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# A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval)

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## Abstract

Nutritional regulatory responses of the generalist caterpillar *Spodoptera littoralis* (Boisduval) were explored, in choice and no-choice experiments, using the Geometric Framework. In the choice experiment, newly moulted final instar larvae were provided with one of three protein-biased foods (PB-food: p35:c7, p28:c5.6 or p21:c4.2) and one of three equal protein–carbohydrate ratio foods (ER-food: p21:c21, p16.8:c16.8 or p12.6:c12.6). On five of the nine treatments, caterpillars independently regulated protein and carbohydrate intake to a mixture of 57 and 43%, respectively. However, when the concentration of the ER-food decreased and that of the paired PB-food increased, caterpillars progressively abandoned regulation and ate more of the PB-food. Despite these regulatory differences, performance (survivorship, growth and development) was similar across all nine treatments. In the no-choice experiment, caterpillars were given one of five foods (p35:c7, p28:c14, p21:c21, p14:c28 and p7:c35). Results indicated that caterpillars moved to a point in protein–carbohydrate space that was consistent with the Equal Distance Rule of compromise, a pattern previously predicted for generalist feeders. The insects on the two extreme foods, p35:c7 and p7:c35, showed reduced pupal mass and longer development, respectively. There was also strong evidence for post-ingestive regulation of nutrient utilisation, notably for protein. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Nutrients; Herbivore; Geometric framework; Lepidoptera; Self-selection

## 1. Introduction

Experimental evidence collected over the past several decades has shown that insects are able to self-select a mixture of nutrients that supports optimal performance (reviewed by Simpson and Simpson, 1990; Waldbauer and Friedman, 1991; Simpson et al., 1995). These findings have further developed into a conceptual and experimental framework for considering nutrient balancing in insects and other animals (Simpson et al., 1995; Raubenheimer and Simpson, 1999). This ‘Geometric

Framework’ identifies fundamental variables in nutritional regulatory responses, including the position of regulated points of intake and growth for multiple nutrients and the balancing rules employed when animals are forced to trade-off undereating some nutrients against overeating others when restricted to suboptimal diets. A central aim has been to seek general principles underlying the design of nutritional regulatory systems by collecting and comparing data from a selection of insect species from different taxa and life histories (e.g. see Simpson and Raubenheimer, 1993a).

To date there have been three studies published using the Geometric Framework on larval Lepidoptera. These investigated the effect of developmental stage, sex, and parasitism on nutrient regulation (Stockhoff, 1993; Telang et al., 2001; Thompson et al., 2001). Here, we provide a complete analysis of the nutritional responses of a caterpillar *Spodoptera littoralis*, in choice and no-choice assays, comparable to those that had been done on locusts (Raubenheimer and Simpson, 1993; Chambers et al., 1995). *S. littoralis* is a polyphagous noctuid, the host

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plants of which include families such as Malvaceae, Cruciferae, Solanaceae, Gramineae, Rosaceae (Hill, 1987).

The aim of the study was first to establish whether final-instar caterpillars of *S. littoralis* regulate their intake of both protein and carbohydrate. Next, we measured the nutrient balancing responses of caterpillars provided with single foods that differed in nutritional composition from the self-selected diet. We also investigated the post-ingestive regulatory responses of the same insects, to establish whether they could differentially utilise ingested protein and carbohydrate and thus regulate growth derived from these nutrients. Finally, the performance consequences of failing to regulate intake and growth were also explored.

## 2. Materials and methods

### 2.1. Insects and experimental chambers

Caterpillars came from a culture at the NERC Centre for Ecology and Hydrology, in Mansfield Road, Oxford, and were reared on a wheat-germ based semi-artificial diet containing 33% protein and 28% carbohydrate (Smith, 1966) and maintained at a constant temperature of  $27 \pm 5^\circ\text{C}$  under a 14L:10D photoregime until they had reached the final (6th) stadium. Caterpillars were brought to the Department of Zoology, Oxford University, just prior to reaching the 6th-stadium, and placed in an incubator (LMS Ltd, Kent, UK) set at  $27 \pm 5^\circ\text{C}$  under a 12L:12D photoregime. Immediately upon moulting to the 6th-instar, individuals were weighed to the nearest 0.1 mg (initial fresh mass) and then placed singly into Petri dishes (9 cm diameter) that had five 1 mm diameter holes perforated on the upper lid that allowed ventilation. They were then returned to the incubator. A total of 12 replicates were used for each treatment.

### 2.2. Synthetic foods

The chemically defined foods used in this experiment were based on those used by Simpson and Abisgold (1985) and have been used in previous *S. littoralis* studies (Simpson et al., 1988; Simmonds et al., 1992). In total, nine foods differing in their content of protein (p) and digestible carbohydrates (c) were prepared: p35:c7, p28:c14, p28:c5.6, p21:c21, p21:c4.2, p16.8:c16.8, p14:c28, p12.6:c12.6 and p7:c35. The protein content of all the foods consisted of a 3:1:1 ratio of casein, peptone and albumen, while sucrose was the digestible carbohydrate. Other components of the food were Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%) and 0.2% of a vitamin mix (Dadd, 1961). The remaining part of the food was filled with non-nutritive bulking agent, cellulose. 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 protocol

Two experiments were performed. The first was a choice experiment where we simultaneously presented the caterpillars with two food types, in which one of three protein-biased foods (PB-food: p35:c7, p28:c5.6 or p21:c4.2) was paired with one of three foods with equal ratios of protein to carbohydrate (ER-food: p21:c21, p16.8:c16.8 or p12.6:c12.6). These combinations resulted in nine treatments. The aim in this experiment was to provide foods that were individually sub-optimal in their P:C ratio, but together were complementary (i.e. allowed an insect to mix a balanced food). Earlier work (Simpson et al., 1988) had indicated that final larval *S. littoralis* self-selected a ratio of 65% protein to 35% carbohydrate. Hence, the two protein to carbohydrate ratios chosen (5:1 and 1:1) comfortably encompass the likely point of intake regulation. The second experiment was a no-choice design. Here, we presented the caterpillars with one of five foods differing in their protein to carbohydrate ratio but containing the same total nutrient concentration (p35:c7, p28:c14, p21:c21, p14:c28 and p7:c35).

On the first day of the 6th-stadium (day 0), individuals in the choice experiment received two blocks of food (one of the three PB-foods and one of the three ER-foods). Each block of food was weighed to the nearest 0.1 mg before being presented to the insects. In general, the fresh mass of an individual block of food was between 1300 and 2000 mg, an amount that slightly exceeds the insect's total daily consumption. This was done to ensure that the insects were never food deprived, but equally were not presented with excessive food which would have made estimation of food consumption prone to error (Schmidt and Reese, 1986). Once the food and caterpillars were inside, the arenas were sealed with a strip of Parafilm to inhibit desiccation of the food block. After 24 h, any food still remaining was collected and replaced with fresh, pre-weighed food. The food that was removed was then transferred to a drying oven ( $50^\circ\text{C}$ ) and weighed to the nearest 0.1 mg after it had reached a constant dry mass. This procedure was repeated each day until the caterpillars entered the pre-pupal stage. At this stage, food changes were halted. The experiment ended when caterpillars had pupated, at which time they were removed and weighed to the nearest 0.1 mg. Pupae were then dried at  $50^\circ\text{C}$  until they reached a constant mass. Following desiccation, the pupae were weighed to the nearest 0.1 mg and lipid extracted in three, 24-h changes of chloroform. At the end of the third chloroform wash they were re-dried and re-weighed to calculate lipid content. The lipid-free car-

cases were analysed for nitrogen content using the micro-Kjeldahl procedure. An identical procedure was used for the no-choice experiment, except that caterpillars were presented with only one block of food.

To estimate daily dry food consumption for individual caterpillars, control arenas were established that only contained pre-weighed blocks of food. These controls were run concurrently with the other treatments and were used to construct a regression equation that was used to back calculate the initial dry mass of the blocks of food presented to the caterpillars.

#### 2.4. Statistical analysis

Various aspects of food and nutrient consumption and insect performance were analysed using analysis of covariance (ANCOVA) and multiple analysis of covariance (MANCOVA). For MANCOVA tests we used Pillai's trace statistic, which is considered to be the most robust to violations of assumptions (Scheiner, 1993). When significant differences among treatments were detected, post-hoc pairwise comparisons and contrasts were performed (when multiple comparisons were made, alpha levels were adjusted by Bonferroni correction). Where necessary, the data were transformed to meet the underlying assumptions of the models. Failure-time analysis was used to test any differences in stadium duration among treatments (Fox, 1993). All statistical analyses were performed using SAS 6.12 (SAS Institute, 1990).

### 3. Results

#### 3.1. The choice experiment

##### 3.1.1. Nutrient and food intake

Results indicate that points of protein and carbohydrate intake were significantly affected by an interaction between the P:C ratios of the PB- and ER-foods (MANCOVA:  $F_{8,192} = 3.44$ ,  $P = 0.001$ ; Fig. 1). Fig. 1a plots the three treatments in which the P:C content of the ER-food was p21:c21 (the most concentrated of those tested). This figure shows that regardless of the P:C content of the PB-food, insects with access to p21:c21 food converged in their nutrient intake to follow a similar protein-carbohydrate intake trajectory. That this trajectory represented an actively regulated response is indicated by the fact that all three differed from the trajectory expected if they were to have fed indiscriminately between the two foods in their choice treatment (Fig. 2a). Fig. 1b plots data from the three treatments in which the ER-food had a P:C content of p16.8:c16.8. Here, two of the treatments continued to follow the same trajectory as in Fig. 1a, but the one paired with the most concentrated PB-food (p35:c7) diverged to follow a

higher P:C intake ratio. In Fig. 1c, where the ER-food was diluted so that it had a P:C ratio of p12.6:c12.6, only insects with access to p21:c4.2 food followed a similar trajectory to that in Fig. 1a. In contrast, larvae on the treatment with p28:c5.6 food diverged to follow a higher P:C ratio, and those insects on the treatment with p35:c7 food showed an even more pronounced divergence in this direction.

In summary, the larvae regulated their ratio of protein to carbohydrate eaten when the ER-food, which contained a lower than optimal P:C ratio, was concentrated. However, as the ER-food became progressively diluted caterpillars were more likely to abandon regulation and increase consumption of the PB-food (which had a P:C ratio of 5:1). The patterns of food intake associated with these nutrient intake trajectories are shown in Fig. 2.

#### 3.2. Performance and nutrient utilisation

During the experiment, two insects out of a total of 108 (one each from treatment p35:c7 vs. p21:c21 and treatment p21:c4.2 vs. p16.8:c16.8) failed to eat any food and were discarded from all analyses. All other insects pupated successfully and no difference in survival occurred across the nine treatments. Pupal dry mass was affected by the mass of the newly moulted final-instar caterpillars (where fresh initial mass was included as a covariate), but not by the food treatment (Table 1). When stadium duration was analysed, using failure time analysis (FTA), there was a significant difference among the nine treatments (FTA:  $df = 8$ ,  $\chi^2 = 27.77$ ,  $P = 0.001$ ). Contrasts revealed that caterpillars reared on the most concentrated food pair (p35:c7 vs. p21:c21) pupated earlier than those on other treatments, but that no other differences among treatments were observed.

A bicoordinate plot for body nitrogen (protein-derived) and lipid (carbohydrate-derived) content under choice conditions is presented in Fig. 3. Lipid content was significantly affected by the ER-food (ANCOVA:  $F_{2,96} = 7.80$ ,  $P < 0.001$ ) but not by the PB-food ( $F_{2,96} = 1.38$ ,  $P = 0.257$ ), while nitrogen content was not influenced by either food type (ANCOVA:  $F_{2,96} = 0.23$ ,  $P = 0.794$  and  $F_{2,96} = 2.54$ ,  $P = 0.084$  for PB- and ER-food, respectively).

Utilisation plots for ingested nitrogen and carbohydrate are shown in Fig. 4. The total amount of nitrogen eaten was estimated by dividing total protein consumption by 6.25. The efficiency of conversion of ingested nutrients into body content was investigated using ANCOVA (Raubenheimer and Simpson, 1994; Simpson and Raubenheimer, 2001). No significant interaction between covariates (protein and carbohydrate intake for nitrogen and lipid analyses, respectively) and main factors was observed. The nitrogen content of pupae was significantly affected both by the concentration of the PB-food and ER-food and by the amount of protein con-

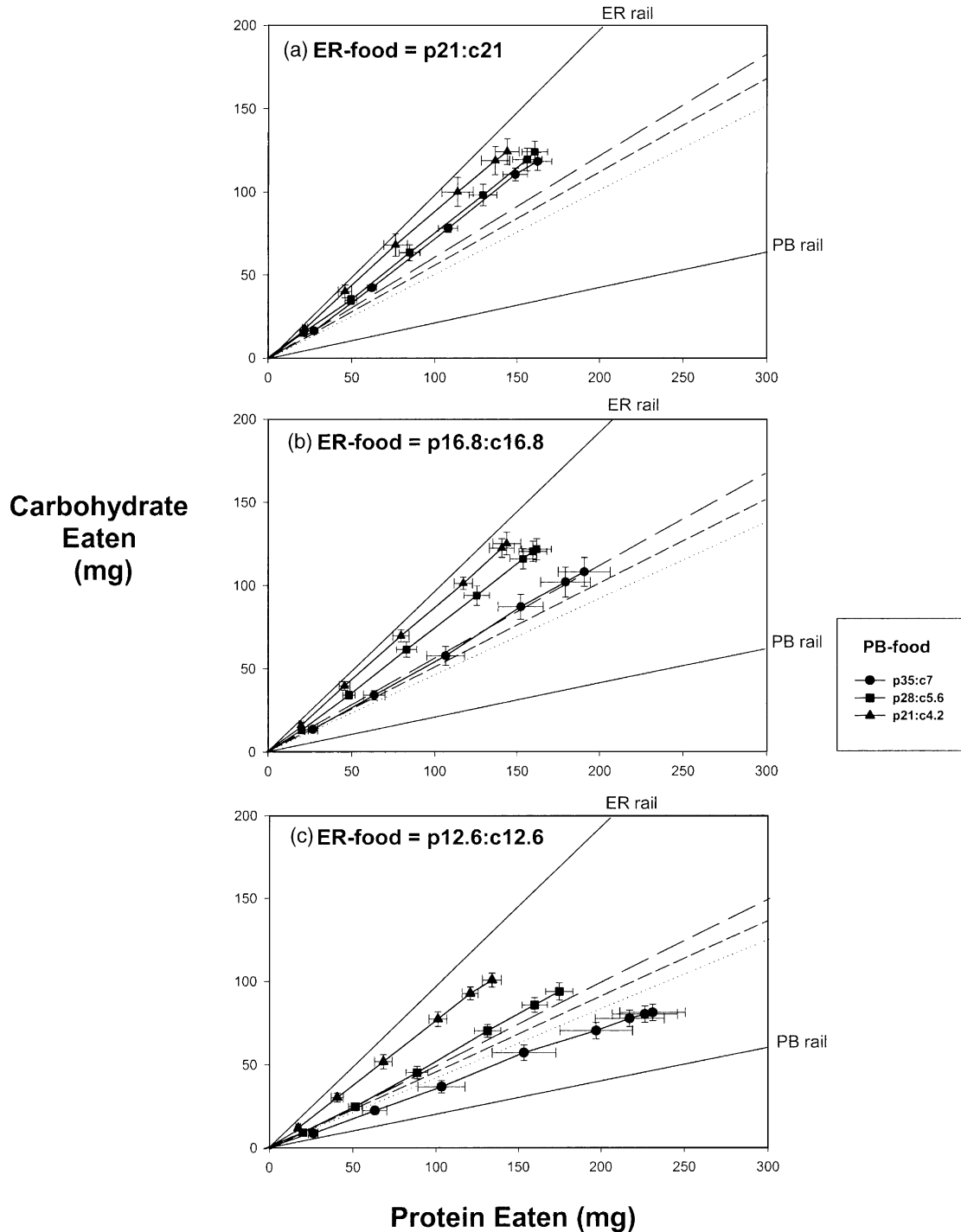


Fig. 1. Bivariate means ( $\pm$ SE) of protein and carbohydrate intake for caterpillars from the choice experiment. Points along each trajectory represent the cumulative intake of protein and carbohydrate over successive days, up to the last day of feeding. The two solid lines indicate the protein-carbohydrate ratio of the protein-biased food (five parts protein to one part carbohydrate) and the equal-ratio food (equal parts protein and carbohydrate). The long-dashed, short-dashed and dotted lines indicate the expected protein-carbohydrate intake trajectories on the treatments with p21:c4.2, p28:c5.6, p35:c7 foods, respectively, if feeding had occurred indiscriminately between the PB- and ER-foods.

sumed (covariate), but there was no significant interaction between the PB- and ER-food (Table 2, Fig. 4a). As the amount of protein (nitrogen) eaten increased, so did pupal nitrogen content. Additionally, as the PB-food became more concentrated, the efficiency with which

ingested protein was converted to body nitrogen decreased (Fig. 4a). Interestingly, the result was the opposite when the regressions were fitted based on the ER-food, such that there was higher conversion efficiency with increasing nutrient concentration of the

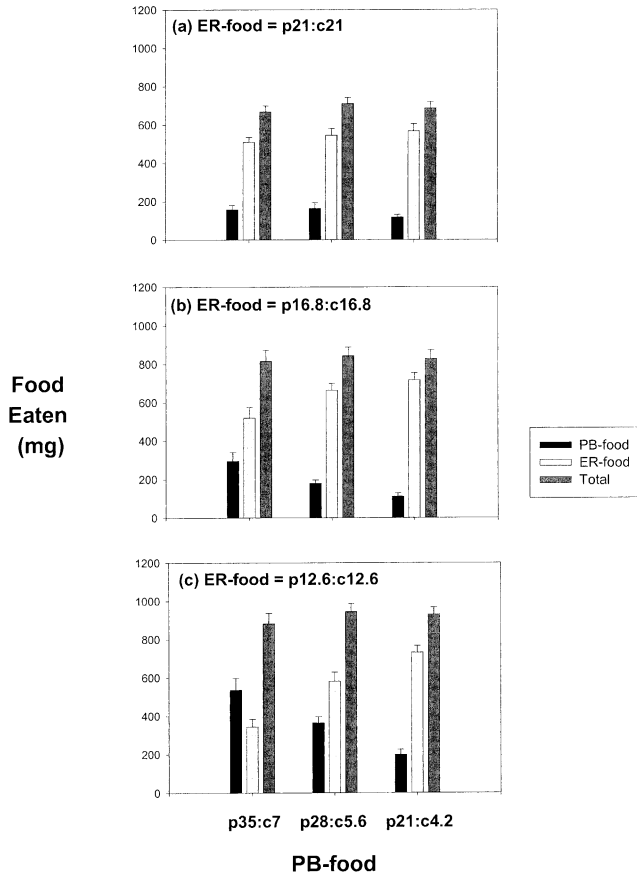


Fig. 2. The mean ( $\pm$ SE) amounts of the PB-food, ER-food and total food eaten by caterpillars during the choice experiment.

Table 1  
ANCOVA results comparing the total food consumption and dry pupal mass for caterpillars from the choice experiment

Source	df	Total food consumption		Pupal mass	
		F-value	P-value	F-value	P-value
PB-food	2	0.56	0.574	0.12	0.891
ER-food	2	22.13	0.000	2.65	0.076
PB-food $\times$ ER-food	4	0.06	0.993	0.76	0.557
Initial mass	1	4.04	0.047	19.16	0.000
Error	96				

There were three different protein-biased foods (p35:c7, p28:c5.6, p21:c4.2) and three equal-ratio foods (p21:c21, p16.8:c16.8, p12.6:c12.6). Initial mass (fresh) was used as a covariate to adjust for size differences between insects.

food (Fig. 4b). The latter result is mainly due to the fact that insects on the lowest ER-food ingested relatively more protein than those on higher ER-food choices (Fig. 1c).

The analysis of the conversion of ingested carbohydrate to body lipid content revealed a significant interaction between the PB- and ER-foods (Table 2). The

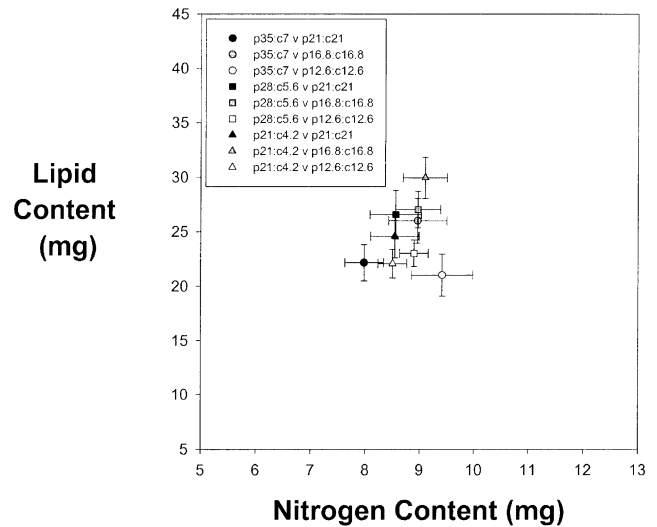


Fig. 3. Bivariate means ( $\pm$ SE) of body nitrogen and lipid content for pupated caterpillars during the choice experiment.

pattern underlying this significant interaction is shown in Fig. 4d. Overall, regardless of the two choice foods, the result showed that the lipid content of the insects increased as a function of carbohydrate intake (Fig. 4c).

### 3.3. The no-choice experiment

#### 3.3.1. Nutrient and food intake

In the no-choice experiment, caterpillars were restricted to one of five foods differing in their P:C ratios. Cumulative protein and carbohydrate intake data across the stadium are shown in Fig. 5 and analysed in Table 3. On days 3 and 5, the array of intake points across the five foods formed a straight line with a slope near  $-1$  (Fig. 5). Polynomial contrasts revealed that the slopes of these lines were linear (Table 3). On all but the two most extreme foods (p7:c35 and p35:c7), larvae ceased feeding by day 6 and pupated 2 days later. Caterpillars on the two extreme foods (especially p7:c35) continued to feed beyond day 6, and hence the final cumulative intake array bent outwards at the extremes, in a quadratic fashion (Table 3, Fig. 5).

As expected from the patterns of the intake arrays, total food consumption across the stadium differed between the five treatments (ANCOVA:  $F_{4,53} = 4.02$ ,  $P = 0.006$ ). Consumption was greatest on the p7:c35 food and lowest on the three more moderately unbalanced foods (p28:c14, p21:c21, p14:c28). Consumption on the p35:c7 food was intermediate between these two groups and did not differ significantly from either of them.

#### 3.3.2. Performance and nutrient utilisation

Only one insect, from the p21:c21 treatment, failed to pupate and was excluded from the experiment. Dry

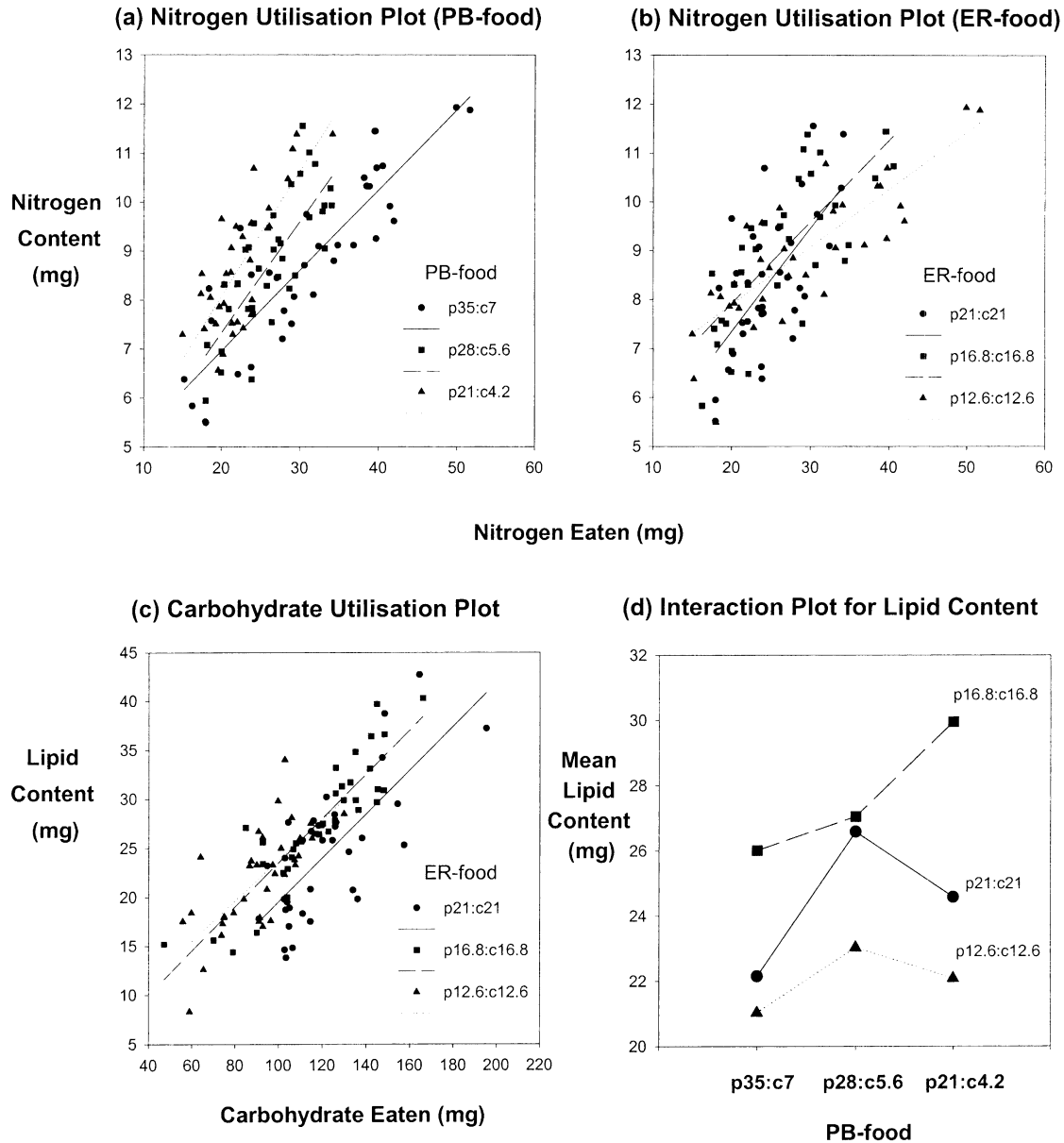


Fig. 4. Utilisation plots exploring the conversion efficiency of ingested nutrients to body content for pupated caterpillars from the choice experiment. In plots (a), (b) and (c) each point indicates an individual insect that pupated, and in each of these plots simple linear regressions are fitted to demonstrate the conversion efficiencies. Plots (a) and (b) show the efficiency at which ingested nitrogen is converted to body nitrogen content. Plot (a) shows this according to the nutrient concentration of the PB-food, while plot (b) shows it according to the nutrient concentration of the ER-food. Plot (c) shows the efficiency at which ingested carbohydrate is converted to body lipid content for three different ER-foods. Finally, plot (d) demonstrates the interaction between the PB- and ER-food on the conversion of ingested carbohydrates to mean body lipid content.

pupal mass was significantly affected both by food composition and by wet initial mass (ANCOVA:  $F_{4,53} = 5.33$ ,  $P = 0.001$  and  $F_{1,53} = 19.01$ ,  $P < 0.001$ , respectively). Post-hoc (Tukey) tests revealed that pupae from the p35:c7 treatments weighed less than those from the other treatments, but that no other differences existed. Stadium duration was also affected by the P:C ratio of the food (FTA:  $df = 4$ ,  $\chi^2 = 29.22$ ,  $P < 0.001$ ). Contrasts showed that caterpillars reared on p7:c35 took significantly longer to pupate compared to

those on the other treatments but that among the four other treatments no difference in developmental time was observed.

Results from MANCOVA analysis indicate that the P:C ratio of the food had a significant impact on nitrogen and lipid content (MANCOVA:  $F_{8,102} = 15.19$ ,  $P < 0.001$ ), which are shown as bicoordinate plots in Fig. 6. Interestingly, nitrogen content was similar across all treatments except for p7:c35, where it was low. In contrast, lipid content differed for each treatment and tended

**Table 2**  
ANCOVA results exploring the conversion of ingested protein to body nitrogen content and of ingested carbohydrates to body lipid content for pupated caterpillars in the choice experiment

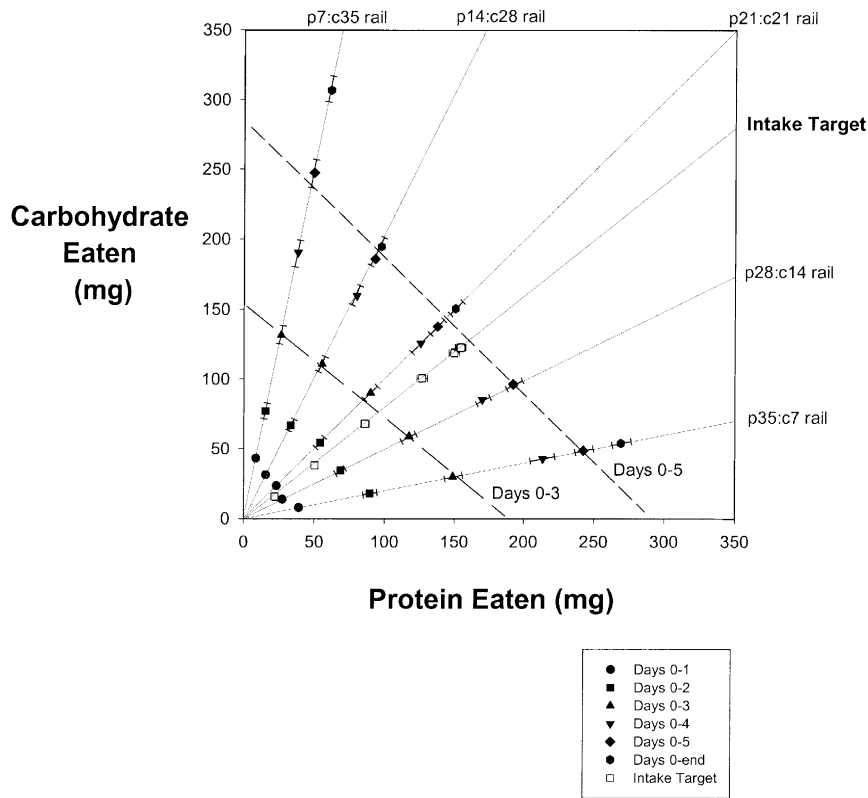
Source	df	Nitrogen content		Lipid content	
		F-value	P-value	F-value	P-value
PB-food	2	24.21	0.000	0.51	0.599
ER-food	2	3.56	0.032	14.08	0.000
PB-food×ER-food	4	0.94	0.446	2.77	0.032
Covariate	1	193.69	0.000	175.34	0.000
Error	96				

There were three different protein-biased foods (p35:c7, p28:c5.6, p21:c4.2) and three equal-ratio foods (p21:c21, p16.8:c16.8, p12.6:c12.6). Total protein ingested was used as the covariate for analysing nitrogen utilisation, while total carbohydrate ingested was used as the covariate for analysing carbohydrate utilisation.

**Table 3**  
MANCOVA results for protein–carbohydrate intake in the no-choice experiment

Source	df	Days 0–3		Days 0–5		Days 0-end	
		Num., Den.	F-value P-value	F-value P-value	F-value P-value		
P:C ratio	8,106	12.40	0.000	14.92	0.000	23.33	0.000
Linear	2,52	528.30	0.000	1124.60	0.000	1699.40	0.000
Quadratic	2,52	0.89	0.417	1.51	0.230	11.16	0.000
Initial mass	2,52	54.50	0.000	17.93	0.000	1.13	0.330

In total there were five food treatments (p35:c7, p28:c14, p21:c21, p14:c28 and p7:c35). The shape of the intake arrays up to day 3, day 5 and the last day of the stadium were examined using linear and quadratic contrasts. Initial mass (fresh) was used as a covariate to adjust for size differences between insects.



**Fig. 5.** Bivariate means ( $\pm$ SE) for protein and carbohydrate intake for pupated caterpillars from the no-choice experiment. Each point along each trajectory represents the cumulative intake of protein and carbohydrate over successive days. Solid lines represent nutrient ‘rails’ for the five food treatments (p7:c35, p14:c28, p21:c21, p28:c14 and p35:c7). White squares represent the cumulative intake target for caterpillars that self-selected (data from the choice experiment). The shape of the intake arrays for cumulative intake up to days 3 (long-dash) and 5 (short-dash) were consistent with the Equal Distance Rule of compromise.

to increase as the concentration of digestible carbohydrate in the food increased.

The efficiency of conversion of ingested protein to body nitrogen content and ingested carbohydrate to body lipid content were also analysed as utilisation plots (Fig. 7). The ANCOVA result for nitrogen utilisation demon-

strated a significant interaction between the covariate (ingested protein) and the main factor (protein:carbohydrate ratio) ( $F_{4,52} = 4.67, P = 0.003$ ). In this respect, it was necessary to fit the different regressions for each treatment combination (Raubenheimer and Simpson, 1994; Simpson and Raub-

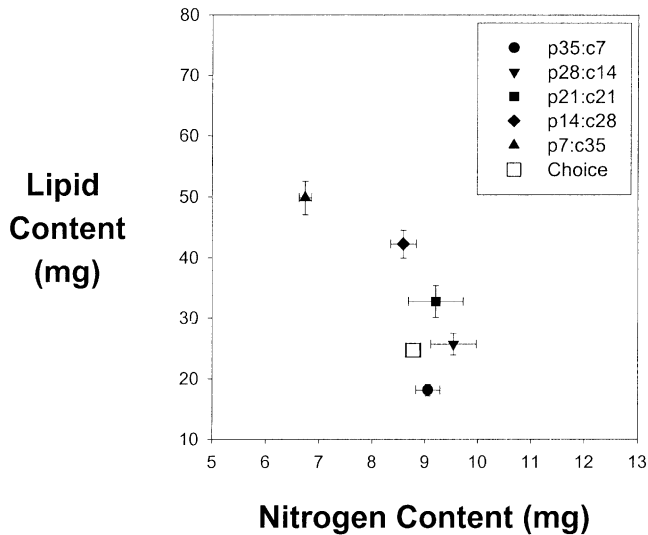


Fig. 6. Bivariate means ( $\pm$ SE) of body nitrogen and lipid content for pupated caterpillars from the no-choice experiment. The white square represents the average body nitrogen and lipid content of the nine treatments in choice experiment.

enheimer, 2001). When simple linear regressions of nitrogen conversion were fitted (Fig. 7a), caterpillars fed on p7:c35, p14:c28, p21:c21 and p28:c14 showed high conversion efficiencies of ingested nitrogen to body mass. However, the efficiency of conversion was considerably reduced on the p35:c7 treatment. Pupal lipid content was significantly affected by the amount of ingested carbohydrate (ANCOVA:  $F_{1,53} = 35.39$ ,  $P < 0.001$ ) and by the P:C ratio of the food (ANCOVA:  $F_{4,53} = 8.57$ ,  $P < 0.001$ ), but there was no covariate-by-treatment interaction (see utilisation plot in Fig. 7b). The higher the carbohydrate content of the food, the lower the efficiency of conversion to body lipid.

## 4. Discussion

### 4.1. The choice experiment

Our result shows that the caterpillars regulated their intake of protein and carbohydrate by differentially selecting between two complementary foods, as has been demonstrated previously for grasshoppers (Chambers et al., 1995), indicating independent regulation of protein and carbohydrate intake. This provides supplementary evidence to the results from previous self-selection studies done with caterpillars (Waldbauer et al., 1984; Simpson et al., 1988; Telang et al., 2001). The self-selected ratio was 57% protein to 43% carbohydrate. This ratio was somewhat lower than that obtained from the earlier work using the same species but with more extreme foods (p42:c0 or p21:c0 paired with p0:c42 or p0:c21) Simpson et al., 1988). Despite this difference, our present results agree well with the general trend of pro-

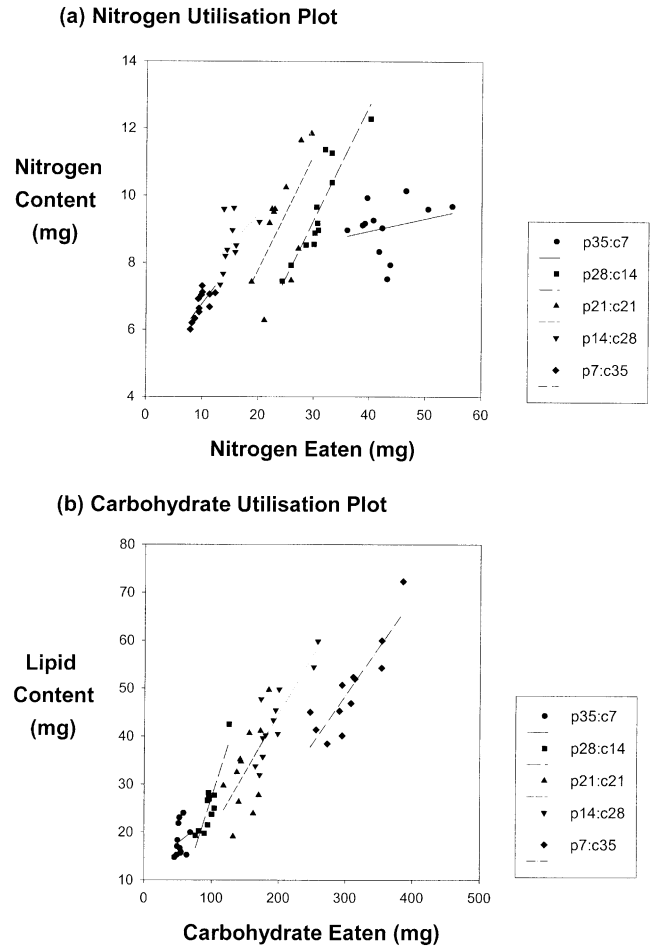


Fig. 7. Utilisation plots exploring the efficiency of conversion of ingested nutrients to body content for pupated caterpillars from the no-choice experiment. In plots (a) and (b) each point represents an individual insect that pupated, and in each of these plots simple linear regressions are fitted to demonstrate conversion efficiencies of ingested nutrients. Plot (a) shows the efficiency of conversion of ingested nitrogen to body nitrogen content according to the five food treatments, while plot (b) shows the conversion efficiencies of ingested carbohydrate to body lipid content for the five food treatments.

tein:carbohydrate requirements, which have been described in previous studies comparing various insect groups. It has been observed that holometabolous insects, which acquire most of their adult protein needs as larvae, such as most Lepidoptera, generally select more protein than carbohydrate under ad libitum choice conditions (Waldbauer et al., 1984), whereas nymphal hemimetabolous insects such as locusts (Chambers et al., 1995) and cockroaches (Cohen et al., 1987; Jones and Raubenheimer, 2001) do the opposite. Such differences correlate with physiological and life-history characteristics such as relative cuticular mass, growth rate, adult diet and possession of symbiotic bacteria (Bernays, 1986; Simpson and Raubenheimer, 1993a). The mechanisms of such regulatory responses include specific modulation of taste receptors responding to amino acids and sugars, possible involvement of CNS neuromodula-

tors and learning (Simpson and White, 1990; Waldbauer and Friedman, 1991; Simmonds et al., 1992; Simpson and Raubenheimer, 1993b, 1996).

A striking finding from the present study was the way that caterpillars progressively abandoned regulation of protein–carbohydrate intake when the concentration of the ER-food fell and concurrently the concentration of the paired PB-food rose. Thus, rather than increase consumption of the ER-food to compensate for dilution, insects preferred to ingest excess protein. Assuming that such nutritional responses reflect associated fitness costs, it could be argued that these data allow us to identify the point at which the costs of increasing consumption of the ER-food to compensate for dilution outweigh the costs of processing excess protein in unbalanced PB-food. It is known that caterpillars increase their consumption rate when the nutrient content in the food is diluted with cellulose or water (Timmins et al., 1988; Slansky and Wheeler, 1989; Wheeler and Slansky, 1991). However, this compensatory response is limited, perhaps by direct physiological constraints such as reduced food retention time in the gut or metabolic effects of ingesting excess cellulose (Martin and Van't Hof, 1988; Slansky and Wheeler, 1989; reviewed by Simpson and Simpson, 1990; Slansky, 1993). Ecological pressures such as increased risk from natural enemies while feeding (Bernays, 1997) may also have resulted in physiological limitations to such compensation. Performance costs of ingesting excess nitrogenous nutrients have been reported for locusts and aphids (Raubenheimer, 1992; Abisgold et al., 1994). A potential counterbalance to these deleterious effects, however, is the fact that at least some caterpillars have a well developed capacity to deaminate protein and subject it to gluconeogenesis (Thompson, 1998; Thompson and Redak, 2000). There was little difference in pupal mass and stadium duration among caterpillars across the nine food choice regimens, which suggests differential use of ingested protein and carbohydrate (Zanotto et al., 1993).

An obvious question is the extent to which caterpillars encounter foods with high protein to carbohydrate ratios in nature. High protein plant tissues such as young leaves and reproductive tissues are one possibility, but another is conspecifics. Cannibalism is commonly observed in this insect and its congeneric species (Scoble, 1992; Pierce, 1995; Chapman et al., 1999). McNeill and Southwood (1978) suggested that cannibalism could be an alternative way to obtain additional nitrogen when it is deficient in the food plant. Several studies experimentally supported this hypothesis by demonstrating higher frequency of cannibalism of larval *Spodoptera* on host plants having lower levels of nitrogen (Al-Zubaidi and Capinera, 1983) and on less preferred host plants (Raffa, 1987). The nutritional benefit of cannibalism remains controversial (Fox, 1975; Chapman et al., 1999), but

there is no escaping that conspecifics provide a high protein food source.

#### 4.2. The no-choice experiment

Being restricted to single unbalanced foods, the caterpillars were not able to reach their intake target in the no-choice experiment. Therefore, they were forced to compromise between the consequences of eating too much of one macronutrient and too little of the other. These trade-offs are represented by the shape of the intake arrays, which in turn ought to reflect the feeding ecology of the animal (Raubenheimer and Simpson, 1999; Simpson et al., 2002). In the present study, the intake array of the generalist caterpillar was closely similar to the pattern previously demonstrated in the gregarious form of the generalist grasshopper *Schistocera gregaria* and termed the Equal Distance Rule (Raubenheimer and Simpson, 1997). By contrast, it has been reported that a grass-specialist locust, *Locusta migratoria*, overeats extremely unbalanced foods to a lesser degree, with the intake array forming an arc rather than a straight line, termed the Closest Distance Rule (Raubenheimer and Simpson, 1993, 1997). A similar pattern has recently been found for the less mobile, ecologically specialized solitary form of *S. gregaria*. The hypothesis has been formulated that generalists such as *S. littoralis* and gregarious phase *S. gregaria*, which likely experience greater nutritional heterogeneity than do specialists, discount the costs of eating excess nutrients in extremely unbalanced foods against the higher probability that they will encounter a complementary food (Raubenheimer and Simpson, 1999; Simpson et al., 2002).

The no-choice experiments strongly suggest that *S. littoralis* is able to regulate protein utilisation post-ingestively. Despite having eaten different amounts of protein, insects on all the treatments, except for the p7:c35 treatment arrived at a similar point of body nitrogen content. This is also the same point, on average, to which insects on choice treatments regulated (Fig. 6). Furthermore, utilisation plots for protein indicated a conspicuous reduction of nitrogen conversion efficiency in larvae fed p35:c7, strongly implying that the caterpillars have regulated their growth by removing excess nitrogen from their body, e.g. as fecal uric acid and possibly ammonium (Harrison and Phillips, 1992; Zanotto et al., 1993; Raubenheimer and Simpson, 1997; Simpson and Raubenheimer, 2001). Nevertheless, insects fed on p35:c7 food became significantly smaller pupae compared with those fed on more balanced foods, with potential implications for adult fecundity (Honek, 1993). Schroeder (1986) explained the smaller weight of pupae in a food with supplementary protein in terms of the elevated metabolic costs of catabolising and excreting excess protein. In addition to this 'metabolic load

hypothesis', it is also likely that severe carbohydrate shortage in p35:c7 treatment would have led to more protein being subjected to gluconeogenesis rather than being incorporated into pupal growth.

There was a general trend for body lipid content to increase as the amount of carbohydrate eaten increased. Nevertheless, there is an indication that the caterpillars regulated body lipid content, as shown by the fact that the efficiency with which ingested carbohydrate was converted to lipid decreased as dietary carbohydrate content increased. This suggests the presence of post-ingestive regulatory mechanism that enables the caterpillar to release overeaten carbohydrate from its body. A possible mechanism for this is via a facultative increase in the rate of respiration, as has been demonstrated for locusts (Zanotto et al., 1993, 1997).

We also observed an effect of dietary imbalance on development time. The caterpillars fed on the extreme p7:c35 food exhibited prolonged stadium duration compared with those on other treatments. The extended development was necessary for the insects to achieve sufficient protein growth and, under natural conditions, would result in an increased risk of predation and delayed reproduction (Nylin and Gotthard, 1998). However, an ameliorating benefit of a high-carbohydrate, low-protein food is better resistance against starvation due to higher body lipid content (Stockhoff, 1991).

Our present data have quantified the nutritional regulatory responses of a generalist caterpillar maintained under various dietary regimes in the laboratory. As discussed here and elsewhere (e.g. Raubenheimer and Simpson, 1999; Simpson et al., 2002), the form of these responses provides insights into the selection pressures that have fashioned their evolution in nature. Such insights in turn help define ecological questions for further research.

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