

# A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker)

Kwang Pum Lee<sup>a,\*</sup>, David Raubenheimer<sup>a</sup>, Spencer T. Behmer<sup>a</sup>, Stephen J. Simpson<sup>a,b</sup>

<sup>a</sup> Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

<sup>b</sup> University Museum of Natural History, University of Oxford, South Parks Road, Oxford OX1 3PW, UK

Received 20 May 2003; received in revised form 28 August 2003; accepted 28 August 2003

## Abstract

In an earlier study, we showed that the ingestive responses of the generalist caterpillar *Spodoptera littoralis* to foods imbalanced in their protein:carbohydrate content is similar to generalist locusts, but differs from that of specialist-feeding locusts. Here we further pursued the comparison by repeating the experiments using a closely related specialist caterpillar, *Spodoptera exempta*. First, caterpillars were allowed to self-compose a diet of preferred protein:carbohydrate balance by mixing between nutritionally complementary foods. Then, they were confined to one of five imbalanced foods, in which we measured the trade-off between over- and under-ingesting the two nutrients. On complementary foods, the caterpillars actively regulated their protein and carbohydrate intake. In the no-choice experiment, those fed excess-protein foods ingested small surpluses of protein compared with generalist feeders, thus showing a pattern of nutrient balancing similar to that observed in specialist locusts. Utilisation data indicated that ingested excesses and deficits were to some extent offset by differential utilisation. Evidence also showed that post-ingestive responses of the specialist *S. exempta* were less flexible than those observed in the generalist *S. littoralis*, a pattern which is again in accordance with comparisons of acridids differing in their host-plant range.

© 2003 Elsevier Ltd. All rights reserved.

**Keywords:** Nutrient regulation; Nutritional heterogeneity hypothesis; Host-plant range; *Spodoptera exempta*; *Spodoptera littoralis*

## 1. Introduction

While some insect herbivores feed on a broad range of host-plant families, the majority are host-plant specialists (Bernays and Chapman, 1994; Schoonhoven et al., 1998). The diets of specialists and generalists differ in aspects of their chemistry, most notably secondary plant metabolites (Ehrlich and Murphy, 1988; Rosenthal and Berenbaum, 1992). Although not explicitly tested, it is also likely that, on average, generalists experience a greater degree of nutritional heterogeneity in their diets than do specialists. This would be the case if the variance in nutritional quality was greatest at higher levels in the hierarchy of food items (plant species, individual plants,

ramets, branches, shoots and leaves), and some evidence for this does exist (Suomela and Ayres, 1994; Suomela et al., 1995, 1997; Suomela, 1996). Such differences would be expected to influence the evolution of strategies for nutrient balancing (Raubenheimer and Simpson, 1999, 2003; Simpson et al., 2002).

In recent years, we have begun an investigation of the differences between specialist and generalist species in responses to macronutrient imbalance. A comparative study conducted with two grasshopper species, one the grass-specialist African migratory locust *Locusta migratoria* (L.) and the other the extreme generalist desert locust *Schistocerca gregaria* (Forskål), revealed significant differences in the regulatory responses to macronutrient imbalance. When restricted to single nutritionally imbalanced foods, gregarious phase *L. migratoria* over-ingested protein to a lesser extent than did the highly polyphagous gregarious form of *S. gregaria* (Raubenheimer and Simpson, 1999, 2003). The same

\* Corresponding author. Tel.: +44-1865-271252; fax: +44-1865-310447.

E-mail address: kwang.lee@zoo.ox.ac.uk (K.P. Lee).

difference in feeding response was also found between the gregarious phase of *S. gregaria* and the more specialised solitary phase, with the solitary insects responding to imbalance in a manner that was more similar to *L. migratoria* than to their gregarious conspecifics (Simpson et al., 2002). These observations suggest that for grasshoppers, host-plant range is a likely predictor of responses to nutritional imbalance, with generalist feeders ingesting larger nutrient excesses than those with narrower host range.

In a previous study (Lee et al., 2002), we investigated various aspects of nutritional regulation in the highly polyphagous cotton leafworm *Spodoptera littoralis* (Boisduval). *S. littoralis* responded to protein:carbohydrate imbalance in a manner that closely resembled that for generalist-feeding locusts (Lee et al., 2002). In the present paper we took the opportunity offered by a closely related grass-feeding specialist, *Spodoptera exempta* (Walker), to further test the relationship between host range and nutritional regulatory responses in insects. We used the same protocols as the earlier studies to measure ingestive, post-ingestive and performance responses of *S. exempta* to foods that are nutritionally balanced and imbalanced with respect to their protein:carbohydrate content. As predicted, *S. exempta* showed patterns of regulation that were similar to specialist grasshoppers, and different from the generalist-feeding caterpillar *S. littoralis*. For the first time this broadens the evidence beyond locusts for distinct patterns of nutrient regulation by generalists and specialists. We discuss the possible reasons underlying this emerging correlation.

## 2. Materials and methods

### 2.1. Insects and experimental arenas

Caterpillars (gregarious phase *S. exempta*) came from a culture at the NERC Centre for Ecology and Hydrology, Oxford, and were reared on a wheat-germ based semi-artificial diet containing approximately 33% protein and 28% carbohydrate (Hoffman et al., 1966) until they had reached their final (6th) stadium. During this pre-experimental rearing period, they were cultured under crowded conditions at a density of 150–200 insects per container (25 × 25 × 45 cm). Upon moulting to the final stadium, caterpillars were weighed to the nearest 0.1 mg (initial fresh mass), and each was placed into its own experimental arena, a 9 cm diameter Petri dish that had five 1 mm diameter perforations in the upper lid (Lee et al., 2002). The insects were maintained throughout the experiment at 27 ± 5 °C under a 12L:12D photoregime.

### 2.2. Synthetic foods

Seven foods differing in their content (%) of protein (p) and digestible carbohydrates (c) were prepared: p35:c7, p28:c14, p21:c21, p21:c4.2, p14:c28, p12.6:c12.6 and p7:c35. The protein content of all the foods consisted of a 3:1:1 mixture of casein, peptone and albumen (a mixture which provides a closely similar amino acid profile to seedling wheat), while sucrose was the digestible carbohydrate. Other nutrient components included micronutrients such as Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%) and a vitamin mix (0.2%) (Dadd, 1961). The remaining portion of the food was comprised of the non-nutritive bulking agent, cellulose. The chemical compositions of the foods were identical to those used during our previous study on *S. littoralis* (Lee et al., 2002), thus allowing a direct comparison of the nutritional regulatory responses between *S. exempta* and *S. littoralis*. The foods were presented to the insects suspended in a 1% agar solution in a 6:1 agar solution:dry ingredients ratio (83% water content).

### 2.3. Experimental design and protocol

Two separate experiments were designed. In the first, caterpillars were given a choice between two nutritionally complementary but imbalanced foods (Lee et al., 2002). One of two protein-biased foods that contained five times higher protein than carbohydrate (PB-food: p35:c7 or p21:c4.2) was paired with one of two foods with equal ratios of protein to carbohydrate (ER-food: p21:c21 or p12.6:c12.6). These combinations resulted in four food-pair treatments. The second experiment was a no-choice design in which we presented the caterpillars with one of five foods differing in their protein to carbohydrate ratio but containing the same total nutrient concentration (P + C% = 42%): p35:c7, p28:c14, p21:c21, p14:c28 and p7:c35.

Both experiments started when the caterpillars had ecdysed to the final larval stadium. Throughout the experimental period, individual insects received either two blocks (choice experiment) or a single block of food (no-choice experiment). Each block was weighed to the nearest 0.1 mg before being presented to the insects. The mass of the blocks was between 1300 and 2000 mg, an amount that slightly exceeds the insects' daily consumption. Hence, caterpillars had continuous access to food, and yet the surplus was minimal thus improving the accuracy of intake estimates (Schmidt and Reese, 1986). Feeding dishes were sealed with a strip of Parafilm to prevent rapid desiccation of the food block. After 24 h, any food still remaining was collected and replaced with a fresh, pre-weighed block. Removed blocks were then dried to constant mass at 50 °C and subsequently weighed to the nearest 0.1 mg. This procedure was

repeated daily until each caterpillar ceased to feed prior to pupation. Stadium duration was measured to within 6 h from pupation. Pupae were dried at 50 °C to constant mass and weighed to the nearest 0.1 mg. Dried pupae were lipid extracted in three, 24 h changes of chloroform. At the end of the third chloroform wash, pupae were re-dried and re-weighed. Lipid content was estimated from their mass change. Finally, analysis of nitrogen content in lipid-free pupae was performed using the micro-Kjeldahl procedure. To estimate daily food intake (dry mass) for individual caterpillars, control arenas were established that contained only pre-weighed blocks of food. These controls were run concurrently with the other experimental arenas and were used to construct a regression equation from which the initial dry mass of the blocks of food was back calculated. Twelve replicates were used in all four treatments in the choice experiment and in treatments p28:c14 and p14:c28 in the no-choice experiment. However, we assigned twenty-five replicates to the remaining three treatments in the no-choice experiment (p35:c7, p21:c21 and p7:c35) to increase the statistical power of comparisons with the other treatments.

## 2.4. Statistical analyses

Analysis of covariance (ANCOVA) was used to examine how the protein and carbohydrate content of the different foods related to intake, growth and utilisation efficiencies (i.e. utilisation indices; Raubenheimer and Simpson, 1992). In addition, multivariate nutrient intake data were analysed by multivariate analysis of covariance (MANCOVA), using Pillai's trace statistic, which is considered to be the most robust to violation of assumptions (Scheiner, 1993). For MANCOVA analyses, the initial fresh mass of the insect was used as a covariate to adjust for size differences (Raubenheimer and Simpson, 1992). The normality and homoscedasticity of the data were inspected with Kolmogorov–Smirnov and Bartlett's tests, respectively, before performing parametric analyses. Where necessary, data were transformed. Accelerated failure-time analysis (PROC LIFEREG using Weibull distribution) was used to test for differences in stadium duration among treatments (Fox, 1993). All statistical analyses were performed using SAS version 6 (SAS Institute, 1990).

## 3. Results

### 3.1. Experiment 1: Selected diet

#### 3.1.1. Nutrient and food intake

To test for effects of food pairing on macronutrient intake by self-selecting insects, we used a MANCOVA with cumulative protein-carbohydrate intake as depen-

dent variable and dilution level of the ER- and PB-foods as factors. Concentrations of the PB- and ER-food significantly affected nutrient intake, but there was no significant effect due to the interaction between the two main factors (Table 1). Fig. 1b shows that the effect of diluting the ER-food was to shift the intake trajectories towards higher protein intake. Fig. 1 also shows that the protein-carbohydrate intakes of all treatments were actively regulated, since their trajectories differed significantly from hypothetical trajectories expected if the caterpillars were to have fed indiscriminately between the two foods in their choice treatment.

Food intake patterns between the PB-food and ER-food were affected by the concentration of the ER-food but not by that of the PB-food (Table 1). There was no significant interaction between the two main factors. In all four treatments, caterpillars ate more of the ER-food than the PB-food (Fig. 2). Total food intake was significantly higher for caterpillars provided with the more dilute ER-food (p12.6:c12.6) than with the concentrated food (p21:c21) (ANOVA:  $F_{1,39} = 36.36$ ,  $P < 0.001$ ). However, the concentration of PB-food had little effect on total food intake ( $F_{1,39} = 0.41$ ,  $P = 0.525$ ).

#### 3.1.2. Performance and utilisation

During the experiment, five insects (one each from treatment p35:c7 vs. p21:c21 and treatment p21:c4.2 vs. p21:c21 and three from treatment p35:c7 vs. p12.6:c12.6) failed to pupate and were discarded from all analyses. There was no difference in stadium duration among treatments (accelerated failure-time analysis:  $\chi^2 = 0.03$ , d.f. = 3,  $P = 0.999$ ). Dry pupal mass was affected by the concentration of the ER-food (ANCOVA:  $F_{1,38} = 9.98$ ,  $P = 0.003$ ) but not by that of the PB-food ( $F_{1,38} = 0.90$ ,  $P = 0.348$ ). In general, caterpillars provided with p21:c21 as the ER-food produced heavier pupae than those with p12.6:c12.6. The initial fresh mass (covariate) had little effect on the pupal mass ( $F_{1,38} = 2.04$ ,  $P = 0.161$ ). No significant interaction was found between the effects of ER-food and PB-food on dry pupal mass ( $F_{1,38} = 0.00$ ,  $P = 0.963$ ).

A bicoordinate plot for nitrogen (protein-derived) and lipid (carbohydrate-derived) pupal content under choice conditions is presented in Fig. 3. The positions of nitrogen-lipid content in the plane were strongly affected by the ER-food, such that those experimental groups given the concentrated ER-food had higher lipid content irrespective of the concentration of the PB-food with which it was paired (lack of statistical interaction, Table 2). In addition, insects provided with p35:c7 had higher body nitrogen content than those with p21:c4.2, but the effect of the PB-food concentration on the overall carcass nitrogen-lipid content was not as great as that of the ER-food. Initial fresh mass had a just significant effect on the nutrient composition of the dry pupae (Table 2).

Utilisation plots (Raubenheimer and Simpson, 1992,

Table 1

MANCOVA results for macronutrient (protein and carbohydrate) and food (PB- and ER-food) intake in the choice experiment. Initial fresh mass was used as a covariate to adjust for size differences between insects

Source	d.f. Num., Den.	Macronutrient Intake		Food Intake	
		F-value	P-value	F-value	P-value
PB-food	2, 37	10.64	0.000	0.93	0.403
ER-food	2, 37	22.73	0.000	30.12	0.000
PB-food × ER-food	2, 37	1.50	0.237	1.40	0.260
Initial mass	2, 37	0.45	0.640	0.92	0.406

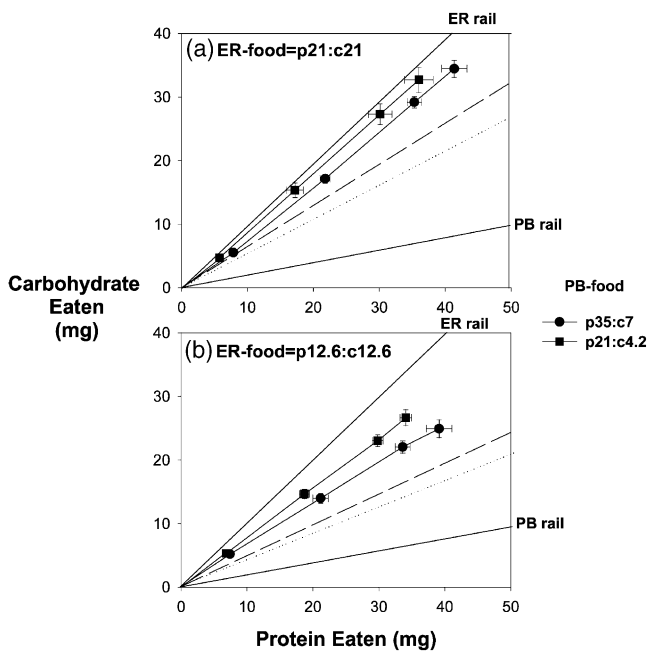


Fig. 1. Bivariate means ( $\pm$ SE) for protein-carbohydrate intake of caterpillars in the choice experiment. Points along each trajectory represent the cumulative intake of protein and carbohydrate over successive days, up to the last day of feeding. The two solid lines represent nutrient 'rails' and indicate the protein:carbohydrate ratio of the PB-food (5:1) and the ER-food (1:1). The long-dashed and dotted lines indicate the expected protein-carbohydrate intake trajectories on the treatments with p21:c4.2 and p35:c7 foods, respectively, if feeding had occurred indiscriminately between two complementary foods, PB- and ER-foods.

1994) showing the conversion of ingested nitrogen to body nitrogen are presented in Fig. 4a and b, where nitrogen eaten is estimated as protein consumption/6.25. The effect of the concentration of each food (PB- or ER-food) on nitrogen utilisation was analysed separately. When the homogeneity of slopes was tested in each, we did not find any significant interaction between ingested nitrogen (covariate) and either of the main factors (ingested nitrogen  $\times$  PB - food:  $F_{1,39} = 2.10$ ,  $P = 0.155$ ; ingested nitrogen  $\times$  ER - food:  $F_{1,39} = 0.28$ ,  $P = 0.601$ ). Subsequent ANCOVA for each food treatment indicated that the amount of nitrogen eaten was the only

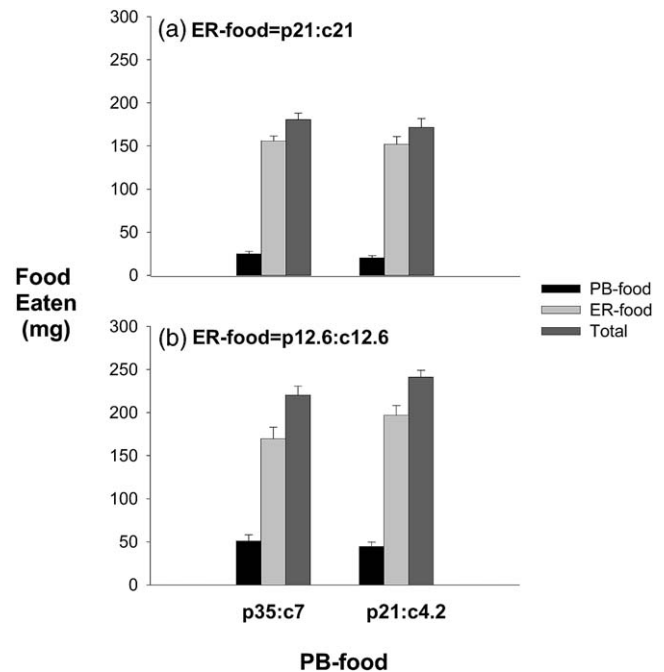


Fig. 2. Mean ( $\pm$ SE) of the PB-, ER-food and the total food eaten by caterpillars in the choice experiment.

factor affecting pupal nitrogen content (for differing PB-food treatment:  $F_{1,40} = 27.95$ ,  $P < 0.001$ ; for differing ER-food treatment:  $F_{1,40} = 38.02$ ,  $P < 0.001$ ), with no residual effect due to the concentration of each food (PB-food:  $F_{1,40} = 0.07$ ,  $P = 0.798$ ; ER-food:  $F_{1,40} = 1.21$ ,  $P = 0.278$ ). Thus, there was no difference among treatments in nitrogen utilisation efficiency.

The conversion of ingested carbohydrate to body lipid content according to the level of PB- and ER-food is shown in Fig. 4c and d, respectively. For the PB-food, we detected a significant interaction between the concentration of the PB-food and the covariate (Fig. 4c; ingested carbohydrate  $\times$  PB - food:  $F_{1,39} = 8.82$ ,  $P = 0.005$ ), whereas no such covariate-by-treatment interaction was found for treatments that differed in their ER-food nutrient content (Fig. 4d; ingested carbohydrate  $\times$  ER - food:  $F_{1,39} = 0.13$ ,  $P = 0.725$ ). When the slopes of regression lines were compared, we observed that body



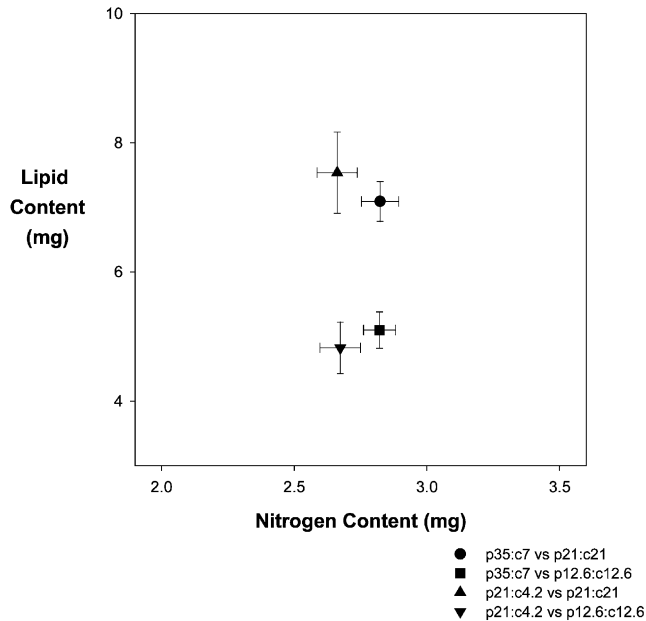


Fig. 3. Bivariate means ( $\pm$ SE) of body nitrogen–lipid content of pupae in the choice experiment.

Table 2  
MANCOVA results for body nitrogen–lipid content in the choice experiment. Initial fresh mass was used as a covariate to adjust for size differences between insects

Source	d.f. Num., Den.	Body Composition	
		F-value	P-value
PB-food	2, 37	3.43	0.043
ER-food	2, 37	18.36	0.000
PB-food $\times$ ER-food	2, 37	0.41	0.664
Initial mass	2, 37	3.50	0.041

lipid content increased more rapidly with carbohydrate intake in the low PB-food (p21:c4.2) than in the high PB-food (p35:c7) (Fig. 4c), while no detectable difference in such rate of conversion was found between the treatments with differing ER-food concentration level (Fig. 4d). ANCOVA on the data presented in Fig. 4d revealed a significant residual effect of diet on lipid growth once carbohydrate intake had been statistically removed, suggesting that the cellulose-mediated dilution effect of ER-food reduced the body lipid content per unit carbohydrate eaten ( $F_{1,40} = 4.49$ ,  $P = 0.040$ ). A similar reduction in the utilisation efficiency of ingested carbohydrate with dietary cellulose content has been demonstrated for *S. littoralis* (K.P. Lee, D. Raubenheimer and S. J. Simpson, submitted), and there we discuss possible reasons for this.

### 3.2. Experiment 2: Imbalanced diets

#### 3.2.1. Nutrient and food intake

In the no-choice experiment, caterpillars were confined to one of five foods differing in their P:C ratios. Cumulative protein–carbohydrate intake data across the stadium are shown in Fig. 5, with the intake points across the five foods on each day connected to describe the shape of intake arrays. As can be seen from Fig. 5, the intake arrays on day 2 and day 3 are distinctly arch-shaped. Polynomial contrasts in MANCOVA revealed significant quadratic effect on the intake arrays at these stages (day 2:  $F_{2,90} = 20.48$ ,  $P < 0.001$ ; day 3:  $F_{2,90} = 28.94$ ,  $P < 0.001$ ). Most caterpillars ceased feeding by day 4 except those confined to extremely carbohydrate-biased food (p7:c35). Caterpillars on this food continued to eat beyond day 4, thus prolonging their development and causing the cumulative intake array to straighten in the later stages of development. Food intake was thus greatest on the p7:c35 food but gradually reduced as the P:C ratio was increased.

#### 3.2.2. Performance and utilisation

Only two insects, one each from treatment p35:c7 and p21:c21, failed to pupate and were discarded from the analyses. Dry pupal mass was significantly affected both by the food P:C ratio (ANCOVA:  $F_{4,91} = 50.27$ ,  $P < 0.001$ ) and by the initial mass ( $F_{1,91} = 10.72$ ,  $P = 0.002$ ). Post-hoc (Tukey) multiple comparisons showed that pupal mass was greatest on p14:c28 and smallest on p35:c7, but no difference was found between the three intermediate-sized treatments (p28:c7, p21:c21 and p7:c35) (Fig. 6). Stadium duration was also influenced by the P:C ratio of the food (Fig. 6), showing that caterpillars reared on p7:c35 took longer to pupate than the others (accelerated failure-time analysis:  $\chi^2 = 126.35$ , d.f. = 4,  $P < 0.001$ ).

Lipid content is plotted against nitrogen content in Fig. 7. Results from MANCOVA analysis indicate that the P:C ratio of the food had a significant impact on pupal nitrogen and lipid content ( $F_{8,182} = 70.58$ ,  $P < 0.001$ ). Nutrient composition was also affected by the initial mass of larvae ( $F_{2,90} = 4.98$ ,  $P = 0.009$ ). When the two extreme treatments (p35:c7 and p7:c35) were excluded from the analysis, there was no difference in body nitrogen content between the three moderately balanced treatments (p28:c14, p21:c21 and p14:c28) (ANCOVA:  $F_{2,44} = 0.82$ ,  $P = 0.449$ ), suggesting regulation of nitrogen growth. In contrast to nitrogen, body lipid content increased with the carbohydrate level in the food ( $F_{4,91} = 171.79$ ,  $P < 0.001$ ).

Utilisation plots were used to analyse the efficiency of conversion of ingested nitrogen to body nitrogen and of ingested carbohydrate to body lipid (Fig. 8). When homogeneity of slopes was analysed for nitrogen utilisation, a significant covariate (ingested nitrogen)-by-treat-

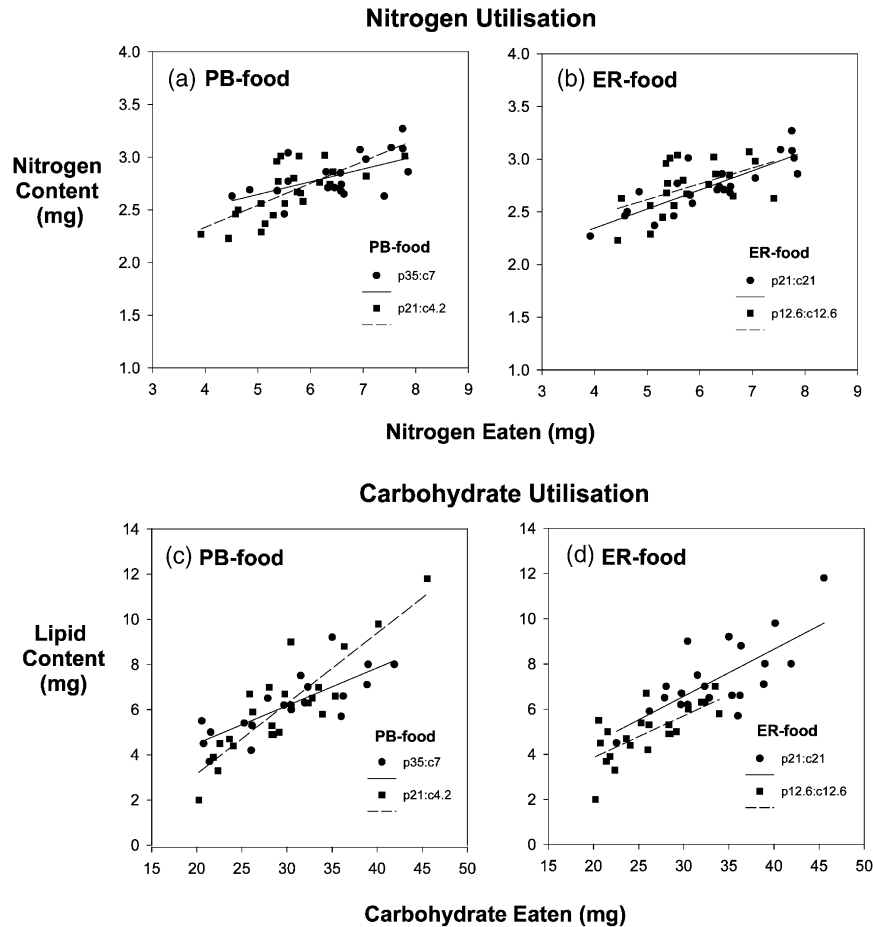


Fig. 4. Utilisation plots describing the conversion of ingested nitrogen to body nitrogen content according to the nutrient concentration of (a) the PB-food and to that of (b) the ER-food in the choice experiment. Similar plots also describe the utilisation of ingested carbohydrate to body lipid content in different levels of (c) the PB- and (d) the ER-food concentration. Each point represents an individual insect that pupated. In each plot, simple linear regressions are fitted to demonstrate the efficiencies at which ingested nutrients are converted to body content.

ment (P:C ratio) interaction was detected ( $F_{4,87} = 6.65$ ,  $P < 0.001$ ). Simple linear regression lines were thus fitted for each P:C ratio to describe the rate of increase in nitrogen growth with nitrogen intake (Fig. 8a). The plot distinguishes three groups in terms of the relationship between nitrogen intake and nitrogen retention: three moderately balanced diets (p28:c14, p21:c21 and p14:c28) formed a central cluster, which was distinct from the two extreme diets. Animals fed the most nitrogen-rich food (p35:c7) had lower nitrogen retention for nitrogen intake values that broadly overlapped with the central cluster, demonstrating a lower nitrogen utilisation efficiency. Those fed the most nitrogen-deficient diet (p7:c35) had roughly similar nitrogen growth to the p35:c7 insects for substantially lower nitrogen intake, suggesting greater efficiency of nitrogen utilisation. Compared with the three moderate diets, the p7:c35 insects both ate and retained less nitrogen, and also showed a markedly steeper slope in the relationship between nitrogen intake and retention. These results are consistent with homeostatic regulation of body nitrogen

growth, where the null hypothesis would be that the data for the four foods form a continuous relationship with an initial phase of high efficiency of utilisation for intakes below the intake target, followed by a phase of low utilisation efficiency as the intake target is exceeded and excess ingested nitrogen is removed from the body (Raubenheimer and Simpson, 1994). The point of inflection in the relationship between intake and growth lies in the region of a nitrogen intake of 4–6 mg with an associated pupal nitrogen content of 2.5–3 mg (Fig. 8a), which comfortably encompasses the results for the self-selecting insects (nitrogen intake of c. 5.6 mg, and pupal content of c. 2.75 mg; Fig. 3).

From Fig. 8b, it appears as if the slope of the line relating carbohydrate eaten to lipid content was slightly steeper for the extreme carbohydrate-limiting diet (p35:c7) than for the other diets, but there was no significant covariate (ingested carbohydrate)-by-treatment (P:C ratio) interaction ( $F_{4,87} = 0.32$ ,  $P = 0.861$ ), suggesting that lipid content increased linearly with the amount of carbohydrate intake across treatments. It is clear that

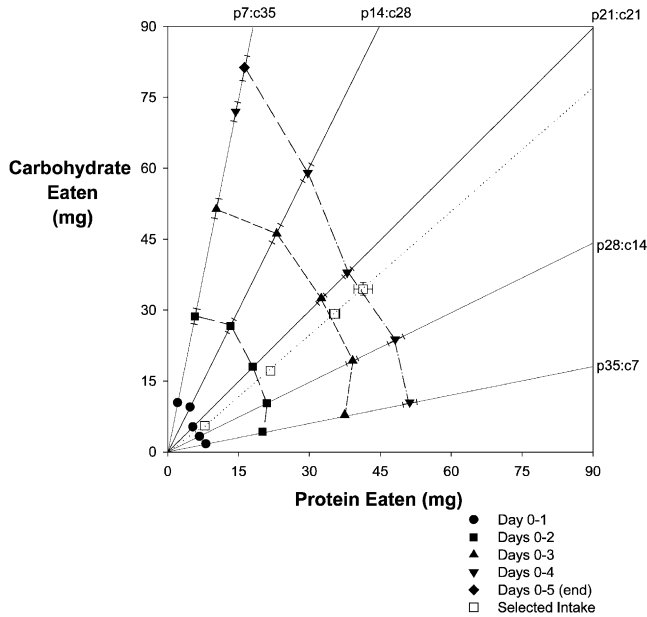


Fig. 5. Means (±SE) of protein-carbohydrate intake of caterpillars in the no-choice experiment where caterpillars were restricted to one of five protein:carbohydrate ratio foods. Solid lines represent nutrient ratios ('rails') for the five food treatments. Each point shows the cumulative nutrient intake over successive days. Open squares indicate the point of self-selected intake of protein and carbohydrate from the choice experiment. The shape of the arrays for cumulative intake up to day 2, 3 and the final feeding day are represented as long-dashed, short-dashed and dash-dotted lines, respectively.

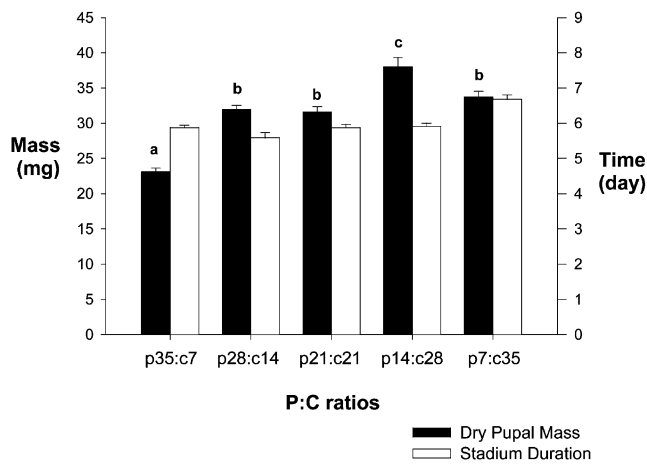


Fig. 6. Means (±SE) of dry pupal mass and stadium duration of caterpillars across five dietary treatments in the no-choice experiment.

the range of body lipid contents across treatments was considerably larger than for nitrogen (Fig. 8a), suggesting tighter regulation for the latter.

#### 4. Discussion

Various aspects of nutrient regulatory responses in the caterpillars of *S. exempta* were investigated in the

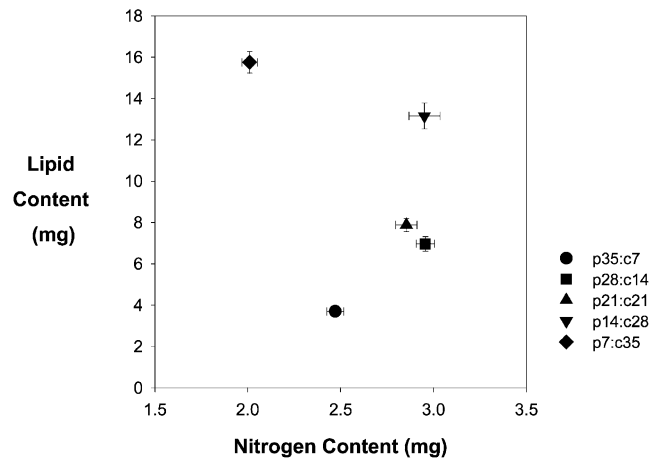


Fig. 7. Bivariate means (±SE) of body nitrogen-lipid content of pupae in the no-choice experiment.

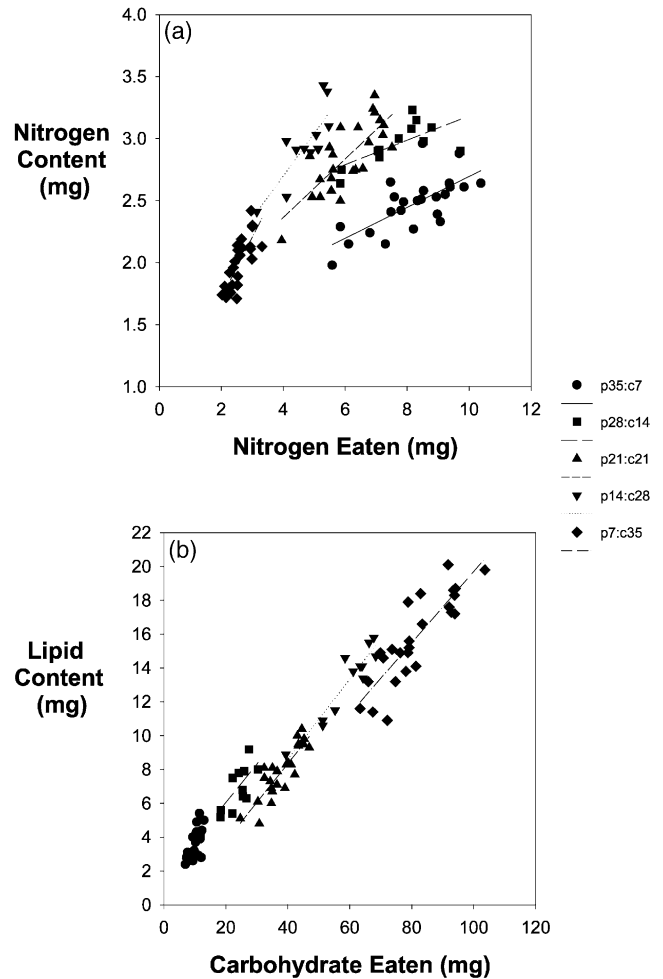


Fig. 8. Utilisation plots describing the conversion of (a) ingested nitrogen to body nitrogen and of (b) ingested carbohydrate to body lipid in the no-choice experiment. Each point represents an individual insect that pupated. In these plots, simple linear regressions are fitted across the five treatments to demonstrate the conversion efficiencies of ingested nutrients to body content.

present study. Here they will be compared with those from the previous results for *S. littoralis* (Lee et al., 2002).

#### 4.1. Dietary self-selection

Data from the choice experiment demonstrate that larval *S. exempta* regulated their intake of protein and carbohydrate through nonrandom selection between two complementary foods. This was not surprising since the presence of such regulation of macronutrient intake had been described for other lepidopterans (Waldbauer et al., 1984; Stockhoff, 1993; Telang et al., 2001), including *S. littoralis* (Simpson et al., 1988; Lee et al., 2002). However, regulation was not complete, i.e. the points of protein–carbohydrate intake did not converge to a statistically indistinguishable point in nutrient space. Insects offered the higher concentration PB-food had slightly higher protein intake regardless of the concentration of the paired ER-food. Similarly, caterpillars reared on higher concentration ER-food ate more carbohydrate than did those on the more diluted ER-food. It seems that diluting food with a large amount of cellulose may have prevented complete compensation for both protein and carbohydrate (K.P. Lee, D. Raubenheimer and S. J. Simpson, submitted).

It is also evident that the self-selected ratio of protein to carbohydrate tended to lie close to the 50:50 ratio present in the ER-foods; i.e. insects fed predominantly from the ER-food (Fig. 2). There is, therefore, the possibility that the intake target actually lies to the left of this rail in nutrient space (i.e. a ratio that contains more carbohydrate than protein): a region that was not encompassed by the two food ratios offered in the choice experiments (ER and PB). We based the expectation that the intake target ratio is protein-biased on existing data from other larval Lepidoptera (Waldbauer et al., 1984; Simpson et al., 1988; Telang et al., 2001; Lee et al., 2002), but perhaps *S. exempta* are an exception. This possibility needs to be borne in mind in future experiments of this sort on larval Lepidoptera.

When the composition of the average selected P:C ratio for *S. exempta* in treatments that had p21:c21 as the ER-food was analysed, we found that the ratio for *S. exempta* (c. 54%: 46%) was slightly lower than that demonstrated for *S. littoralis*, which we previously showed selected a mixture of 57% protein and 43% carbohydrate when tested under the same conditions (Lee et al., 2002). Counterbalancing this difference in protein intake, *S. littoralis* appeared to utilise ingested protein less efficiently than did *S. exempta* as indicated by the fact that less ingested nitrogen (3.44 mg) was required to compose a unit body nitrogen mass (1 mg) in the latter species than in the former (5.06 mg). There are interesting similarities here with the finding that the grass-specialist locust *L. migratoria* selected a target diet

lower in protein than did the grass-generalist *S. gregaria*, but utilised the ingested nitrogen more efficiently (Raubenheimer and Simpson, 2003). Unlike *L. migratoria*, *S. gregaria* was able to maintain body lipid content on foods containing excess protein relative to carbohydrate, or even devoid of carbohydrates, demonstrating that they are capable of de-aminating amino acids and channeling the carbon skeletons into energy metabolism (via gluconeogenesis). Raubenheimer and Simpson (2003) postulated that this capacity for gluconeogenesis might explain the selection of higher nitrogen intake by *S. gregaria*, since it imparts a premium for the generalist species on obtaining the dual-purpose macronutrient protein (which, unlike carbohydrate, can be used both as a source of reduced carbon and nitrogen). Could the same be true in the comparison between generalist and specialist caterpillars? While the present data are strongly suggestive, a direct test will require biochemical measurements of the gluconeogenic capacities of the two caterpillar species.

More extreme than the difference in the proportion of protein in the selected diet of the generalist and specialist caterpillars was the manner in which they responded to the dietary dilution of the ER-food. As described in our previous study (Lee et al., 2002), *S. littoralis* gradually shifted their protein–carbohydrate intake from their intake target (P57%:C43%) to a position closer to the protein axis when the concentration of the ER-food fell and, concurrently, the concentration of the paired PB-food rose. In other words, rather than increasing consumption on a diluted food containing a close-to-optimal balance of protein to carbohydrate, they switched to feeding on less-dilute, protein-biased foods. In contrast, when the same experiment was conducted with *S. exempta*, no such pronounced divergence in protein–carbohydrate intake was found in *S. exempta* caterpillars. In this case, the caterpillars consistently ate more of the ER-food than the PB-food across all four treatments. One possible explanation for this, which would accord well with the above discussion on the position of the intake target, is that the generalist-feeding *S. littoralis* is more adept at de-aminating protein and subjecting the carbon skeleton to gluconeogenesis than the specialist *S. exempta* (Thompson, 1998; Thompson and Redak, 2000).

One point which needs further explanation is the discrepancy between larval performance and the diet choice; dry pupal mass was greatest on p14:c28 while the self-selected ratio of protein to carbohydrate was proximate to p21:c21. This further suggests that the intake target for *S. exempta* is carbohydrate-biased, in contrast with other species of larval Lepidoptera studied so far (see above).



#### 4.2. Responses to imbalanced diets

When restricted to a range of single foods (the no-choice experiment), there was a marked difference in the macronutrient balancing rules between *S. exempta* and *S. littoralis*. In contrast to the linear intake array seen in *S. littoralis* and gregarious *S. gregaria*, the array of *S. exempta* was closely similar to the arc-shape patterns of *L. migratoria* and of solitary *S. gregaria* (Raubenheimer and Simpson, 1999, 2003; Simpson et al., 2002). This pattern is anticipated by the nutritional heterogeneity hypothesis, which predicts that the response adopted in trading-off excess of one macronutrient against a deficit of the other is determined by the probability of encountering foods of complementary nutritional composition (see Simpson et al., 2002). The caterpillars of the generalist *S. littoralis* not only feed upon a variety of host-plant families but also a wide range of plant tissues, including leaf, stem, bud, flower and fruit, which vary greatly in nutritional composition (Hill, 1987). In contrast, caterpillars of *S. exempta* feed exclusively on grasses, and mainly on the upper leaves of these plants (Simmonds and Blaney, 1986). Hence, it seems most probable that larval *S. exempta* will, on average, experience less nutritional heterogeneity than will the highly polyphagous *S. littoralis*.

Such differences in balancing rules between the two species are highlighted in Fig. 9 which plots nutritional errors (excesses and deficits in relation to the self-selected intake target). The nutritional error plot for *S. littoralis* is almost identical to that described for gregarious *S. gregaria* (Simpson et al., 2002). In contrast, the plot of *S. exempta* was similar to that of the more specialist solitary phase of *S. gregaria*, with the exception that *S. exempta* ingested greater relative excesses of carbohydrate than did the specialist grasshoppers (Simpson et al., 2002; Raubenheimer and Simpson, 2003). It is important to note that these conclusions from the error plot in Fig. 9a remain essentially unchanged even if the position of the intake target for *S. exempta* is shifted to a ratio that is carbohydrate-biased (e.g. P45%:C55%).

A possible explanation for this difference in the extent to which carbohydrate is overeaten by *S. exempta* and solitary *S. gregaria* concerns the mobility of the two animals. *S. exempta* larvae used for this study were in the gregarious phase, the adults of which are known to have higher migratory potential than the adults of the solitary phase (Riley et al., 1983; Parker and Gatehouse, 1985; Woodrow et al., 1987). Larval feeding is the major stage for accumulating lipid reserves for flight (Gunn and Gatehouse, 1987; Woodrow et al., 1987), although these can be supplemented through adult nectar feeding (Mensah and Gatehouse, 1998). It has, in addition, been reported that under the same feeding condition the gregarious phase of *S. exempta* larvae contain higher lipid reserves than does the solitary phase

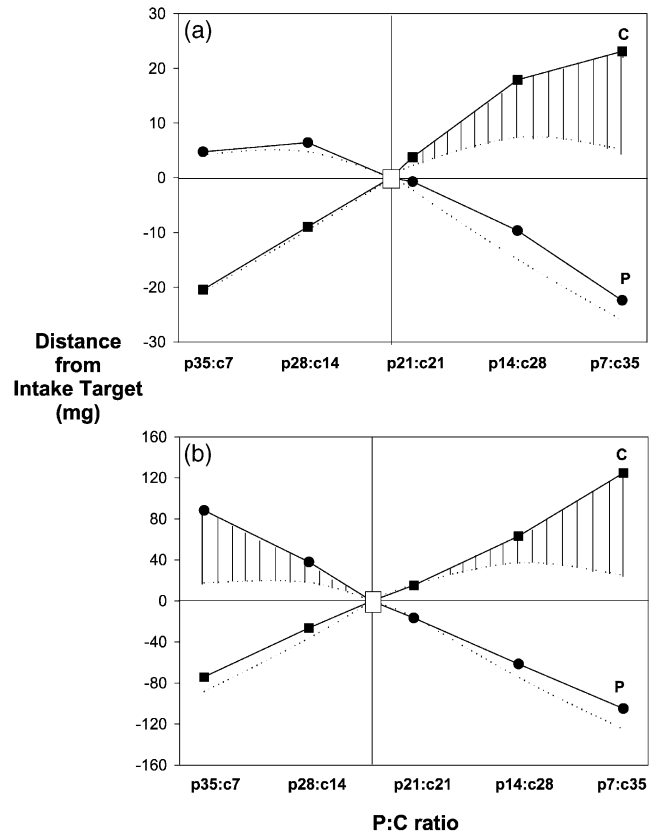


Fig. 9. Error plots comparing the nutrient error (extent to which protein or carbohydrate were over- or under-eaten) relative to the intake target (solid lines) across the five dietary treatments in (a) *S. exempta* and in (b) *S. littoralis* (latter data adapted from Lee et al., 2002). Nutrient intake data up to day 3 and day 5 were used for each insect, respectively. Dotted lines represent the hypothetical minimum nutritional error (the minimum sum of the absolute distance to the self-selected nutrient intake for the two nutrients). The hatched regions thus illustrate the amount of nutrient over-eaten relative to the minimum. Open squares, filled circles and filled squares stand for the self-selected point of intake, protein and carbohydrate, respectively.

(Gunn and Gatehouse, 1987). It might be, therefore, that the ability of these animals to tolerate large excesses of ingested carbohydrate is related to their role as accumulators of fuel reserves for migration (Zera and Larsen, 2001; Zhao and Zera, 2002). Interestingly, the gregarious phase of the specialist-feeding African migratory locust, *L. migratoria*, showed a similar pattern of regulation to *S. exempta*, with a greater reluctance to over-ingest protein than carbohydrate (Raubenheimer and Simpson, 2003). We are pursuing the possible link between migratory behaviour and carbohydrate nutrition further by comparing the regulatory responses of non-migratory, solitary *S. exempta* and *L. migratoria* with their gregarious counterparts.

Analysis of the body nitrogen content of *S. exempta* showed that the insects maintained on the two extreme diets had significantly less body nitrogen than those on the three more moderate foods. Lower nitrogen content in the most extremely carbohydrate-biased food treat-

ment (p7:c35) was mainly due to low nitrogen intake of the insects on this food, as shown on the consumption axis of the utilisation plot presented in Fig. 8a. For the caterpillars on the most extremely protein-biased food (p35:c7), reduced body nitrogen content resulted from reduced conversion efficiency of ingested nitrogen into body nitrogen (i.e. high rates of nitrogen excretion), since nitrogen growth was low relative to animals on the more moderate foods despite similar levels of nitrogen intake (Fig. 8a). A likely explanation for the low nitrogen utilisation efficiency of these animals is that the deficit of carbohydrate in the p35:c7 food caused them to channel a portion of ingested protein to carbohydrate metabolism via gluconeogenesis (Thompson, 1998; Thompson and Redak, 2000), thus generating high levels of nitrogenous wastes.

In our earlier experiment (Lee et al., 2002), the generalist-feeding *S. littoralis* similarly showed reduced nitrogen utilisation efficiency of the p35:c7 food. However, unlike *S. exempta* they experienced no loss of body nitrogen content on this food relative to animals on the self-selecting treatments (Fig. 10). The reason for this is that the generalist *S. littoralis* ingested a greater excess of protein than did *S. exempta* (see Fig. 7 in Lee et al.,

2002), and were therefore able to allocate excess ingested protein to carbohydrate metabolism, rather than sacrifice body nitrogen content to maintain energy metabolism. This provides a clear illustration of how the interaction of behaviour and physiology might be differentially shaped by selection in association with key aspects of the nutritional environment, such as breadth of host range.

This result also suggests a compatible reason from the nutritional heterogeneity hypothesis as to why generalist feeders might over-ingest protein to a greater extent than insects with narrow host ranges. Like the generalist-feeding locust *S. gregaria* (Raubenheimer and Simpson, 2003), *S. littoralis* are able to divert the carbon components of excess ingested protein to energy metabolism while excreting the nitrogenous residues. In so doing, they are simultaneously reducing the ingested protein surplus and, effectively, supplementing the carbohydrate deficit incurred on high-protein foods.

## 5. Conclusions

Comparison of the results of this study on a specialist-feeding caterpillar *S. exempta* with those of Lee et al. (2002) on the closely related generalist *S. littoralis* are remarkably consistent with previous comparisons of generalist and specialist-feeding grasshoppers (Simpson et al., 2002; Raubenheimer and Simpson, 2003). In both cases the generalist selected a diet richer in protein, but utilised protein less efficiently, and when confined to imbalanced foods ingested a greater excess of protein than did the specialist. Greater ingestion of protein was associated with the physiological ability to use amino acids both as a source of nitrogen and, via gluconeogenesis, reduced carbon. Such physiological flexibility makes adaptive sense if viewed as part of an evolved suite of behavioural and physiological responses to high levels of nutritional heterogeneity in the environment of generalist feeders.

Finally, the present study introduces the possibility that the patterns of ingestive trade-offs might be influenced by ecological factors other than host range, such as migratory capacity. Together, these data provide further evidence for the role of the ecological environment in influencing the nutritional responses of herbivorous insects, and emphasise the need to broaden the study of the ways that regulatory systems have been shaped in different evolutionary circumstances.

## Acknowledgements

We thank Tim Carty for providing insects and John Castle for nitrogen analysis. We also appreciate com-

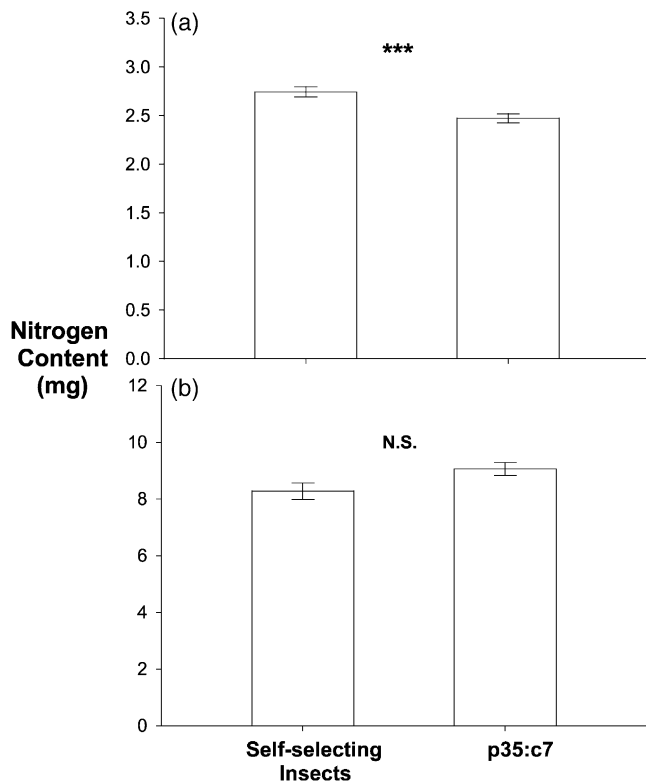


Fig. 10. Carcass nitrogen content (means  $\pm$  SE) of self-selecting insects vs. those fed the most extreme surplus-protein food (p35:c7) for (a) *S. exempta* and (b) *S. littoralis*. Data for the latter are taken from Lee et al. (2002). In each species, an unpaired *t*-test was carried out to compare the difference in nitrogen content between the treatments (*S. exempta*:  $t = 3.87$ , d.f. = 44,  $P < 0.001$ ; *S. littoralis*:  $t = -1.81$ , d.f. = 33,  $P = 0.079$ ).

ments on the manuscript from two anonymous reviewers.

## References

- Bernays, E.A., Chapman, R.F., 1994. Host-Plant Selection by Phytophagous Insects. Chapman and Hall, New York.
- Dadd, R.H., 1961. The nutritional requirements of locusts. IV. Requirements for vitamins of the B complex. *Journal of Insect Physiology* 6, 1–12.
- Ehrlich, P.R., Murphy, D.D., 1988. Plant chemistry and host range in insect herbivores. *Ecology* 69, 908–909.
- Fox, G.A., 1993. Failure-time analysis: emergence, flowering, survivorship, and other waiting times. In: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*. Chapman and Hall, New York, pp. 253–289.
- Gunn, A., Gatehouse, A.G., 1987. The influence of larval phase on metabolic reserves, fecundity, and lifespan of the African armyworm moth, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 77, 651–660.
- Hill, D.S., 1987. *Agricultural Insect Pests of Temperate Regions and Their Control*. Cambridge University Press, Cambridge.
- Hoffman, J.D., Lawson, F.R., Yamamoto, R.T., 1966. Tobacco hornworms. In: Smith, C.N. (Ed.), *Insect Colonization and Mass Production*. Academic Press, London, pp. 479–486.
- 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.
- Mensah, B.A., Gatehouse, A.G., 1998. Effect of larval phase and adult diet on fecundity and related traits in *Spodoptera exempta*. *Entomologia Experimentalis et Applicata* 86, 331–336.
- Parker, W.E., Gatehouse, A.G., 1985. The effect of larval rearing conditions on flight performance in females of the African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 75, 35–47.
- Raubenheimer, D., Simpson, S.J., 1992. Analysis of covariance: an alternative to nutritional indices. *Entomologia Experimentalis et Applicata* 62, 221–231.
- Raubenheimer, D., Simpson, S.J., 1994. The analysis of nutrient budgets. *Functional Ecology* 8, 783–791.
- 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 diet breadth. *Journal of Experimental Biology* 206, 1669–1681.
- Riley, J.R., Reynolds, D.R., Farmery, M.J., 1983. Observations of the flight behaviour of the armyworm moth, *Spodoptera exempta*, at an emergence site using radar and infra-red optical techniques. *Ecological Entomology* 8, 395–418.
- Rosenthal, G.A., Berenbaum, M.R. (Eds.), 1992. *Herbivores: Their Interaction with Secondary Plant Metabolites*, 2nd ed. Academic Press, San Diego, California.
- SAS Institute, 1990. *SAS/STAT User's Guide*, Version 6.0. SAS Institute, Cary, North Carolina.
- Scheiner, S.M., 1993. MANOVA: multiple response variables and multispecies interactions. In: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*. Chapman and Hall, New York, pp. 94–112.
- Schmidt, D.J., Reese, J.C., 1986. Source of error in nutritional index studies of insects on artificial diet. *Journal of Insect Physiology* 32, 193–198.
- Schoonhoven, L.M., Jermy, T., van Loon, J.J.A., 1998. *Insect-Plant Biology: From Physiology to Evolution*. Chapman and Hall, London.
- Simmonds, M.S.J., Blaney, W.M., 1986. Effects of rearing density on development and feeding behaviour in larvae of *Spodoptera exempta*. *Journal of Insect Physiology* 32, 1043–1053.
- Simpson, S.J., Simmonds, M.S.J., Blaney, W.M., 1988. A comparison of dietary selection behaviour in larval *Locusta migratoria* and *Spodoptera littoralis*. *Physiological Entomology* 13, 225–238.
- Simpson, S.J., Raubenheimer, D., Behmer, S.T., Whitworth, A., Wright, G.A., 2002. A comparison of nutritional regulation in solitary and gregarious phase nymphs of the desert locust, *Schistocerca gregaria*. *Journal of Experimental Biology* 205, 121–129.
- Stockhoff, B.A., 1993. Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. *Journal of Insect Physiology* 39, 677–686.
- Suomela, J., 1996. Within-tree variability of mountain birch leaves causes variation in performance for *Epirrita autumnata* larvae. *Vegetation* 127, 77–83.
- Suomela, J., Ayres, M.P., 1994. Within-tree and among-tree variation in leaf characteristics of mountain birch and its implications for herbivory. *Oikos* 70, 212–222.
- Suomela, J., Kaitaniemi, P., Nilson, A., 1995. Systematic within-tree variation in mountain birch leaf quality for a geometrid, *Epirrita autumnata*. *Ecological Entomology* 20, 283–292.
- Suomela, J., Suominen, O., Torvi, M., 1997. Variation in quality of mountain birch and tea-leaved willow for mammal and insect herbivores: Differences among trees, among sites and between tree species. *Ecography* 20, 224–232.
- 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.
- Thompson, S.N., 1998. Long-term regulation of glucogenesis by dietary carbohydrate and relevance to blood sugar level in an insect *Manduca sexta* L. *International Journal of Biochemistry and Cell Biology* 30, 987–999.
- Thompson, S.N., Redak, R.A., 2000. Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta* L. *Biochimica et Biophysica Acta* 1523, 91–102.
- Waldbauer, G.P., Cohen, R.W., Friedman, S., 1984. Self-selection of an optimal nutrient mix from defined diets by larvae of the corn earworm *Heliothis zea* (Boddie). *Physiological Zoology* 57, 590–597.
- Woodrow, K.P., Gatehouse, A.G., Davies, A.D., 1987. The effect of larval phase on flight performance of African armyworm moths, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 77, 113–122.
- Zera, A.J., Larsen, A., 2001. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, *Gryllus firmus*. *Journal of Insect Physiology* 47, 1147–1160.
- Zhao, Z., Zera, A.J., 2002. Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capacity in a wing-polymorphic cricket. *Proceedings of the National Academy of the Sciences of the United States of America* 99, 16829–16834.