Nutrient utilization by caterpillars of the generalist beet armyworm, *Spodoptera exigua*

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Abstract. Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), caterpillars are destructive crop pests responsible for considerable annual economic losses. These generalist herbivores are challenged with a diversity of dietary choices that can impact their survival, development and fecundity. In the present study, dietary choices of *S. exigua* caterpillars, based on the protein to digestible carbohydrate (P:C) ratio of the food, and the impact of nutritionally unbalanced foods on caterpillar performance are assessed. In choice experiments, individual third-instar caterpillars are offered simultaneously a P-biased and a C-biased food until pupation. Caterpillars feed nonrandomly and select a slightly P-biased diet (22P:20C). In no-choice experiments, second instar caterpillars are reared until pupation on diets ranging in P:C ratio from extremely P- to extremely C-biased. High mortality and delayed development are observed on the C-rich, P-poor diets, highlighting the potential deleterious effects of excess carbohydrates and the importance of protein for growth and development. Diet-dependent differences in pupal weight or pupal lipid reserves are not observed. This contrasts with closely-related *Spodoptera* species where pupal mass and lipid stores increase on C-rich, P-poor diets. On the extremely P-biased diet, performance is similar to that of individuals reared on the self-selected diet, suggesting that these caterpillars may efficiently be deaminating excess amino acids to generate carbon skeletons, which are shunted into lipid biosynthesis. *Spodoptera exigua* caterpillars exhibit flexible and efficient pre-ingestive nutrient intake regulation and post-ingestive utilization, allowing these generalist feeders to cope with the heterogeneous diets they may encounter.

Key words. Beet armyworm, carbohydrate, larval performance, Lepidoptera, lipid, nutritional quality, plant–insect interactions, protein, self-selection, *Spodoptera exigua*.

Introduction

Herbivorous insects are faced with dietary diversity and must make feeding decisions to achieve a nutritionally balanced food intake that leads to optimal performance. A suboptimal nutrient diet can adversely affect lepidopteran survivorship, growth, development and fecundity (Barton Browne, 1993; Auger, 1995; Awmack & Leather, 2002). Actively-feeding caterpillars respond to their environment and, when given choices, often regulate their nutrient intake and utilization to optimize their performance (Lee et al., 2003, 2004a, b; Simpson et al., 2004; Bede et al., 2007). This regulation of nutritional balance may occur on many levels, such as through selecting and ingesting appropriate plant tissue or through nutrient digestion, absorption and utilization (Mattson, 1980; Waldbauer & Friedman, 1991; Awmack & Leather, 2002).

Nutrients required by caterpillars for development include lipids, salts, vitamins and sterols (Schiff et al., 1988; Stockhoff, 1993; Awmack & Leather, 2002; O’Brien et al., 2002). However, much research on nutritional quality and self-selection has focused on a key variable in their diet that significantly impacts insect performance: the ratio of protein...
to digestible carbohydrates (P:C). Caterpillars allowed to select the dietary ratio of P:C often have superior growth, development and fecundity compared with those fed suboptimal diets with either a P- or C-bias or which are dilute (Waldhauser et al., 1984; Manuwoto & Scriber, 1985; Karowe & Martin, 1989; Telang et al., 2001; Lee et al., 2002, 2003, 2004a, b, 2006; Simpson et al., 2004; Raubenheimer et al., 2005; Thompson & Redak, 2005). Caterpillars reared on these unbalanced diets may exhibit either smaller pupal mass (C-poor), longer developmental time (P-poor) or higher mortality (C-rich or P-poor).

In nature, caterpillars, particularly those with a wide dietary breadth (generalists), are exposed to variation in the nutritional composition of their diet. Even within a single plant, there are differences in the P:C content between older leaves (high in total carbohydrates and low in protein), mature photosynthetic leaves (high sucrose levels due to active photosynthesis and moderate protein levels due to photosynthetic enzymes such as Rubisco), and younger leaves and reproductive tissues (high in protein) (Mattson, 1980; Lincoln, 1993). For most caterpillars, plants supply sufficient digestible carbohydrates, but protein may be limiting or of unacceptable quality (Mattson, 1980; Scriber & Slansky, 1981; Horie & Watanabe, 1982; Karowe & Martin, 1989; Selton, 1996; Elser et al., 2000; Lavoie & Oberhauser, 2004; Throop & Lerdau, 2004). For some nutrients, caterpillars may be able to synthesize lacking compounds. For example, insects may be able to derive required carbohydrate skeletons from amino acids through deamination and gluconeogenesis (Thompson & Lee, 1988; Thompson et al., 2003). For other limiting nutrients, particularly protein, phytophagous insects may adjust intake by compensatory feeding. However, because plants are often limiting in their protein levels and contain sufficient or excess carbohydrates, this overcompensatory feeding may lead to a greater P:C imbalance (Mattson, 1980; Scriber & Slansky, 1981; Lincoln, 1993; Elser et al., 2000; Lavoie & Oberhauser, 2004; Throop & Lerdau, 2004).

Therefore, excess sugars and insufficient protein in the plant diet pose a realistic problem for fast-growing caterpillars (Waldhauser et al., 1984; Lee et al., 2002, 2004a). How do phytophagous insects cope with temporal and spatial variations in nutritional quality of plants? Many studies on caterpillar nutritional self-selection have focused on members of the Noctuidae family and, in particular, of the genus Spodoptera. This family includes some of the most destructive lepidopteran crop pests. They also provide an excellent framework for comparative studies because Noctuid caterpillars vary greatly in diet breadth from Spodoptera exempta, which is a specialist on 25 plant species mostly in the Poaceae, to the generalist Spodoptera littoralis, which feeds on over 140 different plant species (Brown, 1962; Ellis, 2004; Pogue, 2006).

Caterpillars of the beet armyworm, Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae), are a classic example of a polyphagous herbivore confronted with a diversity of potential host plants; this Lepidopteran accepts more than 130 host plants representing over 30 different families (Pogue, 2006). Not only are S. exigua caterpillars major agricultural pests, responsible for annual economic losses, particularly in the southern U.S.A. and Asia, but their management is confounded by their migratory dispersal and rapidly evolving resistance to conventional pesticides (Adamczyk et al., 2003; Shimada et al., 2005). Understanding their feeding decisions is necessary for the development of appropriate and effective control strategies.

In the present study, the regulation of nutrient acquisition by S. exigua caterpillars is investigated. In addition, the consequences of dietary imbalance in terms of performance criteria such as survivorship, growth, development and pupal mass, which is positively related to fecundity in this species (Greenberg et al., 2001; Tisdale & Sappington, 2001), and pupal lipid reserves using the Geometric Framework of nutrient utilization is examined (Raubenheimer & Simpson, 1993; Simpson & Raubenheimer, 1993). The S. exigua caterpillars are reared on defined artificial diets, which allows manipulation of the P:C ratios and removal of feeding cues, such as leaf physical properties, or plant defensive compounds, such as deterrents. By contrast to many studies that focused on feeding performance in only late-larval instars (Waldhauser et al., 1984; Karowe & Martin, 1989; Telang et al., 2001, 2003; Lee et al., 2002, 2004a, b; Thompson & Redak, 2005; Despland & Noseworthy, 2006), the present study is a longer-term developmental study: caterpillars were allowed to feed from third (choice experiment) or second (no-choice experiment) instar to pupation. Insect sex was identified at the pupal stage to determine any sex-related differences in nutrient choice or performance. As insect herbivores are often limited by the low protein quantity or quality of their host plants, it is particularly interesting to understand the nutrient utilization strategies used by generalist caterpillars to cope with unbalanced C-biased diets.

Materials and methods

Caterpillar rearing

Eggs of the beet armyworm were obtained from the GAST Insect Rearing Facility (Stonerville, Mississippi) and AgriPest Inc. (Zebulon, North Carolina). The laboratory colony from these eggs was reared for multiple generations on an artificial wheat germ-based diet (Bio-Serv, Frenchtown, New Jersey) in a growth chamber under an LD 16:8 h photoperiod (28–40% RH at 28.5 °C) to allow for a standard nutritional history (Warbrick-Smith et al., 2006). Adult moths, which eclosed from collected pupae, were allowed to mate and oviposit in glass jars on paper towels.

Synthetic foods

Artificial foods with defined ratios of protein to digestible carbohydrates (P:C) were prepared according to Simpson & Abisgold (1985). Protein [casein (BioServ, Frenchtown, NJ), peptone (Matheson, Coleman and Bell, East Rutherford, NJ) and albumen (BioServ); 3:1:1 (w:w:w)] and digestible carbohydrates [dextrose (BioServ) and glucose (Sigma, St. Louis, MO); 1:1 (w:w)] made up 42% of the diet on a dry weight basis. The P:C ratio was manipulated to generate five...
Caterpillars were allowed to feed block of food was never completely eaten. The Petri dish was the nearest 0.1 mg. Foods were replenished as needed and the 28P : 14C. Food blocks of approximately 1 – 2 g were weighed to ment 2: 28P : 14C versus 14P : 28C; treatment 3: 7P : 35C versus Each treatment consisted of a protein-biased and a carbohy- towel and presented with two chemically defined artificial foods. were enclosed in a Petri dish lined with a sterile, moist paper nutrients and vitamin components, were weighed to the nearest 0.1 mg, mixed and ground to obtain a smooth powder. Food was suspended in a 2% agar solution (w/v) and used in both choice and no-choice experiments.

Choice studies

Newly-moulted, preweighed third-instar S. exigua caterpillars were enclosed in a Petri dish lined with a sterile, moist paper towel and presented with two chemically defined artificial foods. Each treatment consisted of a protein-biased and a carbohy- drate-biased food: treatment 1: 35P : 7C versus 14P : 28C; treat- ment 2: 28P : 14C versus 14P : 28C; treatment 3: 7P : 35C versus 28P : 14C. Food blocks of approximately 1–2 g were weighed to the nearest 0.1 mg. Foods were replenished as needed and the block of food was never completely eaten. The Petri dish was sealed with Parafilm to reduce the drying of the food block. Caterpillars were allowed to feed ad libitum and were restricted to their arena until pupation. Caterpillar survivorship was moni- tored. Frass was collected throughout the experiment. Twelve hours after pupation, any remaining food, the frass and the pupa were transferred to a drying oven (45 ± 2°C) until a constant mass was reached. The dry weight of food, frass and pupal masses were weighed to the nearest 0.1 mg. The experiment was repeated three times with 7–15 individuals per treatment.

To determine food consumption, preweighed blocks of food were used as controls. The foods were placed under the same conditions for the duration of the experiment and dried in a drying oven and dried to a constant mass. From the linear regression of fresh versus dry weight of the control foods, the initial dry mass of the blocks of food given to the cater- pillars was determined. From this, the amount of food ingested and, therefore, the protein and carbohydrate ingested by each caterpillar was calculated.

No-choice experiments

No-choice experiments were established in a similar manner to the choice experiments except that second instar cater- pillars were restricted to one defined synthetic food containing a P : C ratio of either 35P : 7C, 28P : 14C, 22P : 20C, 14P : 28C or 7P : 35C. Performance criteria, such as mortality, growth and development, final pupal weight and lipid content as described below, were determined for each caterpillar. Performance criteria, except for mortality, were analyzed only for those individuals surviving to pupation. This experi- ment was repeated twice with 14–16 individuals per diet.

After the moult to the third stadium, individuals were weighed and reweighed every 24 h until pupation. The develop- mental stage was noted every 12 h. Blocks of food were placed in a drying oven (40°C) until a constant dry mass was reached and weighed to the nearest 0.1 mg. As described above, the amount of food remaining was determined and the protein and carbohydrate intake was calculated. Twelve hours after pupation, pupae were collected and freeze-dried to determine their biomass to the nearest 0.1 mg when a constant dry weight was reached.

Sex determination

The sex of the insects was determined at the pupal stage according to genital aperture, which is present on the ventral side of the pupa on the posterior segments anterior to the anus (Scoble, 1992).

Lipid extraction

Pupal lipid content of insects in the no-choice experiments was determined in accordance with the protocol of Lee et al. (2004b) with modifications based on Zera et al. (1994). Pre-weighed, freeze-dried pupae were crushed using glass homogenizers. Chloroform was added to the ground pupa for 24 h at room temperature and then removed. Three chloroform washes were performed. Samples were dried overnight at 40°C to ensure complete evaporation of the solvent and reweighed to determine the amount of extracted lipids.

Statistical analysis

Statistical analysis was performed using spss version 14 and 15 (SPSS Inc., Chicago, IL). Food consumption and cat- erpillar performance were analyzed using general linear models, in particular, analysis of variance (ANOVA), analysis of covariance (ANCOVA) and multiple analysis of covariance (MANCOVA). Caterpillar survivorship was analyzed by Fisher’s exact test. For nutrient utilization, ANCOVAs were used to analyze the effects of the treatments and the appropriate covariate. The ANCOVA model including the interaction term was used beforehand to test if the equal-slope assumption of ANCOVA was met. If not, data were interpreted graphically by fitting individual regressions through data for each treatment. Statistical differences were determined by Tukey post-hoc tests. Significant differences (P ≤ 0.05) are indicated in graphs and tables by different alphabetical letters.

Results

Choice experiment: selection of nutritional P : C ratio

Third-instar caterpillars of the beet armyworm, S. exigua, were allowed to feed ad libitum on two defined synthetic foods with determined P : C ratios (7P : 35C versus 28P : 14C; 28P : 14C versus 14P : 28C; 35P : 7C versus 14P : 28C). At pupation, the ingested protein and carbohydrate was deter- mined (Fig. 1). The solid lines (rails) represent nutrient intake if caterpillars fed randomly within each of the three choice
Choice experiment: survivorship and pupal biomass

Survival of caterpillars in these choice experiments was high (> 95%) and did not differ significantly (Fisher’s exact test, \( P = 0.44 \)). Pupal biomass, also, was not significantly affected by treatment (two-way ANCOVA, covariate: initial fresh weight, \( F_{2,99} = 0.03, P = 0.98 \)) or by gender (two-way ANCOVA, covariate: initial fresh weight, \( F_{1,99} = 0.23, P = 0.63 \)).

No-choice experiment: food consumption

Total food consumption over the entire developmental period did not vary between diets (Fig. 2A) (ANOVA, diet: \( F_{4,49} = 2.49, P = 0.053 \)). However, the rate of food consumption decreased with increasing carbohydrate bias in the diet scenarios. Caterpillars ate the same amount of protein and carbohydrate on all treatments (MANCOVA, covariate: initial fresh weight, treatment: \( F_{2,99} = 0.62, P = 0.648 \)). Insect gender did not affect food consumption (MANCOVA, covariate: initial fresh weight, sex: \( F_{1,99} = 0.65, P = 0.62 \)). These results show that the caterpillars selected a protein-biased target nutrient intake of 22P: 20C, and defended it in the face of dietary variability.

Fig. 1. Nutrient intake of *Spodoptera exigua* caterpillars given a choice between two defined artificial foods. Third-instar caterpillars were allowed to feed *ad libitum* on two foods (△, 35P: 7C versus 14P: 28C; ■, 28P: 14C versus 14P: 28C; ▼, 7P: 35C versus 28P: 14C) containing different protein to digestible carbohydrate (P: C) ratios. At pupation, the ingested protein and carbohydrate were calculated and nutrient intake was plotted as a bivariate mean ± SE. Lines indicate, for each case, the nutrient trajectory if caterpillars had fed equally from the two foods provided to them (- - -, 35P: 7C versus 14P: 28C; — — —, 28P: 14C versus 14P: 28C; — — — —, 7P: 35C versus 28P: 14C). Overlapping bivariate means that do not overlap the rails indicate that caterpillars regulated their protein and carbohydrate intake to attain a self-selected ratio of 22P: 20C (multiple analysis of covariance, covariate initial fresh weight, treatment: \( F_{2,99} = 0.621, P = 0.648 \)).

Fig. 2. Nutrient intake of *Spodoptera exigua* caterpillars. Second-instar caterpillars were restricted to defined diets with varying ratios of protein to digestible carbohydrate (P: C) (●, 35P: 7C; ■, 28P: 14C; ▲, 22P: 20C; ◊, 14P: 28C; ▼, 7P: 35C) and allowed to feed until pupation. (A) Total consumption. Bivariate means ± SE indicate total nutrient intake (mg) over development from second instars to pupa. Statistical differences in the total amount of food ingested were not observed [analysis of variance (ANOVA)], diet: \( F_{4,49} = 2.49, P = 0.053 \). (B) Protein (P) and digestible carbohydrate (C) intake rate. Bivariate means ± SE of total nutrient intake per day. The straight line represents the intake if caterpillars were eating until they were satiated. Diet significantly affected the rate of food intake (ANOVA, diet: \( F_{4,49} = 3.41, P = 0.014 \)).

Relative to insects on diets closest in composition to their intake target (22P: 20C and 28P: 14C), composition rate is increased on the C-poor (35P: 7C) diet and reduced on the C-rich diets (14P: 28C; 7P: 35C). Therefore, caterpillars ate less of the carbohydrate extreme diet, either to minimize error in carbohydrate consumption or as a result of the poor performance due to lower food quality. Nonetheless, over their entire development, they followed the equal distance rule of compromise (Raubenheimer & Simpson, 1999), meaning that
they consumed the same total amount of food across all treatments. Caterpillar gender did not affect either the total dietary consumption or consumption rate (ANOVA, sex: $F_{1,69} = 1.67, P = 0.20; F_{1,69} = 0.56, P = 0.45$, respectively).

**No-choice experiment: survivorship**

Diet affected caterpillar mortality (Fisher’s exact test, $P < 0.001$) (Fig. 3). On all diets, a small percentage of the caterpillars did not survive until pupation. On most diets, caterpillar survivorship was over 67%; however, there was higher mortality when caterpillars were reared on the C-biased diets (14P: 28C and 7P: 35C).

**No-choice experiment: development**

The P:C ratio of the diet impacted significantly on *S. exigua* caterpillar development rate (Fig. 4). The duration of the second larval stadium was not affected by diet (MANOVA, second instar: $F_{1,91} = 0.52, P = 0.72$) but the duration of third, fourth and fifth stadia increased with increasingly C-biased diets (MANOVA, third instar: $F_{1,91} = 6.57, P = 0$; fourth instar: $F_{1,91} = 3.83, P = 0.007$; fifth instar: $F_{1,91} = 2.93, P = 0.026$), such that the overall time to pupation increased as diets became more C-rich and P-poor (MANOVA, $F_{1,91} = 4.78, P = 0.002$). Time to pupation included rare instances of a sixth larval instar, which occurred in less than 3.3% of caterpillars. This supernumerary instar was observed more frequently in caterpillars reared on the 7P: 35C diet than on the other diets and was only observed in female caterpillars. No difference in larval development was attributed to caterpillar gender (MANOVA, sex $F_{1,91} = 0.23, P = 0.63$).

**No-choice experiment: pupal mass**

Pupal dry weight did not vary between diets (Fig. 5A) (ANOVA, $F_{1,91} = 0.84, P = 0.50$). Caterpillar gender, also, did not affect pupal mass (ANOVA, $F_{1,91} = 0.24, P = 0.63$).

**No-choice experiment: nutrient utilization**

Pupal lipid content showed a strong, positive relationship with pupal mass ($r^2 = 0.92$); larger pupae contained higher amounts of lipid stores (Fig. 5B). Unexpectedly, pupal biomass and lipid content did not significantly vary between diets (ANOVA, diet: $F_{4,91} = 0.84, P = 0.50$ and $F_{4,91} = 1.28, P = 0.28$, respectively) and pupal lipid content was not related to the amount of carbohydrate consumed (ANOVA, covariate: carbohydrate ingested, covariate effect: $F_{1,69} = 2.37, P = 0.13$) (Fig. 6A). Indeed, six out of the ten largest pupae were from caterpillars fed on P-biased diets. The amount of digestible carbohydrates consumed by caterpillars restricted to P-biased diets (35P: 7C and 28P: 14C) was hypothetically sufficient to account for the pupal lipids accumulated by these caterpillars (Woodring et al., 1979).

Lean pupal mass, which is correlated closely with caterpillar nitrogen content (Raubenheimer & Simpson, 2003), was affected by both protein consumed (ANOVA, covariate: proteins consumed, covariate effect: $F_{1,69} = 35.06, P < 0.001$) and by the interaction between the covariate and diet (ANOVA, covariate: proteins consumed: interaction: $F_{4,69} = 6.62, P < 0.001$). Regression lines representing the relationship between the lean pupal mass and the amount of proteins ingested suggests a diminishing slope with increasing dietary protein content (Fig. 6B). This implies an increase in protein utilization efficiency on P-limited diets and, thus, regulation of protein growth. Regardless of the diet reared on, caterpillars showed similar pupal lipid content ($F_{1,69} = 2.06, P = 0.10$). Therefore, on the balanced and P-biased diets, excess amino acids may either be egested or shunted toward lipid biosynthesis.

**Discussion**

**Selection of dietary protein to digestible carbohydrate ratio**

When given a choice between P- or C-biased foods, *S. exigua* caterpillars do not eat randomly but select a target intake of 22P : 20C. Selection of a P-biased diet is characteristic of generalist caterpillar species, such as *S. littoralis* (24P: 18C) and *Heliothis virescens* (80P : 20C) (Waldbauer et al., 1984; Telang et al., 2001; Lee et al., 2002; Simpson et al., 2002). In comparison, oligo- and monophagous caterpillars, such as *Manduca sexta*, *Heliothis subflexa* and *S. exempta*, select diets with equal P:C contents or slightly C-biased; these specialists are thought to have evolved feeding behaviour that matches
the nutrient content of their host plant (Lee et al., 2004b; Thompson & Redak, 2005; Lee et al., 2006). Dietary self-selection is not indicative of all caterpillar species; specialist forest tent caterpillars, *Malacosoma disstria*, do not select specific nutrient ratios (Despland & Noseworthy, 2006).

**Fig. 4.** Caterpillar development on defined artificial foods. Second instar *Spodoptera exigua* caterpillars were placed on synthetic foods varying in the ratio of protein to digestible carbohydrate (P:C) (35P:7C, 28P:14C, 22P:20C, 14P:28C, 7P:35C). The duration of (A) second stadium, (B) third stadium, (C) fourth stadium, and (D) fifth stadium was calculated from 12-h interval observations. (E) Total developmental data, as well as time spent in the supernumary sixth instar and prepupal stage. A sixth instar was extremely rare (3%). The bars represent the mean ± SE time (days) between molts. Significant differences (Tukey’s post-hoc tests, $P \leq 0.05$) in caterpillar development are indicated by alphabetical letters.

**Effects of dietary protein to digestible carbohydrate ratio on caterpillar performance**

*Spodoptera exigua* caterpillars do not adjust their total food intake when exposed to unbalanced diets, but instead follow the ‘equal distance rule of compromise’, which also
represents the feeding strategy of generalist caterpillars, such as *H. virescens* and *S. littoralis* (Raubenheimer & Simpson, 1999, 2004; Lee et al., 2002, 2006), and generalist locusts (Raubenheimer & Simpson, 2003). Therefore, depending on the diet they are restricted to, these caterpillars need to cope with tremendous differences in their protein and/or carbohydrate intakes.

On unbalanced diets, caterpillars face an excess of one component and a deficiency in another. On the high C-, P-poor diet, many generalist caterpillar species, including *S. littoralis*, often counteract protein limitations by compensatory feeding, which may result in increased pupal lipid accumulation, presumably derived from the excess consumed carbohydrates (Lindroth et al., 1991; Telang et al., 2001; Lee et al., 2002). It is, therefore, unexpected that *S. exigua* caterpillars do not change their dietary intake based on the P-content of the diet. Instead, on these diets (14P:28C and 7P:35C), a carbohydrate threshold is attained, which results in feeding cessation and equal food consumption is only achieved by extending larval development. This is reminiscent of feeding by caterpillars of the generalist *H. virescens* where reduced nutrient intake is also observed on C-biased diets (Lee et al., 2006).

One possible metabolic fate of ingested carbohydrate is to be converted into trehalose in the fat body and released into the haemolymph. Threshold haemolymph trehalose levels are implicated in mediating food switching behaviour (Friedman et al., 1991; Thompson, 2003; Thompson et al., 2005) and, therefore, high carbohydrate diets may result in the cessation of feeding observed by *S. exigua* caterpillars. In locusts, haemolymph amino acid levels may also affect feeding behaviour (Simpson & Simpson, 1990). When fed P-biased foods, locusts selectively egest lysine, which allows the insects to bypass the negative effect of excess dietary protein on feeding (Zanotto et al., 1994), enabling continued food intake to acquire sufficient carbohydrate nutrients. Therefore, in comparison to specialist caterpillars, which tend to select an equal or C-biased diet (Lee et al., 2004b, 2006; Thompson & Redak, 2005), *S. exigua* caterpillars likely have higher haemolymph amino acid and/or lower trehalose thresholds, which are involved in the regulation of feeding behaviour (Bede et al., 2007). An alternate explanation is that the amino acid/ trehalose thresholds is the same
between different caterpillar species and differences lie in post-ingestive processing and egestion (Telang et al., 2003). Either of these mechanisms would allow these generalist caterpillars to maximize their utilization of protein sources, an adaptive strategy because caterpillar diet is often protein-limited.

*Spodoptera exigua* caterpillars reared on C-biased diets show mortality costs, similar to other Lepidopteran species (Raubenheimer et al., 2005). Mortality increases when *Spodoptera eridania* caterpillars are reared on diets with protein levels lower than 21% or on nitrogen-deficient corn (Manuwo & Scriber, 1985; Karowe & Martin, 1989). Other studies with *Spodoptera* species do not demonstrate significant dietary effects on survival, but such research focused only on the final instar (Lee et al., 2002, 2004a, b). This stresses the importance of examining caterpillar feeding over the entire developmental period to detect cumulative effects.

For caterpillars, there are trade-offs in larval growth between rapid development, which limits exposure to dangers and may lead to increased reproductive cycles during a season, and the need to accumulate sufficient resources (Price et al., 1980; Williams, 1999). *Spodoptera exigua* caterpillars on C-biased diets exhibit delayed development. Similar trends of longer developmental time of caterpillars reared on protein-limited diets are also observed in *H. virescens*, *M. disstria*, *S. eridania*, *S. littoralis*, *S. exempta*, *Spodoptera frugiperda*, *Samea multiplicalis* and *M. sexta* (Manuwo & Scriber, 1985; Whitford et al., 1992; Wheeler & Halpern, 1999; Petersen et al., 2000; Telang et al., 2001; Lee et al., 2002, 2003, 2004a, 2006; Raubenheimer et al., 2005; Thompson & Redak, 2005; Despland & Noseworthy, 2006).

In the present study, delayed development is not observed in the first stadium included in the experiment (L2), but the duration of all subsequent stadia increases and, very rarely, supernumary instars also occur.

**Post-ingestive mechanisms of nutrient regulation**

Lipids, which adults need for flight or egg development in females, are a critical resource for the adult moth and are predominantly obtained from carbohydrates ingested during the larval stage (Wheeler, 1996; Tisdale & Sappington, 2001). On C-poor diets, many Lepidopteran species (e.g. *S. eridania*, *S. littoralis* and *S. exempta*) obtain lipids for pupal stores from dietary proteins through the deamination and gluconeogenesis of excess amino acids to supply carbon backbones for triglycerides (Lee et al., 2002, 2003, 2004a). However, pupal mass of these caterpillars is lower when fed a P-biased diet, reflecting the increased energy requirements for amino acid gluconeogenesis (Karowe & Martin, 1989; Lee et al., 2002, 2004b). By contrast, *S. exigua* caterpillars reared on the extreme P-biased diets perform similarly to those reared on the diet containing the optimal P:C ratio; on these C-poor diets, beet armyworm caterpillars are the same pupal size and have the same lipid reserves as those raised on a diet containing more digestible carbohydrates. Many of the largest pupa in the present study are from caterpillars reared on the P-rich, C-poor diet. Without labelling studies, direct conversion efficiency of amino acids to lipids cannot be determined absolutely. However, based on empirical conversions of protein to triglycerides, caterpillars reared on all diets ingested sufficient resources not only to account for pupal lipid pools, but also lean growth mass (Woodring et al., 1979; Wolman et al., 1980; Thompson & Redak, 2000). Therefore, *S. exigua* caterpillars appear to be extremely efficient at carbohydrate utilization, as well as processing excess amino acids, and do not appear to suffer the huge metabolic costs evident in related *Spodoptera* species. In *S. exigua*, pupal size and adult fecundity are closely associated (Tisdale & Sappington, 2001). Therefore, this efficient metabolism of excess proteins has ecological implications and has been witnessed in the field where adult female egg production increases when larvae are fed on well nitrogen-fertilized plants (Al-Zubaidi & Capinera, 1983).

**Conclusions**

In choice experiments, *S. exigua* caterpillars selected a slightly P-biased P:C ratio in their diet that supported good performance, in terms of survivorship, development, pupal mass and pupal lipid content, in the no-choice experiments. This implies that through diet switching, *S. exigua* caterpillars are able to select an optimal diet. Caterpillars constrained to a C-biased diet showed increased mortality and delayed growth but, contrary to previously studied species, no increase in food consumption or lipid accumulation is observed, suggesting differences between *Spodoptera* species in the mechanisms for coping with protein limitation relative to carbohydrate intake. In addition, even with dietary restriction, these caterpillars manage to attain the same pupal mass as caterpillars in choice experiments. Caterpillars on an extremely P-biased diet, contrary to previously studied species, do not have smaller pupal size or lower lipid reserves, suggesting highly efficient mechanisms for converting excess amino acids to attain carbon skeletons. Because *S. exigua* pupal size is correlated closely with egg production and output, this has important implications for caterpillar fecundity (Tisdale & Sappington, 2001).

Generalist insects faced with dietary heterogeneity generally have more flexible metabolic strategies to deal with nutrient imbalances compared with mono- or oligophagous insects that specialize on a host plant species (Lee et al., 2002; Simpson et al., 2002; Raubenheimer & Simpson, 2003, 2004). Within the genus *Spodoptera*, the generalist *S. littoralis* is more willing to overeat either protein or carbohydrate when placed on an unbalanced diet than the specialist *S. exempta* and shows more flexible post-ingested responses to nutrient imbalance, including increases in the efficiency of utilization of the limiting nutrient (Lee et al., 2002, 2003). *Spodoptera littoralis* caterpillars are able to compensate for a P-biased diet by metabolizing and utilizing the carbohydrate skeleton of the amino acids and excreting the excess as nitrogenous waste. In comparison, caterpillars of the grass-specialist *S. exempta* are less able to cope with excess protein and the bodily nitrogen content of caterpillars reared on a
P-biased diet is actually lower than those that ingested a P-poor diet (Lee et al., 2003). Hence, S. littoralis exhibits lower fitness costs to being constrained to an unbalanced diet than S. exempta. As expected, S. exigua caterpillars demonstrate similar nutritional regulatory mechanisms to S. littoralis because both are generalist herbivores. Indeed, species with broader diet breadths are more likely to encounter unbalanced foods and are therefore expected to possess flexible pre- and post-ingestive regulatory mechanisms (Simpson et al., 2002). However, the present study shows lower fitness costs on extreme diets in S. exigua than were observed previously in S. littoralis, suggesting more efficient metabolic mechanisms for coping with extremely biased diets in S. exigua than in the closely-related and ecologically similar S. littoralis.

An important question that remains concerns the extent to which nutrient ratios affect beet armyworm behaviour and performance on natural host plants. In most natural environments, caterpillar herbivores are faced with a carbohydrate-rich, protein-limited plant diet (Mattson, 1980; Elser & Scriber, 1983; Capinera et al., 2006). Al-Zubaidi & Capinera (1983) observed that adult female S. exigua lay more eggs as the nitrogen fertilization of their larval host plant, sugarbeet, increases. Spodoptera exigua grow to smaller pupal size and have 15% fewer eggs when reared on cotton, Gossypium hirsutum, than on pigweed, Amaranthus retroflexus, which contains almost twice the amount of free amino acids (Ali & Gaylor, 1992; Greenberg et al., 2001; Showler, 2001). Interestingly, early-instar S. exigua caterpillars show no preference between the two host plants, whereas third-instar caterpillars select pigweed strongly over cotton (Showler, 2001), suggesting that the behavioural self-selection exhibited in the present study improves as the caterpillars become larger and more mobile. Protein limitation thus appears to be a significant constraint on beet armyworm in the field, and fitness consequences can include reduction in pupal mass as well as the increased mortality and decreased development rate. Cannibalism, which is rampant in S. exigua, has been proposed as a possible means for caterpillar herbivores on low quality plants to obtain much needed protein (Mattson, 1980; Al-Zubaidi & Capinera, 1983; Simpson et al., 2006). Obviously, plants contain multiple nutrients, all of which contribute to overall caterpillar performance (Simpson et al., 2004). From the present study, it is evident that P:C nutrient intake is critical for S. exigua survival, development and pupal biomass and lipid reserves. These caterpillars appear to have multiple behavioural and metabolic strategies to cope with nutritionally unbalanced diets, which contribute to their effectiveness as generalist herbivores and, as a result, as agricultural pests.

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