grasshoppers (Dadd 1960), modified later by Simpson and Abisgold (1985) and then modified further for caterpillars by Simpson, Simmonds and Blaney (1988)]. The protein portion of the synthetic diet was a 3:1:1 mixture of casein, peptone and albumen; the digestible carbohydrate portion was entirely sucrose. Other nutrients in the synthetic diet included Wesson's salt (1.92%), cholesterol (0.4%), linoleic acid (0.4%), ascorbic acid (0.24%) and a vitamin mix (0.16%), with the remaining portion being non-nutritive cellulose. These combined dry ingredients were suspended in a 1% agar solution, in a 1:6 dry/wet ratio. Finally, to each treatment, formaldehyde (0.1 mL per 200 mL), methylparaben (0.4 g per 200 mL) and a microbial inhibitor (0.5 mL per 200 mL; see Roeder *et al.* 2010) were added to suppress the growth of mould and other microbial agents; these components were added shortly after the dry ingredients and agar solution were mixed, while still in a semi-liquid form.

### LARVAL PROTOCOL

Individual newly hatched neonates were randomly allocated to one of the seven diet treatments. There were sixty replicates per treatment, and all treatments were run concurrently. Blocks of diet, each weighing approximately 1000 mg, were placed in individual cups; each diet block was replaced with a fresh diet block of equivalent size every 3 days. After caterpillars reached the 4th stadium, cup lids were perforated with small holes to reduce

humidity levels, plus allow an increased flow of oxygen in, and carbon dioxide out. For each cup, two measures of performance were recorded: (i) whether larvae pupated (survival to the pupal stage) and (ii) for those that pupated, the length of time it took to become a pupa.

#### PUPAL PROTOCOL

Five days after the larvae pupated, their mass and sex were recorded. Pupae were then split into two groups from each treatment. The first group, which was set aside for mating experiments, contained 65% of the pupating individuals; this group contained an equal number of randomly selected males and females. These pupae were transferred to new cups that contained a small square of damp paper towel (this increased eclosion success). Two variables were recorded for these individuals: (i) eclosion and (ii) the number of days between pupation and eclosion. The remaining 35% of the pupae were frozen and set aside for lipid extractions. Frozen pupae were dried to constant mass at 70 °C, weighed to the nearest 0.1 mg and lipid extracted in three, 24-h changes of chloroform before being re-dried and re-weighed (Loveridge 1973).

#### ADULT PROTOCOL

Overall survival success was calculated by subtracting 35% of the pupating individuals from the starting 60 individuals for each diet. We did this because 35% of the individuals that pupated were removed for lipid analysis (a destructive process). By resetting the starting population size, we were able to accurately score eclosion success. The number of eclosing adults was then divided by the revised population number, which allowed us to calculate an average total survival percentage for each diet. For individuals that successfully eclosed, development time was recorded in days from hatch to eclosion.

Upon eclosion, a single male and female from the same diet treatment were randomly paired and placed into an egg-laying chamber for 6 days. These chambers were composed of two components. The first was a capped 50-mL plastic centrifuge tube, standing upright, which held the mating pair. The second was a 1.5-mL Eppendorf tube, filled with a 10% sucrose solution, pushed through a hole that was drilled in the cap of the larger tube. A hole at the bottom of the 50-mL tube prevented leaked sucrose solution from accumulating. Inside each egg-laying chamber, there was a paper strip for females to lay their eggs. This strip loosely covered the inside of the larger tube in a single layer and was changed every 2 days. Adult moths were monitored daily, and when a death occurred, the date was recorded, and the dead moth was removed. Eggs were counted on each sheet and then placed into separate sealed 8 oz. squat polypropylene containers to monitor offspring viability for each mating pair. Egg viability was calculated by dividing the total number of hatchlings by the number of eggs laid.

#### STATISTICAL ANALYSIS

Logistic regressions were used for survival to the pupal stage, from the pupal to the adult stage, and for total overall survival; odds ratios were used to make comparisons between treatments. Survival analyses were used for developmental time to pupation, from pupation to eclosion, and for the total time from neonate to eclosion; post hoc contrasts were used to make comparisons between treatments. ANOVA was used to compare pupal wet and dry mass, as well as the body lipid content (%); post hoc tests were made using a Tukey's test. Finally, logistic regression was used to analyse: (i) the number of mating pairs and (ii) the number

of egg producing pairs. ANOVA was used to compare egg production and egg viability of egg producing pairs. All analyses were run using JMP®, version 7.0.2 (SAS Institute Inc., Cary, NC, USA).

## Results

#### LARVAL PERFORMANCE

Larval survival (the per cent of individuals pupating) was significantly different between treatments (Logistic Regression: d.f. = 6,  $\chi^2 = 18.06$ ,  $P = 0.006$ ). It was highest on the p17.5:c24.5 diet, although survival on the p14:c28 and p21:c21 diets did not differ statistically compared with this diet. Survivorship was lowest on the most protein-biased diet (p31.5:c10.5), but it did not differ statistically compared with the other protein-biased diets (p28:c14 and p24.5:c17.5), or the most carbohydrate-biased diet (p10.5:c31.5) (Fig. 1a).

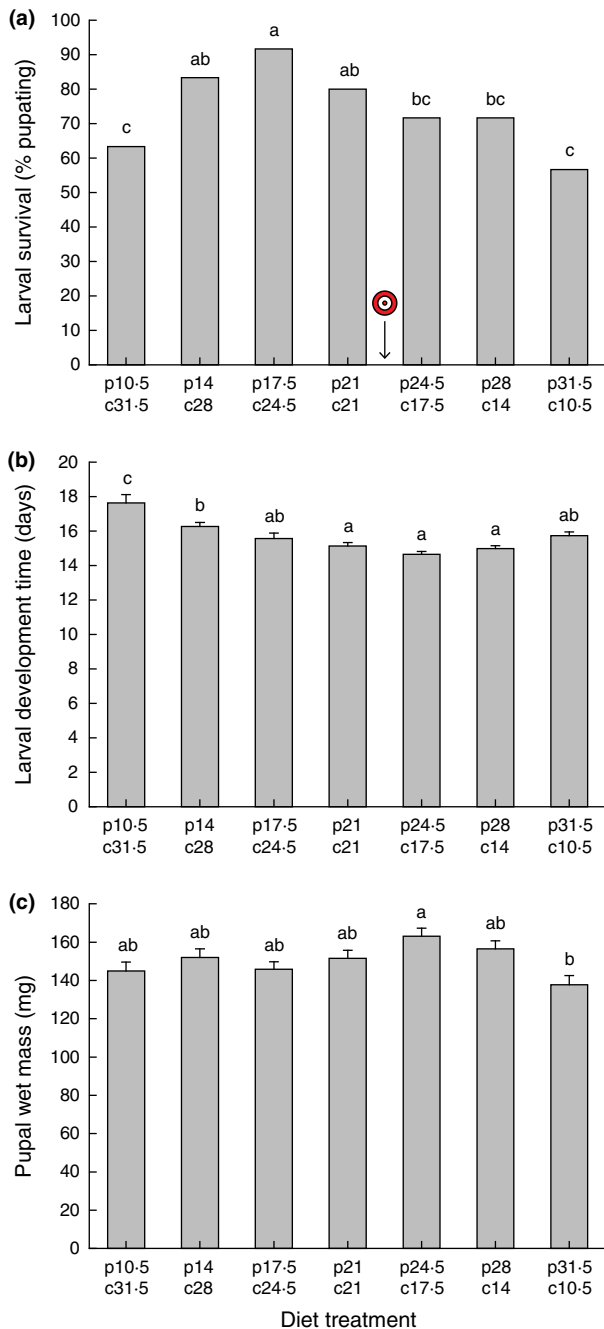
Significant differences in the development time between treatments were also observed (Survival Analysis: d.f. = 6,  $\chi^2 = 72.52$ ,  $P < 0.001$ ). Larval development was fastest on the p24.5:c17.5 diet, but there was no statistical difference between this treatment and treatments with at least 17.5% protein (Fig. 1b). Development was slowest on the most carbohydrate-biased diet.

#### PUPAL PERFORMANCE

With respect to pupal wet mass, a significant treatment effect (ANOVA:  $F_{6,301} = 3.40$ ,  $P = 0.003$ ) was observed, but neither sex, nor the treatment-by-sex interaction, were significant (ANOVA:  $F_{1,301} = 3.48$ ,  $P = 0.063$ , and  $F_{6,301} = 1.58$ ,  $P = 0.152$ , respectively). Pupal wet mass was highest on the p24.5:c17.5 diet and lowest on the most protein-biased treatment (p31.5:c10.5). For the remaining five diets, pupal mass did not differ statistically from the p24.5:c17.5 or p31.5:c10.5 diets (Fig. 1c).

Survival from the pupal to adult stages differed significantly between treatments (Logistic Regression: d.f. = 6,  $\chi^2 = 68.17$ ,  $P < 0.001$ ). It was best on the balanced (p21:c21) and slightly imbalanced diets (p17.5:c24.5 and p24.5:c17.5) and then dropped off in a symmetric fashion as the diets became more nutritionally imbalanced in both directions (Fig. 2a). However, there were strong sex differences. Male pupal survival was best on the p24.5:c17.5 diet, intermediate on the balanced (p21:c21) and slightly carbohydrate-biased (p17.5:c24.5) diets and then dropped off greatly on diets with extreme p/c imbalances (Fig. 2a). In contrast, female survival was equally high on all but the most carbohydrate-biased diet.

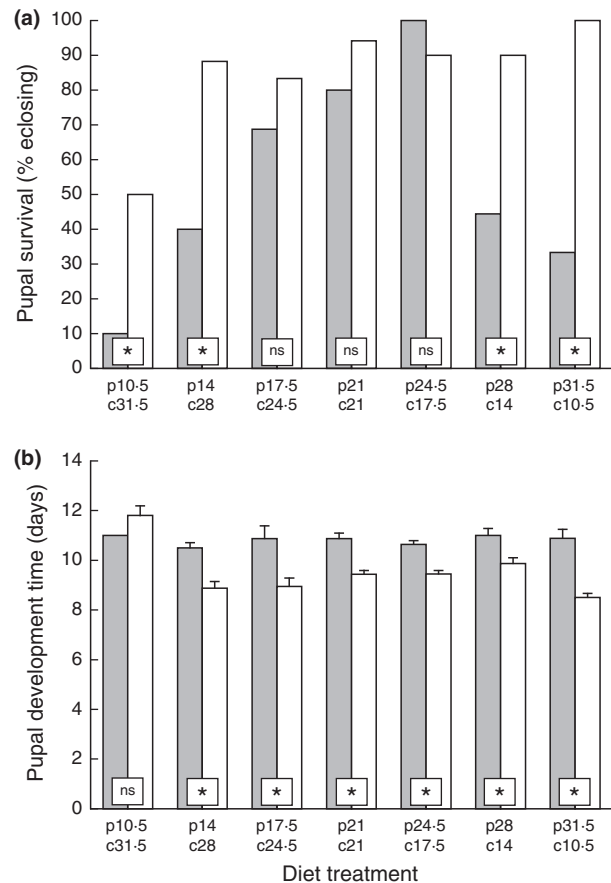
Pupal development time (measured from pupation to eclosion) also differed significantly between the diets (Survival Analysis: d.f. = 6,  $\chi^2 = 97.61$ ,  $P < 0.001$ ), although this was mostly the result of longer development on the most carbohydrate-biased diet (Fig. 2b). Additionally, females developed faster than males; the single exception



**Fig. 1.** Larval performance on diets with different protein/carbohydrate ratios. Panel (a) shows survival (to pupation), measured as a per cent. Panel (b) shows the mean ( $\pm$ SEM) development time for larvae that pupated. Panel (c) shows the mean ( $\pm$ SEM) pupal wet mass. Different letters above the bars indicate statistically significant differences between treatments ( $P < 0.05$ ). The target on panel (a) shows the self-selected protein/carbohydrate intake target for *H. virescens* caterpillars (as reported in Telang *et al.* 2001 and in Lee *et al.* 2006).

was on the most carbohydrate-biased diet, where male and female development time was similar (Fig. 2b).

Finally, the lipid content of pupae (calculated on a dry mass basis) differed significantly between treatments (ANOVA:  $F_{6,90} = 31.93$ ,  $P < 0.001$ ). It was highest on the two most carbohydrate-biased diets, intermediate on diets

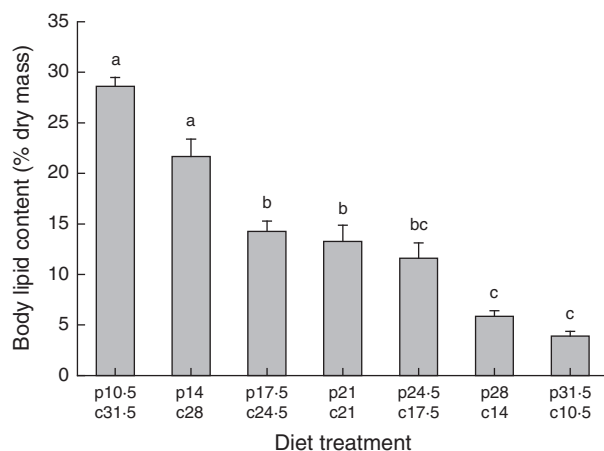


**Fig. 2.** Pupal performance on diets with different protein/carbohydrate ratios. Panel (a) shows survival, measured as the per cent that eclosed. Panel (b) shows the mean ( $\pm$ SEM) development time for pupae that eclosed. Males (grey bars) and females (white bars) are shown separately for each treatment. The boxes between bars indicate whether there were statistically significant differences between the sexes (\* = significant; ns = not significant).

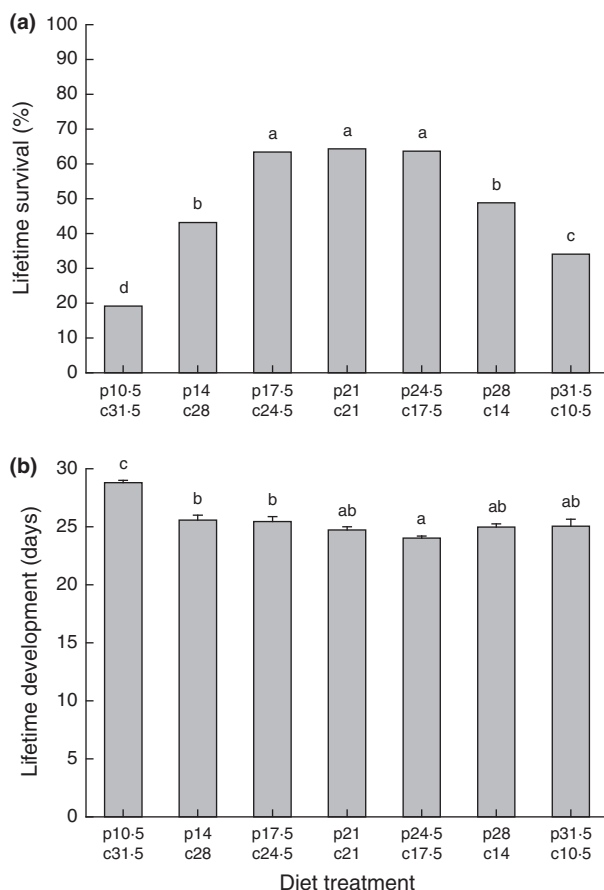
with equal or slightly imbalanced p/c ratios and lowest on the two most protein-biased diets (Fig. 3). A significant sex effect (ANOVA:  $F_{6,90} = 6.86$ ,  $P = 0.010$ ) was also observed with females having a higher average lipid content. There was no significant treatment-by-sex interaction (ANOVA:  $F_{6,90} = 0.31$ ,  $P = 0.933$ ).

#### CUMULATIVE PERFORMANCE AND ADULT REPRODUCTION

Survival from hatch to eclosion was significantly different across the seven treatments (Logistic Regression: d.f. = 6,  $\chi^2 = 33.91$ ,  $P < 0.001$ ). It was equally high on the diets with balanced (p21:c21) and slightly imbalanced p/c ratios (p17.5:c24.5 and p24.5:c17.5), but then steadily declined as the p/c ratios of the diets became more imbalanced (Fig. 4a). Development time from hatch to eclosion was also significantly different across the treatments (Survival analysis: d.f. = 6,  $\chi^2 = 37.69$ ,  $P < 0.001$ ; Fig. 4b). It was fastest on the p24.5:c17.5 diet and slowest on the most carbohydrate-biased diet (p10.5:c31.5). Lifetime develop-



**Fig. 3.** Pupal lipid levels on diets with different protein/carbohydrate ratios. The data are expressed as per cent dry mass of individual pupa, and are presented as means ( $\pm$ SEM). Different letters above the bars indicate statistically significant differences between treatments ( $P < 0.05$ ).



**Fig. 4.** Cumulative performance on diets with different protein/carbohydrate ratios. Panel (a) shows survival, from hatch to eclosion, measured as a per cent. Panel (b) shows the mean ( $\pm$ SEM) development time for individuals from hatch to eclosion. Different letters above the bars indicate statistically significant differences between treatments ( $P < 0.05$ ).

ment on the p14/c28 and p17.5:c24.5 diets was significantly slower compared with the p24.5:c17.5 diet. The remaining three diets, which all had protein content equal to, or  $>21\%$ , developed at rates similar to the p24.5:c17.5 diet.

The total number of mating pairs from each treatment, the number of mating pairs producing eggs, the mean egg production per egg producing pair and the mean egg viability for egg producing pairs are shown in Table 1. The number of mating pairs and egg-producing pairs was highly variable due to the different survival rates across the treatments. Both the number of mating pairs (Logistic Regression: d.f. = 6,  $\chi^2 = 31.38$ ,  $P < 0.001$ ) and the number of egg producing pairs (Logistic Regression: d.f. = 6,  $\chi^2 = 22.14$ ,  $P = 0.001$ ) were statistically different across treatments. When egg production was analysed, no significant treatment effect was observed (ANOVA:  $F_{5,30} = 1.70$ ,  $P = 0.165$ ). All mating pairs that produced eggs generated viable offspring, but egg viability was significantly affected by the p/c ratio of a diet (ANOVA:  $F_{5,30} = 4.08$ ,  $P = 0.006$ ).

## Discussion

For capital-breeding insects, larval food quality impacts both larval growth and adult reproduction (Boggs 1997; Casas *et al.* 2005; Wessels, Jordan & Hahn 2010). Among the Lepidoptera, there is a general trend for larvae to self-select foods in such a way as to ingest more protein than carbohydrate, or at a minimum maintain a balanced protein/carbohydrate intake (reviewed by Behmer 2009). In the field, the protein/carbohydrate content of vegetative tissues can vary within and between plants (Bernays & Chapman 1994; Schoonhoven, van Loon & Dicke 2005; Clissold, Sanson & Read 2006; Clissold *et al.* 2009; Behmer & Joern 2012), so a caterpillar can regulate its protein/carbohydrate intake by feeding on different leaves within a plant, or if plant density is relatively high, caterpillars can regulate their protein/carbohydrate intake by eating a mixed diet of leaves from different plants (e.g. Singer & Stireman 2001; Singer & Bernays 2009). However, where there is little opportunity for dietary self-selection, caterpillars will be eating within a relatively narrow range of protein/carbohydrate ratios (Raubenheimer & Simpson 1999). The current study suggests that larval performance may not be overly sensitive to food p/c ratio, except when food p/c ratio differs widely from the self-selected p/c ratio. However, as food protein/carbohydrate content becomes increasingly imbalanced relative to the self-selected p/c ratio, pupal performance, most notably eclosion success, decreases. Additionally, males, compared with females, are more sensitive to protein/carbohydrate imbalances. Finally, when food protein/carbohydrate effects are explored at the population level, by combining larval and pupal performance with reproductive output (to generate estimates of population size and generation time), it becomes clear that there is a specific p/c ratio that is functionally optimal and that

**Table 1.** Egg production data for moths from the seven different diets. Values are reported for number of mating pairs, number of egg producing pairs, mean ( $\pm$ SEM) egg production, and mean ( $\pm$ SEM) egg viability [egg production and egg viability were calculated for each egg producing pair (not all mated pairs produced eggs)]. No egg production was observed on the p10.5:c31.5 treatment, as no male caterpillars on this treatment eclosed

Treatment	Number of mating pairs	Number of egg producing pairs	Mean egg production	Mean egg viability (%)
p10.5 : c31.5	0	–	–	–
p14 : c28	6	5	166 $\pm$ 33	66.3 $\pm$ 3.7 b
p17.5 : c24.5	11	5	173 $\pm$ 53	75.1 $\pm$ 3.7 ab
p21 : c21	12	9	230 $\pm$ 32	82.5 $\pm$ 2.8 a
p24.5 : c17.5	11	9	162 $\pm$ 25	81.4 $\pm$ 2.8 a
p28 : c14	8	6	199 $\pm$ 52	81.7 $\pm$ 3.4 ab
p31.5 : c10.5	4	2	358 $\pm$ 60	65.1 $\pm$ 5.9 ab

Different letters next to the mean egg viability data indicate significant differences ( $P < 0.05$ ).

small deviations away from this optimal ratio can have strong negative repercussions.

Multiple studies have measured caterpillar larval performance (survival, mass gain, development time) on foods with different p/c ratios, but generally, they have only examined performance across a single stadium (Telang *et al.* 2001; Lee *et al.* 2002, 2003; Raubenheimer & Simpson 2003; Lee, Raubenheimer & Simpson 2004; Lee, Simpson & Raubenheimer 2004; Despland & Noseworthy 2006; Lee, Behmer & Simpson 2006; Lee 2007, 2010; Lee, Kwon & Roh 2012). These studies (employing a range of diets similar to ones used in the current study) showed high survival (usually 100%), plus similar developmental time and mass gain, across the diets tested. There were exceptions, though; development was longer on strongly carbohydrate-biased foods (e.g. p7:c35), and body mass was smaller on foods with extreme protein/carbohydrate imbalances (e.g. p7:c35 or p35:c7). Our study suggests that short-term experiments (e.g. those listed immediately above) can capture the global effects of food p/c ratio on larval development time and mass gain but likely underestimate effects on survival. However, even our survival data need to be interpreted cautiously, because the critical comparison must be made with respect to an animal's self-selected p/c ratio, which is functionally optimal in most instances (Behmer & Joern 2008; Behmer 2009). Using choice experiments, Telang *et al.* (2001) and Lee, Behmer & Simpson (2006) both identified the self-selected protein/carbohydrate intake target for last stadium *H. virescens*, which aligns between our p21:c21 and p24.5:c17.5 treatments. In our study, though, differences in performance (survival, growth and development time) relative to these diets were not significant. Thus, for caterpillars, food protein/carbohydrate content does not have large effects on traits linked to larval development.

However, it is becoming well established that suboptimal nutrition during development can significantly affect physiological processes later in life (Desai & Hales 1997; Metcalfe & Monaghan 2001; Criscuolo *et al.* 2008). Our pupal eclosion results (especially survival) provide another example of this phenomenon, with one notable

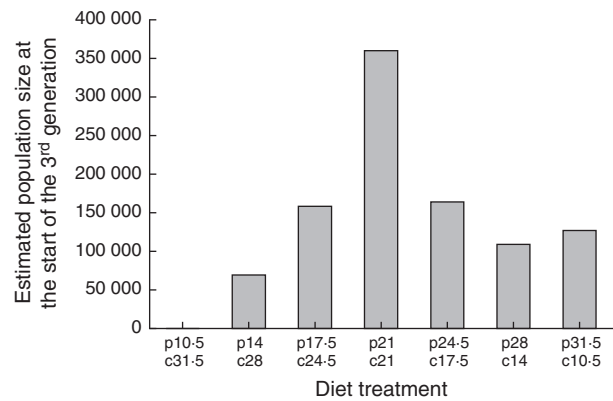
difference – females, compared with males, appear to be more resilient to deviations from an optimal p/c ratio. In the studies by Telang *et al.* (2001) and Lee (2010), it has shown that male caterpillars prefer diets with a balanced p/c ratio while female caterpillars self-select a slightly protein-biased diet. Our eclosion results suggest that balanced diets are functionally best for males. But why are male pupae, compared with females, so sensitive to protein/carbohydrate imbalances?

Where dietary protein and carbohydrates are imbalanced relative to requirements, animals (including caterpillars) will eat greater amounts of food to increase their intake of nutrients that are in deficit (Behmer 2009; Simpson & Raubenheimer 2012). Thus, caterpillars on foods with high p/c ratios (e.g. p28:c14 and p31.5:c10.5) will ingest large amounts of protein to acquire carbohydrates that are in deficit [as shown in *H. virescens* (Lee, Behmer & Simpson 2006)]. This compensatory behaviour can, however, come at a cost. For example, ants self-select carbohydrate-biased diets, but when they are maintained on protein-biased diets, they suffer high mortality rates (Dusutour & Simpson 2009, 2012; Cook *et al.* 2010); it was suggested that for worker ants, protein is toxic when ingested in excess of requirements (although the toxicology of this phenomenon is poorly understood). Perhaps too much dietary protein is also detrimental for male caterpillars? Lee (2010) has shown that female caterpillars on high-protein diets, compared with males, are more efficient at utilizing ingested protein for growth. Additionally, females, in contrast to males, sequester metabolized protein (absorbed across the gut lumen, and into the haemolymph, in the form of amino acids) using storage proteins (Telang *et al.* 2001). This serves the following two purposes: first, metabolic costs are reduced because less nitrogenous waste is produced, processed and excreted; second, during the adult stage, females can mobilize amino acids from storage proteins to produce vitellin, the primary protein found in egg yolk (Chapman, Simpson & Douglas 2013).

Caterpillars, including *H. virescens*, also overeat foods that are carbohydrate-biased, in an attempt to redress

protein deficits (Behmer 2009). In most insects, sugar ingested in excess of requirement is converted to lipids and stored (Warbrick-Smith *et al.* 2006). Thus, as a consequence of overeating carbohydrate-biased foods to meet protein needs, lipid levels on our two most carbohydrate-biased diets (p10.5:c31.5 and p14:c28) were very high. High lipid content in females is not necessarily a bad thing, because lipids are an important egg component, with triacylglycerol comprising about 40% of a terminal oocyte (Chapman, Simpson & Douglas 2013). In contrast, males do not have a great need for excess lipids (with the potential exception of migration). It is also the case that male caterpillars, compared with females, have reduced carbohydrate utilization efficiency (Lee 2010). Our low eclosion success for males on the two most carbohydrate-biased diets suggests that there is a tangible metabolic cost associated with males when they consume excess carbohydrates.

To our knowledge, this is the first study to quantify the effects of food p/c ratio on reproductive output in a Lepidoptera; the reason for the paucity of data relates to the failure of earlier nutritional defined artificial diets (specifically ones with modified protein-carbohydrate content) to adequately support larval growth through to eclosion, so that reproductive ability of individuals could be measured (Cohen 2003). Reproduction can be measured in a number of ways, and our results show that food p/c ratio significantly affects the number of available mating pairs, the reproductive success of these mating pairs, and egg viability. However, the cost of eating foods with a suboptimal p/c ratio is most easily observed at the population level. To visualize this, we used our data for survival success, egg production and offspring viability to create an estimate of how large a population of *H. virescens* could grow if it was maintained on foods with different p/c ratios (as also done in Behmer & Grebenok 1998; Jing, Grebenok & Behmer 2012). Here, each treatment was designated a starting population of 100 individuals, in a 1:1 sex ratio, with no assumed mortality from biotic or abiotic factors (a very simplified case). To determine generational growth, the starting population for each p/c ratio was multiplied by its lifetime survival and then divided in half to account for gender. The resulting values were then multiplied to their corresponding dietary mean egg production and viability values. This was performed for two successive generations to create an estimated population size for each p/c ratio at the start of the third generation. The results are shown in Fig. 5 and suggest that for *H. virescens* a balanced p/c ratio (p21:c21) is optimal, which also aligns closely to the self-selected p/c ratio for *H. virescens* caterpillars (Telang *et al.* 2001; Lee, Behmer & Simpson 2006). In contrast, populations showed dramatic drops as the food p/c ratio became increasingly imbalanced. Taken as a whole, this suggests that intake targets derived from late stadium caterpillars accurately reflect what is optimal in terms of lifetime fitness for caterpillars, and that food p/c ratios, especially those that differ from what is considered



**Fig. 5.** Estimated population size, at the end of the third generation, for *H. virescens* on diets with different protein/carbohydrate ratios (see the Discussion for details). Population sizes were calculated using survival to eclosion, egg production, and egg viability for each treatment.

optimal, can have significant consequences for insect herbivores, particularly capital-breeding insect herbivores.

It is well established that food nutrient quality (especially food p/c ratio) significantly affects insect herbivores, but our study demonstrates how a more complete understanding can be gained by tracking food p/c ratio effects through multiple life stages. Our study also highlights the importance of separately tracking dietary effects on males and females. Our combined results, including the effects at the population level, suggest that even small departures from an optimal p/c ratio can have dramatic effects, and this study, together with recent field work tracking nutrient variability in grass and forb samples (Joern, Provin & Behmer 2012), suggests that subtle changes in protein/carbohydrate content of available host plants may have a much greater impact on insect herbivore populations, and by extension communities, than has been previously recognized.

## Acknowledgements

We would like to thank past and current members of the Behmer Lab (A. Boswell, S. Cook, M. Le Gall, T. Gates, P. Lenhart and X. Jing) for assistance during the study, and for feedback on earlier versions of the manuscript. This research was partially supported by an NRI-CSREES-USDA grant (#2007-35302-18185) to S.T.B.

## References

- Beckerman, A.P., Uriarte, M. & Schmitz, O.J. (1997) Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 10735–10738.
- Behmer, S.T. (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology*, **54**, 165–187.
- Behmer, S.T. & Grebenok, R.J. (1998) Impact of dietary sterols on life-history traits of a caterpillar. *Physiological Entomology*, **23**, 165–175.
- Behmer, S.T. & Joern, A. (2008) Coexisting generalist herbivores occupy unique nutritional feeding niches. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 1977–1982.
- Behmer, S.T. & Joern, A. (2012) Insect herbivore outbreaks viewed through a physiological framework: insights from Orthoptera. *Insect Outbreaks*

- Revised (eds P. Barbosa, D.K. Letourneau & A.A. Agrawal), pp. 1–29. Blackwell Publishing Ltd, Oxford, UK.
- 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., Cox, E., Raubenheimer, D. & Simpson, S.J. (2003) Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Animal Behaviour*, **66**, 665–675.
- Bernays, E.A. & Chapman, R.F. (1994) *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, New York, USA.
- Boggs, C.L. (1997) Reproductive allocation from reserves and income in butterfly species with differing adult diets. *Ecology*, **78**, 181–191.
- Burton, R.L. (1970) A low-cost artificial diet for the corn earworm. *Journal of Economic Entomology*, **63**, 1969–1970.
- Casas, J., Pincebourde, S., Mandon, N., Vannier, F., Poujol, R. & Giron, D. (2005) Lifetime nutrient dynamics reveal simultaneous capital and income breeding in a parasitoid. *Ecology*, **86**, 545–554.
- Chambers, P., Sword, G., Angel, J.E., Behmer, S. & Bernays, E.A. (1996) Foraging by generalist grasshoppers: two different strategies. *Animal Behaviour*, **52**, 155–165.
- Chapman, R.F., Simpson, S.J. & Douglas, A.E. (2013) *The Insects: Structure and Function*, 5th edn. Cambridge University Press, Cambridge, UK.
- Chown, S. & Nicolson, S.W. (2004) *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford.
- Clements, K.D., Raubenheimer, D. & Choat, J.H. (2009) Nutritional ecology of marine herbivorous fishes: ten years on. *Functional Ecology*, **23**, 79–92.
- Clissold, F.J., Sanson, G.D. & Read, J. (2006) The paradoxical effects of nutrient ratios and supply rates on an outbreaking insect herbivore, the Australian plague locust. *Journal of Animal Ecology*, **75**, 1000–1013.
- Clissold, F.J., Sanson, G.D., Read, J. & Simpson, S.J. (2009) Gross vs. net income: how plant toughness affects performance of an insect herbivore. *Ecology*, **90**, 3393–3405.
- Cohen, A.C. (2003) *Insect Diets: Science and Technology*. CRC Press, Boca Raton, FL.
- Cook, S.C., Eubanks, M.D., Gold, R.E. & Behmer, S.T. (2010) Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs. *Animal Behaviour*, **79**, 429–437.
- Crisuolo, F., Monaghan, P., Nasir, L. & Metcalfe, N.B. (2008) Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. *Proceedings. Biological Sciences/The Royal Society*, **275**, 1565–1570.
- Dadd, R.H. (1960) Observations on the palatability and utilization of food by locusts, with particular reference to the interpretation of performances in growth trials using synthetic diets. *Entomologia Experimentalis Et Applicata*, **3**, 283–304.
- Danner, B.J. & Joern, A. (2003) Stage-specific behavioral responses of *Ageneotettix deorum* (Orthoptera: Acrididae) in the presence of lycosid spider predators. *Journal of Insect Behavior*, **16**, 453–464.
- Desai, M. & Hales, C.N. (1997) Role of fetal and infant growth in programming metabolism in later life. *Biological Reviews of the Cambridge Philosophical Society*, **72**, 329–348.
- Despland, E. & Noseworthy, M. (2006) How well do specialist feeders regulate nutrient intake? Evidence from a gregarious tree-feeding caterpillar. *Journal of Experimental Biology*, **209**, 1301–1309.
- Dussutour, A. & Simpson, S.J. (2009) Communal nutrition in ants. *Current Biology*, **19**, 740–744.
- Dussutour, A. & Simpson, S.J. (2012) Ant workers die young and colonies collapse when fed a high-protein diet. *Proceedings. Biological Sciences/The Royal Society*, **279**, 2402–2408.
- Felton, A.M., Felton, A., Raubenheimer, D., Simpson, S.J., Foley, W.J., Wood, J.T. et al. (2009) Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behavioral Ecology*, **20**, 685–690.
- Fielding, D.J. & DeFoliart, L.S. (2007) Growth, development, and nutritional physiology of grasshoppers from subarctic and temperate regions. *Physiological and Biochemical Zoology*, **80**, 607–618.
- Hawlena, D. & Schmitz, O.J. (2010) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *American Naturalist*, **176**, 537–556.
- Jing, X.F., Grebenok, R.J. & Behmer, S.T. (2012) Plant sterols and host plant suitability for generalist and specialist caterpillars. *Journal of Insect Physiology*, **58**, 235–244.
- Joern, A. & Behmer, S.T. (1997) Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). *Oecologia*, **112**, 201–208.
- Joern, A. & Behmer, S.T. (1998) Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis. *Ecological Entomology*, **23**, 174–184.
- Joern, A., Provin, T. & Behmer, S.T. (2012) Not just the usual suspects: insect herbivore populations and communities are associated with multiple plant nutrients. *Ecology*, **93**, 1002–1015.
- Jönsson, K.I. (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, **78**, 57–66.
- Karason, W.H. & Martínez del Río, C. (2007) *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins*. Princeton University Press, Princeton.
- Lee, K.P. (2007) The interactive effects of protein quality and macronutrient imbalance on nutrient balancing in an insect herbivore. *Journal of Experimental Biology*, **219**, 3236–3244.
- Lee, K.P. (2010) Sex-specific differences in nutrient regulation in a capital breeding caterpillar, *Spodoptera litura* (Fabricius). *Journal of Insect Physiology*, **56**, 1685–1695.
- Lee, K.P., Behmer, S.T. & Simpson, S.J. (2006) Nutrient regulation in relation to diet breadth: a comparison of *Heliothis* sister species and a hybrid. *Journal of Experimental Biology*, **209**, 2076–2084.
- Lee, K.P., Kwon, S.T. & Roh, C. (2012) Caterpillars use developmental plasticity and diet choice to overcome the early life experience of nutritional imbalance. *Animal Behaviour*, **84**, 785–793.
- Lee, K.P., Raubenheimer, D. & Simpson, S.J. (2004) The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiological Entomology*, **29**, 108–117.
- Lee, K.P., Simpson, S.J. & Raubenheimer, D. (2004) A comparison of nutrient regulation between solitary and gregarious phases of the specialist caterpillar, *Spodoptera exempta* (Walker). *Journal of Insect Physiology*, **50**, 1171–1180.
- Lee, K.P., Behmer, S.T., Simpson, S.J. & Raubenheimer, D. (2002) A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). *Journal of Insect Physiology*, **48**, 655–665.
- Lee, K.P., Raubenheimer, D., Behmer, S.T. & Simpson, S.J. (2003) A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker). *Journal of Insect Physiology*, **49**, 1161–1171.
- Loveridge, J.P. (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.
- Metcalfe, N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, **16**, 254–260.
- Price, P.W., Denno, R.F., Eubanks, M.D., Finke, D.L. & Kaplan, I. (2011) *Insect Ecology: Behavior, Populations and Communities*. Cambridge University Press, Cambridge, UK.
- Raubenheimer, D. & Simpson, S.J. (1999) Integrating nutrition: a geometrical approach. *Entomologia Experimentalis et Applicata*, **91**, 67–82.
- Raubenheimer, D. & Simpson, S.J. (2003) Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology*, **206**, 1669–1681.
- Roeder, K.A., Kuriachan, I., Vinson, S.B. & Behmer, S.T. (2010) Evaluation of a microbial inhibitor in artificial diets of a generalist caterpillar, *Heliothis virescens*. *Journal of Insect Science*, **10**, 197.
- Schmitz, O.J. & Suttle, K.B. (2001) Effects of top predator species on direct and indirect interactions in a food web. *Ecology*, **82**, 2072–2081.
- Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. (2005) *Insect-Plant Biology*. Oxford University Press, Oxford.
- Simpson, S.J. & Abisgold, J.D. (1985) Compensation by locusts for changes in dietary nutrients – behavioural mechanisms. *Physiological Entomology*, **10**, 443–452.
- Simpson, S.J. & Raubenheimer, D. (2012) *The Nature of Nutrition: A Unifying Framework from Animal Adaptation to Human Obesity*. Princeton University Press, Princeton, New Jersey.
- Simpson, S.J., Simmonds, M.S.J. & Blaney, W.M. (1988) A comparison of dietary selection behavior in larval *Locusta migratoria* and *Spodoptera littoralis*. *Physiological Entomology*, **13**, 225–238.
- Singer, M.S. & Bernays, E.A. (2009) Specialized generalists: evolutionary ecology of polyphagous woolly bear caterpillars. *Tiger Moths and Woolly Bears: Behavior, Ecology, and Evolution of the Arctiidae* (ed. W.E. Conner), pp. 103–114. Oxford University Press, Oxford.



- Singer, M.S., Bernays, E.A. & Carriere, Y. (2002) The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. *Animal Behaviour*, **64**, 629–643.
- Singer, M.S. & Stireman, J.O. (2001) How foraging tactics determine host-plant use by a polyphagous caterpillar. *Oecologia*, **129**, 98–105.
- Telang, A., Booton, V., Chapman, R.F. & Wheeler, D.E. (2001) How female caterpillars accumulate their nutrient reserves. *Journal of Insect Physiology*, **47**, 1055–1064.
- Telang, A., Buck, N.A., Chapman, R.F. & Wheeler, D.E. (2003) Sexual differences in postingestive processing of dietary protein and carbohydrate in caterpillars of two species. *Physiological and Biochemical Zoology*, **76**, 247–255.
- Villalba, J.J. & Provenza, F.D. (2005) Foraging in chemically diverse environments: energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. *Journal of Chemical Ecology*, **31**, 123–138.
- Villalba, J.J., Provenza, F.D. & Bryant, J.P. (2002) Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? *Oikos*, **97**, 282–292.
- Warbrick-Smith, J., Behmer, S.T., Lee, K.P., Raubenheimer, D. & Simpson, S.J. (2006) Evolving resistance to obesity in an insect. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 14045–14049.
- Wessels, F.J., Jordan, D.C. & Hahn, D.A. (2010) Allocation from capital and income sources to reproduction shift from first to second clutch in the flesh fly, *Sarcophaga crassipalpis*. *Journal of Insect Physiology*, **56**, 1269–1274.
- Wright, G.A., Simpson, S.J., Raubenheimer, D. & Stevenson, P.C. (2003) The feeding behavior of the weevil, *Exophthalmus jekelianus*, with respect to the nutrients and allelochemicals in host plant leaves. *Oikos*, **100**, 172–184.

Received 5 August 2013; accepted 28 January 2014

Handling Editor: Goggy Davidowitz