How well do specialist feeders regulate nutrient intake? Evidence from a gregarious tree-feeding caterpillar

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Summary

Nutritional regulation is a powerful mechanism used by generalist feeders to obtain the balance of nutrients they require from nutritionally diverse, perhaps unbalanced, foods. We examined nutritional regulation in a species with a narrow individual diet breadth: the forest tent caterpillar, *Malacosoma disstria*. Fourth instar caterpillars were provided with artificial foods consisting of different ratios of protein to digestible carbohydrate in no-choice, choice and compensatory feeding experiments. In the no-choice test, caterpillars were confined to a single food source of varying protein/carbohydrate ratio for the duration of the fourth larval stadium. Caterpillars performed best on equal-ratio and slightly protein-biased diets. Significant reductions in performance were only observed on extremely protein- or carbohydrate-biased diets. Daily consumption of the three acceptable intermediate diets was consistent with volumetric regulation, but the timing of the moult to the next instar appeared linked instead to protein intake. In the choice test, caterpillars were provided with two complementary foods, one biased toward protein and the other toward carbohydrate, for the duration of the stadium. The caterpillars fed randomly from the two food sources presented to them, except for the extremely protein-biased diet (P:C ratio of 35:7), which they avoided. The compensatory feeding experiment tested whether forest tent caterpillars deprived of either protein or digestible carbohydrate would select a food containing the deficient nutrient. Insects were conditioned on either protein-only, carbohydrate-only, protein-and-carbohydrate or no-nutrient foods, then offered a choice between protein-only and carbohydrate-only foods. Unlike previously studied generalist feeders, our caterpillars did not compensate for protein deficiency and showed only very weak evidence of compensation for carbohydrate deficiency. Forest tent caterpillars are colonial trail-laying forest folivores that are generally confined to a single host plant and hence do not experience much diversity in food nutrient ratios. We show that forest tent caterpillars do not independently regulate protein and carbohydrate intake. These findings are consistent with predictions that nutritional regulation abilities should be less important in animals with narrower diet breadths.

Key words: *Malacosoma disstria*, Lepidoptera, protein, carbohydrate, compensatory feeding, nutrition, feeding behaviour, dietary self-selection, diet breadth.

Introduction

Many animals, including insects, spiders, fish, hens, rats and even humans, are able to select a balanced nutrient intake from multiple food sources with different nutrient concentrations (Berthoud and Seeley, 1999; Mayntz et al., 2005; Simpson et al., 2003; Simpson et al., 1995). Among insects, nutritional regulation has been examined in depth in the locust (Simpson and Raubenheimer, 2000) and has also been demonstrated in several larval lepidopterans (Lee et al., 2002; Stockhoff, 1993; Telang et al., 2003; Thompson et al., 2001). The Geometric Framework is a conceptual tool (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993) used to investigate the regulation of multiple nutrients. Most research has focussed on the two macro-nutrients most important to herbivorous insects, protein and digestible carbohydrate. The nutrient ratio that promotes optimal growth and development can be identified by feeding insects on a range of diets with different relative concentrations of protein and carbohydrate. One can then determine whether insects self-select this ratio by allowing them to choose between two complementary food sources – in effect testing whether they consistently consume the same amount of nutrients and thus defend an intake target (sensu Simpson and Raubenheimer, 1993) in the face of diet variability. A powerful mechanism for defending an intake target is independent regulation of the nutrients involved (Simpson et al., 1995). If the intake of a given nutrient is regulated independently of other food constituents, an insect conditioned on food deficient in that nutrient will compensate

by selecting a complementary food containing the missing nutrient.

Sophisticated physiological and behavioural mechanisms for defending protein and carbohydrate intake targets, including independent regulation of these nutrients, have been shown in several insects, including *Manduca sexta* L. (Lepidoptera) (Thompson and Redak, 2000), *Spodoptera littoralis* Boisduval (Lepidoptera) (Simpson et al., 1988), *Heliothis zea* Boddie (Lepidoptera) (Friedman et al., 1991; Schiff et al., 1989), *Locusta migratoria* L. (Orthoptera) (Simpson et al., 1988) and *Schistocerca gregaria* Forskål (Orthoptera) (Raubenheimer and Simpson, 2003). However, caterpillars of the polyphagous arctiid *Grammia geneura* Strecker do not compensate for protein deficiencies (Bernays et al., 2004). Instead, long-term dietary regulation seems to occur indirectly via switching between host plants based on their secondary chemistry (Singer et al., 2002). Nutritional regulation abilities thus vary among caterpillars. These differences are predicted to depend on diet breadth and feeding ecology (Raubenheimer and Simpson, 2003; Simpson and Raubenheimer, 2001).

We examined nutritional regulation in the forest tent caterpillar, *Malacosoma disstria* Hübner. This caterpillar is a gregarious outbreaking forest pest. In Canada, its main host plants are trembling aspen (*Populus tremuloides* Michx.) and sugar maple (*Acer saccharum* Marsh). It is an early-season feeder whose life cycle is synchronized with host phenology: larvae emerge at the same time as the growth flush of their host, when leaves are high in nutrients (Fitzgerald, 1995). Caterpillars live in colonies and use pheromone trails to travel together between food sources and temporary bivouacs (Fitzgerald, 1995). During outbreaks, defoliation by the forest tent caterpillar is not random. Specific trees and parts of trees are attacked more often than others, which may at least in part be due to variation in nutritional value of foliage (Lévesque et al., 2002). For instance, in sugar maple, sun leaves have higher concentrations of both nitrogen and digestible sugars than do shade leaves (Lévesque et al., 2002). Forest tent caterpillars have been shown both to prefer sun leaf extracts over shade leaf extracts (Panzuto et al., 2001), and to grow and develop better on sun leaves even under controlled conditions (Fortin, 1994; Lévesque et al., 2002). While differences in allelochemistry are clearly involved in forest tent caterpillar food choice and performance (Hemming and Lindroth, 1995; Hemming and Lindroth, 2000), it is probable that differences in nutrient content also contribute to this preference for sun leaves (Panzuto et al., 2001).

We conducted three experiments to find out the optimal protein to carbohydrate ratio for forest tent caterpillars and to determine whether caterpillars regulate nutrient intake to this ratio. First, in a no-choice experiment caterpillars were reared on a single artificial food of known protein and carbohydrate content to determine the effects of nutrient ratios on food consumption and biological performance. Second, in a choice experiment caterpillars were given a choice of two food sources, differing in protein and carbohydrate content, to test whether

they selected a diet corresponding to the optimal nutrient ratio as defined by the first experiment. Third, a compensation experiment tested whether caterpillars deprived of either protein or carbohydrate compensated for these deficiencies by selecting the missing nutrient when given a choice between protein-only and carbohydrate-only foods. Artificial foods were used because they enabled us to chemically manipulate and standardize nutrient content. Furthermore, artificial foods deliberately exclude allelochemicals to focus on the dynamics of the essential nutrients.

Materials and methods
Experimental insects

Forest tent caterpillars from egg masses from the Canadian Forest Service Great Lakes Forestry Centre (Sault-Ste-Marie, Ontario) were reared on a standard wheat germ-based meridic artificial diet (Addy, 1969; Grisdale, 1985) under a controlled light and temperature regime: 18 h:6 h light:dark cycle and 22°C. Experimental insects were chosen randomly and removed from the culture within 24 h of moulting to the fourth instar. Before entering an experiment, caterpillars were deprived of food for 2 h to ensure all undigested material was expelled, and were weighed to within 0.1 mg. A 2-h period of food deprivation is within the normal inter-meal duration (Peters and Despland, 2006).

Artificial foods

The chemically defined foods used in the experiments were based on the Simpson and Abisgold diet (Simpson and Abisgold, 1985) and were used in previous work on *S. littoralis* (Simmonds et al., 1992; Simpson et al., 1988). Foods varied in their protein and carbohydrate content. The protein content of the food consisted of a 3:1:1 ratio of casein, peptone and albumen, and the carbohydrate portion was made up of a 1:1 ratio of sucrose and dextrin. 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 mixture. Cellulose, a non-nutritive bulk agent, was added to fill the remaining part of the food. The foods were presented to the insects in a 1% agar solution in a 6:1 agar solution:dry ingredients ratio. The diet therefore contained about 85% water. By comparison, aspen leaves are ~90% water and contain ~12–24% protein and 8–20% digestible carbohydrate by dry mass (Hemming and Lindroth, 1999; Lorenzetti, 1993; Miller, 1987). All experimental foods were novel to the insects.

Experiment 1: no-choice rearing

Caterpillars were reared for the duration of the fourth larval stadium on one of the following foods: 7:35, 14:28, 21:21, 28:14 and 35:7, where the first number indicates the percentage protein in the food (by dry mass) and the second number is the percentage digestible carbohydrate. For each treatment, 20–30 insects were placed individually in 10 cm diameter Petri dishes with a 0.3×1×1 cm block of one of the four foods and a 2×2 cm square of paper towel moistened with 2 ml of distilled

water to prevent dehydration. Petri dishes were sealed with Parafilm. Dishes were randomly arranged in a growth chamber at 22°C and 70% humidity in a 18 h:6 h light:dark cycle. Additional pieces of each food were set up in Petri dishes without insects and dried at the end of each experiment to establish a wet-to-dry mass regression. The food was replaced every 4 days to prevent excess drying out. When food was removed, it was labelled and placed in a drying oven for 48 h at 30°C. The initial dry mass of food pieces was estimated from the wet:dry mass regression and compared to the final dry mass to determine the amount consumed by each insect. Caterpillars were removed from the experiment at the moult to the fifth instar. Stadium duration, mass gain and food consumption over the stadium were recorded for each individual to evaluate performance.

Experiment 2: rearing on a choice of two foods

Insects were reared for the duration of the fourth larval stadium as above, except that each insect was given a choice between two food sources, one biased toward carbohydrate and the other biased toward protein. The treatment combinations were: 21:21 and 14:28, 21:21 and 28:14, 14:14 and 14:28, 14:14 and 28:14, 14:28 and 28:14, 14:28 and 35:7, 7:35 and 28:14, 7:35 and 35:7 (protein:carbohydrate). For each of the eight treatments, 20–30 insects were used. Consumption of the two food types was recorded independently. Performance was evaluated, as above, at the end of the experiment.

Experiment 3: compensatory feeding

Individual insects, 25 per treatment, were fed one of four conditioning diets (PC=21:21, P=21:0, C=0:21 or O=0:0) for a conditioning period of either 16 or 48 h. Long conditioning periods were used because of the long inter-meal interval in this species; preliminary trials with 4–12 h conditioning periods showed no evidence of compensatory feeding. Following the conditioning period, insects were deprived of food for 2 h and weighed to determine the mass change during the conditioning period.

Individuals were then placed in the centre of fresh Petri dishes, with moistened paper towel and a piece of each of the P and C foods. The two cubes of food were located on opposite sides of the Petri dish on the outside edge where the insects travel (alternating sides for the P and C foods). The dishes were placed in the growth chamber for the P/C choice period which lasted 24 h. At the end of the choice period, insects were removed, food deprived for 2 h and weighed. Food consumption during both the conditioning and choice periods was determined from a wet-to-dry mass regression as above.

In a second set of trials, additional insects were conditioned for 48 h on the four different diets and then observed on either the P or C food, in order to assess short-term taste responses to the two food types (12 insects per treatment: a total of 96). Caterpillars were observed for 2 h; their behaviour was recorded every 50 s using a hand-held computer and Noldus Observer 5.0 software (Noldus Inc., Wageningen, The Netherlands), noting whether the insect was quiescent, active or feeding. Most of the caterpillars began feeding immediately upon first contact with the food, but the duration of this first feeding bout was very variable (from a few seconds up to 25 min). Meal duration is a good measure of a food's phagostimulatory power because it is little affected by post-ingestive effects (Simpson and Raubenheimer, 2000). The duration of the first meal was compared between the two treatments and four conditioning diets.

Results

Experiment 1: no-choice rearing

Survival differed significantly between treatments (χ^2_4=11.5; *P*=0.02): survivorship was highest on the 28:14 diet, intermediate on the carbohydrate-biased diets, and lowest on the extreme protein-biased 35:7 diet (Fig. 2B). In general, survival was low because of infections with nuclear polyhedrosis virus. This virulent pathogen is common in this species (Kukan and Myers, 1997).

Development time differed significantly between the different diets (survival analysis: Wilcoxon *U*=28; d.f.=4; *P*<0.0001). Insects reached the fifth instar in 7–9 days on the equal-ratio and protein-biased diets, but took over 10 days to complete the fourth stadium on the two carbohydrate-biased diets (see Fig. 2A).

Bicoordinate intake arrays suggest that the daily food consumption rate on the three intermediate diets followed the equal distance rule (sensu Raubenheimer and Simpson, 1999), which predicts a slope of –1 for this array of carbohydrate *versus* protein intake points. Linear regression on these three points gave a slope of –1.04 (R^2=0.98; see Fig. 1A). This implies that daily nutrient consumption (and in this experiment daily food consumption, since the total nutrient content of all foods was identical) was constant between these three treatments. On the extreme protein-biased and carbohydrate-biased diets, consumption rate was lower (see Fig. 1A, Fig. 2C). Analysis of covariance using initial mass as a covariate confirmed that consumption rate differed significantly between treatments ($F_{4,108}$=2.5; *P*=0.04): see Fig. 2C for results of *post-hoc* tests.

Differences in development time combined with those in consumption rate meant that, when measured over the entire stadium, food intake no longer followed the equal distance rule, but was higher than expected on the carbohydrate-biased 14:28 diet (Fig. 1B, Fig. 2D). Total intake was lowest on the 35:7 and 7:35 diets (ANCOVA: $F_{4,107}$=6.83; *P*<0.001).

Diet also affected growth rate ($F_{4,108}$=5.6; *P*<0.0001): insects grew more per day on the 28:14 and 21:21 foods than on the others (Fig. 2E). Because of differences in development time, total growth was highest on the 28:14, 21:21 and 14:28 diets, and lowest on the 35:7 diet (ANCOVA: $F_{4,107}$=6.45; *P*<0.001; Fig. 2F).

Experiment 2: rearing with a choice of two foods

Paired *t*-tests showed that insects fed equally from the two food sources provided to them in all treatments except for the
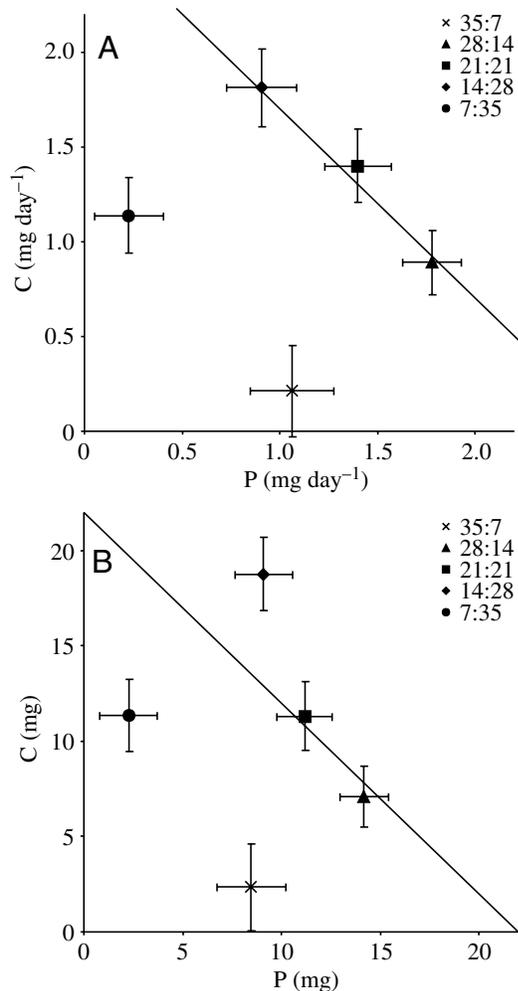
Fig. 1. Experiment 1. Bivariate means (± s.e.m.) of protein (P) and carbohydrate (C) consumption by insects reared on different diets. (A) Consumption rate per day, (B) total consumption over the entire larval stadium. The lines indicate the expectation under the equal distance rule: a straight line of slope –1. Points on this line indicate that insects on these diets consumed the same total amount of nutrients (and hence total amount of food) during the time period in question.

Table 1. *Experiment 2. Paired sample t-tests showing difference in consumption between the two foods presented together in each treatment*

Treatment	t	d.f.	P
21:21 and 28:14	0.74	12	0.47
21:21 and 14:28	−0.76	20	0.45
14:14 and 28:14	−1.51	15	0.15
14:14 and 14:28	−0.38	18	0.71
14:28 and 28:14	−0.01	15	0.99
14:28 and 35:7	5.64	21	*<0.001*
7:35 and 28:14	−0.4	9	0.7
7:35 and 35:7	4.04	7	*0.05*

Significant values (in italics) indicate that the caterpillars did not feed randomly from the two foods presented to them; non-significant values indicate that caterpillars fed randomly from the two foods presented to them.

7:35 and 35:7, and 14:28 and 35:7 treatments (see Fig. 3 and Table 1). In these two cases, the caterpillars ate less of the 35:7 food than of the alternate food. Total intake of protein and of carbohydrate varied between treatments (MANCOVA using initial mass as a covariate: $F_{7,135}=6.48$; $P<0.0001$ and $F_{7,135}=3.95$; $P<0.0001$ for protein and carbohydrate intake, respectively). This indicates that caterpillars did not regulate consumption of either nutrient to a fixed intake target. Thus, caterpillars did not reliably select the same nutrient ratio. Instead, they fed randomly from both foods except for the extremely protein-biased food (35:7), which they avoided (Fig. 3).

Survival analysis showed significant differences in development time between the different diets (Wilcoxon $U=21$; d.f.=7; $P=0.007$): insects on the combination of two extremely biased foods (7:35 and 35:7) developed slower than those on all other treatments. Growth rate also differed significantly between treatments ($F_{7,146}=3.33$; $P=0.002$). Again, values were similar between treatments, except for the combination of two extremely biased foods (7:35 and 35:7), in which growth rate was considerably reduced. Survivorship was lower on the combination of two extreme foods (7:35 and 35:7) than in all the other treatments ($\chi^2_7=37.3$; $P<0.0001$). Thus, random feeding on the two foods available led to different ingestions of protein and carbohydrate but not to differences in performance, except on the combination of two extremely biased foods (35:7 and 7:35), where overall consumption was lowest and intake was most biased toward carbohydrates.

Experiment 3: compensatory feeding

Two-way factorial analysis of covariance using initial mass as a covariate showed that conditioning diet significantly affected mass gain ($F_{3,192}=4.48$, $P=0.005$) and food consumption ($F_{3,192}=25.98$, $P<0.0001$) during the conditioning period (see Fig. 4A,B). Dunnett's *post-hoc* test using the PC diet as a control showed that insects conditioned on the P, C and O diets, both ate and grew less than those on the PC food. The longer conditioning period also increased mass gain ($F_{1,192}=95.9$, $P<0.0001$) and food consumption ($F_{1,192}=77.99$, $P<0.0001$). The interaction terms were also significant (mass gain: $F_{3,192}=4.01$, $P=0.008$; food consumption: $F_{3,192}=15.19$, $P<0.0001$): the increase in growth and consumption during the longer conditioning period was greatest for the PC diet and least for the O diet (Fig. 4A,B).

When given a choice between protein and carbohydrate foods, the insects conditioned on the C, PC and O foods did not deviate significantly from the null expectation of equal consumption of both foods, irrespective of conditioning period (see Fig. 5A,B). Insects conditioned on the P food for either 16 or 48 h appeared to show a slight preference for the carbohydrate food, but in neither case was this statistically
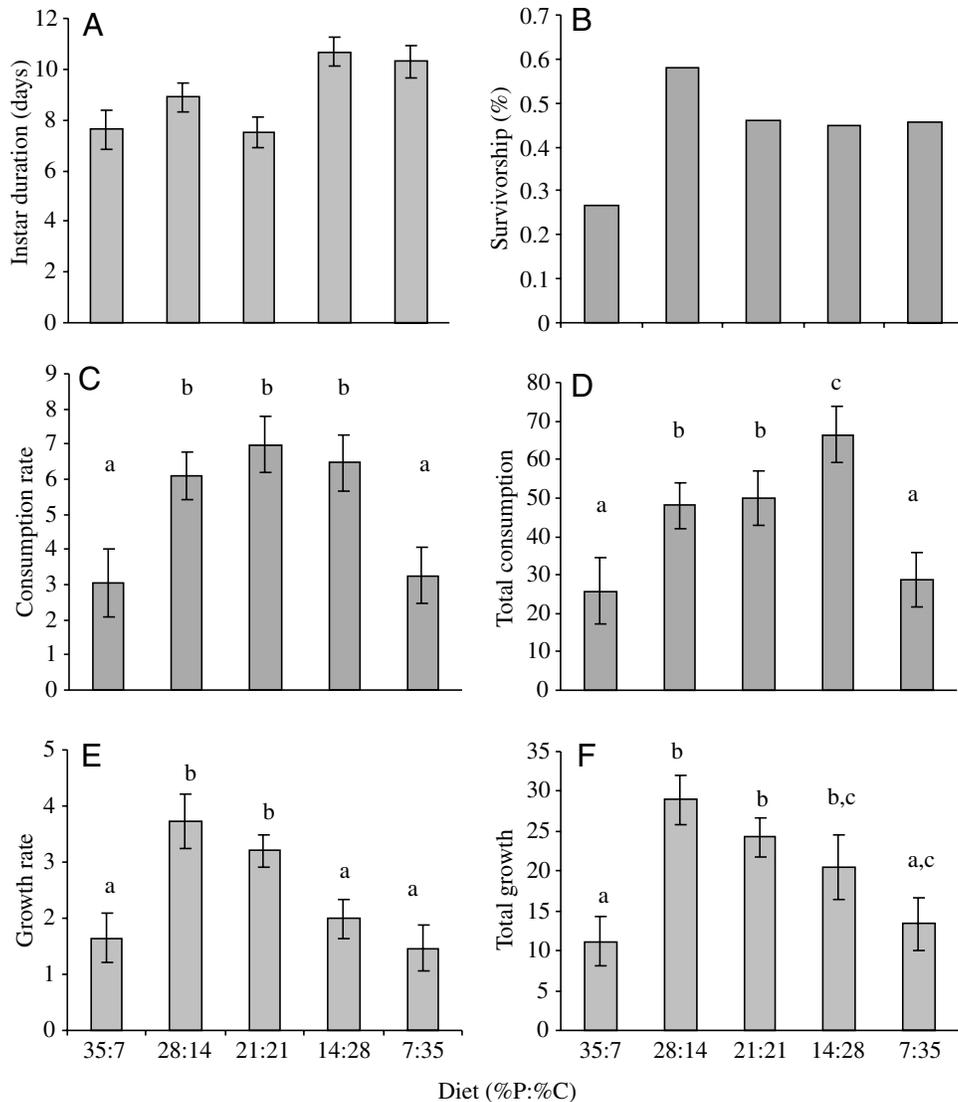
Fig. 2. Experiment 1. Performance on the different diets (P, protein; C, carbohydrate). (A) Mean stadium duration (days ± s.e.m.), (B) proportion survivorship, onsumption rate (mg dry mass day^{-1}), (D) total food consumption (mg dry mass), (E) growth rate (mg wet mass day^{-1}) and (F) total growth during the stadium (mg wet mass). Values in C-F are means ± one standard error. Different letters indicate differences significant at α=0.05 from Tukey *post-hoc* tests following analysis of variance (for variables on which ANOVA was performed).

significant (see Table 2). Total food eaten and mass gained during the choice period were not significantly affected by conditioning diet (two-way ANCOVA $F_{3,192}=0.13$, $P=0.94$ and $F_{3,192}=1$, $P=0.4$ respectively), by conditioning duration (two-way ANCOVA $F_{1,192}=3.26$, $P=0.07$; $F_{1,192}=0.61$, $P=0.44$) or by the interaction between them (two-way ANCOVA $F_{3,192}=0.77$, $P=0.51$; $F_{3,192}=1.26$, $P=0.29$).

Two-way analysis of variance on the behavioural data showed that the identity of the food during the observation period significantly affected the duration of the first meal (two-way ANOVA: $F_{1,88}=19.1$, $P<0.0001$). However, neither the effect of conditioning diet nor the interaction term were significant (two-way ANOVA: $F_{3,88}=0.16$, $P=0.92$ and $F_{3,88}=0.32$, $P=0.81$, respectively). Caterpillars took longer meals on the C food regardless of prior conditioning (Fig. 6).

Thus, although fourth-instar forest tent caterpillars given a choice between protein and carbohydrate foods exhibited an initial preference for carbohydrates (seen in the first 2 h) regardless of prior nutrient deprivation (Fig. 6), over the longer

Table 2. *Experiment 3. Paired sample t-tests of protein versus carbohydrate eaten during the choice period for each insect*

Conditioning time	Conditioning food	t_{11}	P
16 hours	P	–2.177	0.052
	C	–1.690	0.580
	PC	–0.990	0.627
	O	0.833	0.422
48 hours	P	–2.058	0.060
	C	–0.190	0.600
	PC	0.887	0.395
	O	0.980	0.348

P, protein; C, carbohydrate: P=21:0, C=0:21, PC=21:21, O=0:0; %protein:%carbohydrate.

Tests were done separately for each conditioning food/ conditioning period combination. Significant values indicate that the caterpillars did not feed randomly from the two foods presented to them. Non-significant values indicate that the caterpillars fed randomly from the two foods presented to them.

term (24 h), they did not compensate for a deficiency in protein and showed only very weak evidence of compensation for carbohydrate deficiency (Fig. 5A,B). These findings show that forest tent caterpillars do not independently regulate protein and carbohydrate intake.

Discussion

Nutrient ratios and performance

Forest tent caterpillars performed best on the 28:14 and 21:21 foods. Insects on the carbohydrate-biased 14:28 food showed the same consumption rate, but a lower growth rate and delayed development, such that they moulted later. Insects on the extremely carbohydrate-biased 7:35 diet showed a lower consumption rate, suggesting that this food is not recognized as adequate by the caterpillars. They also had a lower growth rate and delayed development, such that they too moulted later but at a smaller size. Insects on the extremely protein-biased 35:7 diet also showed a very low consumption rate and low growth rate, but rapid development, such that they moulted at the same time as the best performing insects, but at a much reduced size (see Fig. 2). The nutrient ratios of these two extreme diets are beyond those usually observed in the foliage of the preferred host plant, trembling aspen (Hemming and Lindroth, 1999; Lorenzetti, 1993; Miller, 1987).

Most other larval lepidopterans studied to-date exhibit a protein-biased intake target (Lee et al., 2002; Lee et al., 2003; Telang et al., 2001). Indeed, as the larval stage of holometabolous insects, caterpillars accumulate most or all of the resources required for adult reproduction and therefore require a higher protein to carbohydrate ratio than do hemimetabolous insects (Lee et al., 2002). Forest tent caterpillars do not feed at all as adults and hence protein acquired during the larval stage is critical to reproductive fitness.

The array of daily intake rates suggests that insects follow the equal distance rule (Raubenheimer and Simpson, 1999) and regulate daily food consumption volumetrically, except on extremely biased pathological diets where consumption is reduced (see Fig. 1A). The intake array for the whole stadium shows that consumption of a fixed amount of food does not determine when feeding ceases and the moult to the next instar occurs. Instead, Fig. 1B shows that the three adequate diets cluster on the P axis, because of a longer stadium duration on the carbohydrate-biased 14:28 diet. This suggests that protein intake plays a more important role in the timing of moulting than does carbohydrate intake.

Diet self-selection and nutrient regulation

Forest tent caterpillars presented with a choice between two food sources did not consistently feed to the same intake target. In similar choice experiments, *L. migratoria* regulated intake to a consistent nutrient ratio over a wide range of combinations of foods (Simpson and Raubenheimer, 2000). *S. littoralis* and *Spodoptera exempta* regulated intake from a choice of an equal-ratio and a protein-biased food, unless the equal-ratio food was too dilute. In this case, the caterpillars fed more on the protein-
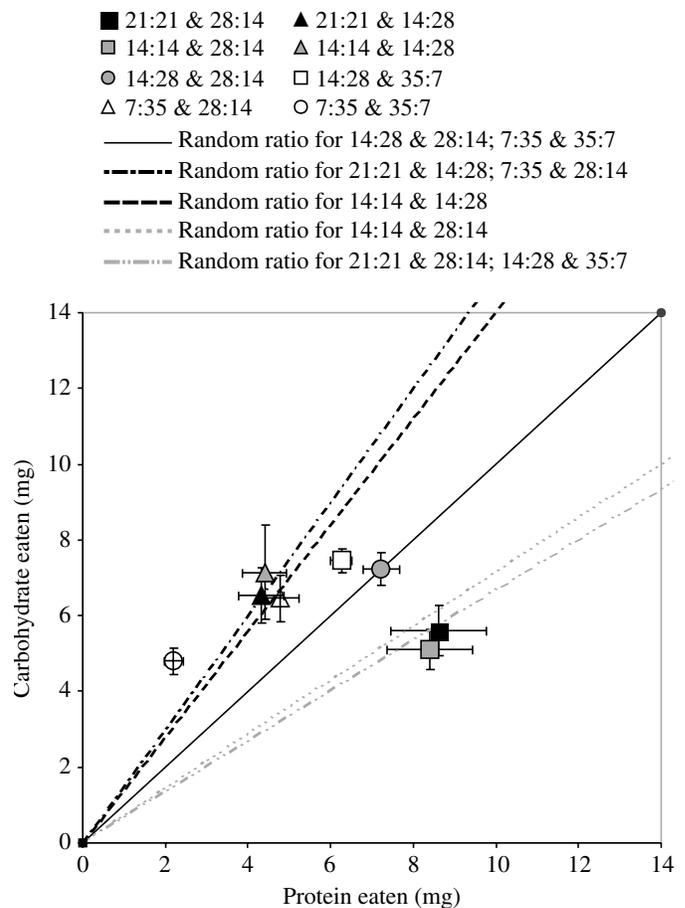

Fig. 3. Experiment 2. Bivariate means (± s.e.m.) of protein and carbohydrate consumption by insects on the different treatments. The lines show expected trajectories if the insects had eaten randomly from the two foods presented to them. The amounts eaten are not significantly different from these expected trajectories in all treatments except for treatments 14:28 and 35:7, and 7:35 and 35:7 (protein:carbohydrate).

biased food (Lee et al., 2002; Lee et al., 2003). When given a choice of foods, *H. virescens* larvae also adjusted their intake of protein and carbohydrate to meet their nutrient needs, unless the two foods were close to the intake target, in which case the caterpillars fed equally from the two sources (Telang et al., 2001). Nutrient regulation has also been demonstrated to varying degrees in *Heliothis zea* Boddie (Waldbauer et al., 1984) and *Lymantria dispar* L. (Stockhoff, 1993).

The behavioural capacity to self-select a diet corresponding to an intake target is thus variable among insects. In general, caterpillars seem to compensate less effectively for protein deficiency than they do for carbohydrate deficiency (Friedman et al., 1991; Simpson et al., 1990) and to regulate protein intake less tightly than do locusts (Bernays et al., 2004; Thompson, 2003). Forest tent caterpillars appear to be among the least adept compensators. Indeed, independent regulation for protein, as shown by compensatory feeding in response to deprivation, was not
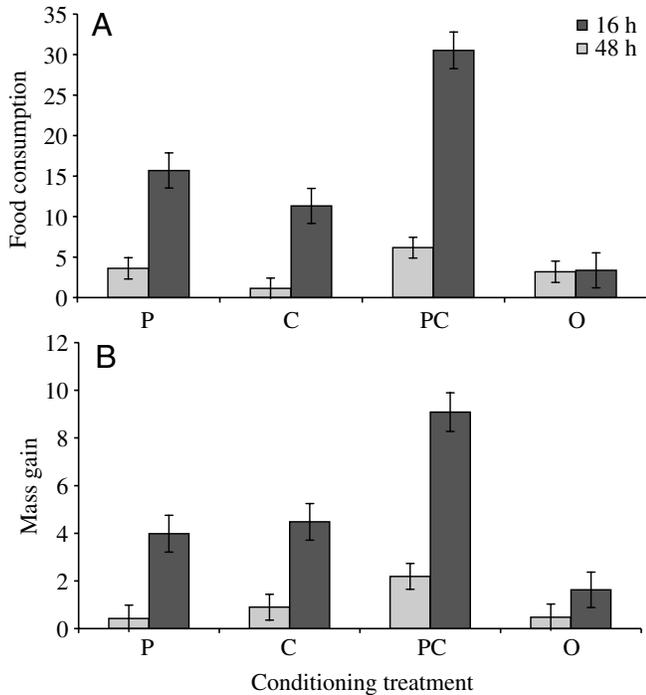
Fig. 4. Experiment 3. Caterpillar performance during the conditioning period. Estimated marginal means from the analysis of covariance (± s.e.m.) for (A) food consumption (mg dry mass), (B) growth (mg wet mass). PC=21:21, P=21:0, C=0:21, O=0:0; % protein:% carbohydrate.
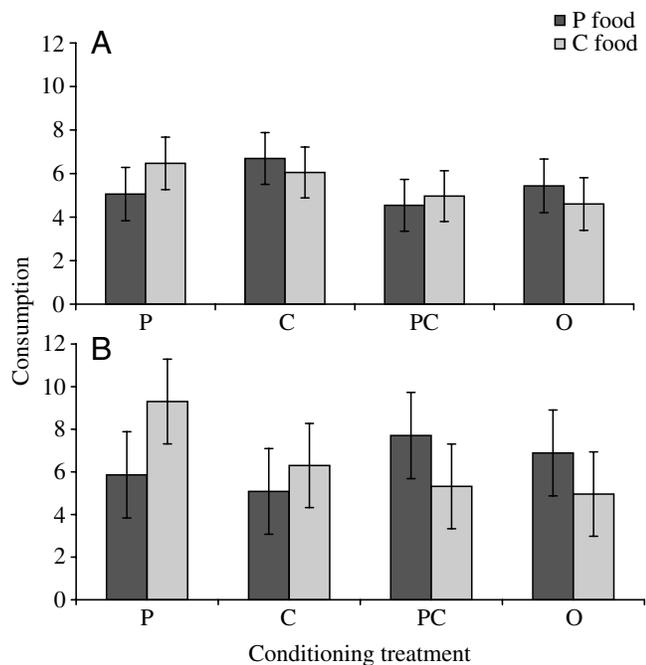

Fig. 5. Experiment 3. Estimated marginal means from the analysis of covariance for consumption of the two food types during the choice period (mg dry mass, ± s.e.m.). (A) Insects conditioned for 16 h; (B) insects conditioned for 48 h. The *x*-axis represents the conditioning diet (PC=21:21, P=21:0, C=0:21, O=0:0; % protein:% carbohydrate), and the different shading of the bars represent the two foods (PC=21:0, C=0:21; % protein:% carbohydrate) offered during the choice period.
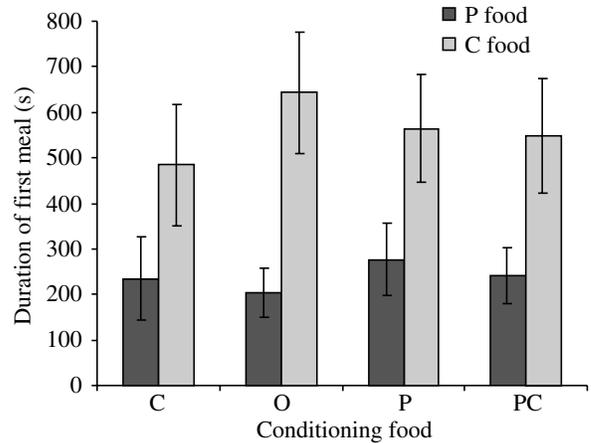

Fig. 6. Experiment 3. Mean duration of first meal (s; ± s.e.m.) of insects offered either protein only (21:0, P) or carbohydrate only (0:21, C) foods following conditioning on different diets. The *x*-axis shows the conditioning food (PC=21:21, P=21:0, C=0:21 or O=0:0), and the bars represent the food presented during the observation period.

observed in the present experiment. Compensation for carbohydrate deficiency was statistically non-significant and weak, making it difficult to reach a conclusion about regulation of carbohydrate intake. Fig. 1A (experiment 1) suggests that, on adequate diets, regulation of intake occurs simply in terms of amount of food consumed. On inadequate diets, caterpillars eat less and, presumably, spend more time searching for other food sources.

For nutritional regulation to occur, an animal needs first to be able to measure its internal nutritional state, and second to modulate its responses to food sources in accordance with that state. In caterpillars, the measure of internal state influencing food intake is the concentration of trehalose (the main storage sugar in insects) in the haemolymph (Thompson, 2003). In *H. zea*, haemolymph trehalose concentrations increased when caterpillars ate carbohydrate and decreased when they ate protein foods, leading the insects to switch between the two foods (Friedman et al., 1991; Schiff et al., 1989). Haemolymph trehalose concentration then modulated feeding behaviour via changes in gustatory responses to nutrient stimuli (Thompson, 2003).

In experiments on compensatory feeding, many caterpillars, including the forest tent caterpillar, show an initial preference for carbohydrate food, irrespective of prior nutritional deficiencies. In compensatory feeding experiments with *S. littoralis* (Simmonds et al., 1992; Simpson et al., 1988) and *Grammia geneura* (Bernays et al., 2004), the first feeding bout on the carbohydrate food was longer, irrespective of conditioning treatment. However, over a few hours, *S. littoralis* compensated for both protein and carbohydrate deficiencies (Simmonds et al., 1992; Simpson et al., 1988) whereas *G. geneura* compensated only for carbohydrate deficiency (Bernays et al., 2004). These differences in nutrient regulation can be explained by differences between these two species in the underlying sensory mechanisms: in *S. littoralis*, caterpillars

deficient in protein or carbohydrates exhibited increased gustatory sensitivity to amino acids or sucrose, respectively (Simmonds et al., 1992; Simpson et al., 1990). *G. geneura*, by contrast, exhibited changes in taste sensitivity to sugars but not to amino acids in response to nutrient deprivation (Bernays et al., 2004).

It is not known whether forest tent caterpillar electrophysiological responses change with nutritional state; however, Panzuto et al. (Panzuto et al., 2001) demonstrated that only the sugar-sensitive cell (and not the amino-acid-sensitive cell) responds differently to preferred and less-preferred leaf extracts, suggesting that the sugar cell is the main chemosensory neuron used in food selection by the forest tent caterpillar. In this case, forest tent caterpillars would not be expected to show increased taste responses to protein-rich foods following protein deprivation, as was indeed seen in our behavioural observations (experiment 3; Fig. 6).

However, this does not mean that forest tent caterpillars are unable to respond to the protein content in food. Indeed, food protein content can influence feeding via differences in post-ingestive processing, even if protein is not independently regulated via changes in haemolymph composition and gustatory sensitivity (Bernays et al., 2004). For instance, *G. geneura* shows post-ingestive effects of protein content in foods (Bernays et al., 2004; Singer et al., 2002), despite its lack of short-term compensatory feeding. Forest tent caterpillars also responded to protein in food sources: during the conditioning period of experiment 3, larvae fed the PC diet consumed substantially more than those fed the C diet (Fig. 4A). Similarly, in experiment 1, caterpillars ate less of the extremely protein-poor 7:35 food than of more balanced foods. A possible mechanism was described in *M. sexta*, where caterpillars fed equal-ratio protein:carbohydrate diets had lower haemolymph trehalose levels than those fed carbohydrate-biased diets and hence consumed more food (Thompson and Redak, 2000).

Regulation and nutritional ecology

Independent regulation of different nutrients is expected to be more important in mobile, polyphagous species encountering a wide variety of food sources varying in nutrient content than in animals with a more restricted diet (Scriber and Slansky, 1981; Simpson and Raubenheimer, 2001; Slansky and Scriber, 1985). Indeed, stronger compensation for dietary imbalances was observed in the generalist *S. littoralis* than in the specialist *S. exempta* (Lee et al., 2003; Lee et al., 2005). Similarly, generalist locusts tolerated, and compensated for, more extreme dietary imbalances that did a more specialist locust species (Raubenheimer and Simpson, 2003). Comparing specialist and generalist forms of the same locust or armyworm species showed the same trend of more liberal regulation in the generalist feeder (Lee et al., 2005; Simpson et al., 2002).

Forest tent caterpillars are synchronized with their host phenology, and are usually confined to a single plant – individual caterpillars therefore experience a restricted diet. Although leaves may vary in protein to carbohydrate ratio according to age and position in the crown, protein and digestible carbohydrates tend to be correlated in host tree foliage (Lindroth, 1991) and are higher in the leaves preferred by forest tent caterpillars (Lévesque et al., 2002). Any effect of different nutrient ratios is hence confounded by differences in total nutrient content. The present study shows that the forest tent caterpillar does not regulate protein and carbohydrate intake independently. However, the initial taste preference for the carbohydrate food irrespective of conditioning (experiment 3; Fig. 6) and the low consumption of the 35:7 food in both no-choice and choice situations (experiments 1, 2; Figs 1A, 2C, 3) suggest the use of taste responses to sugars in food selection, consistent with previous electrophysiological (Panzuto et al., 2001) and behavioural (Lorenzetti, 1993; Panzuto et al., 2001) findings. Furthermore, the higher consumption rate of balanced than of extremely carbohydrate-biased foods (experiment 3, Fig. 4A; experiment 1, Fig. 1A, Fig. 2C) suggests post-ingestive effects favouring the consumption of foods containing protein. These simple mechanisms could be sufficient for forest tent caterpillars to attain their nutrient requirements within the range of food choices normally available.

Moreover, forest tent caterpillars are gregarious and, when foraging, selectively follow pheromone trails rather than move over bare ground. Fourth instar larvae tend to forage more independently than do younger larvae, but they nonetheless use pheromone trails when available (Colasurdo and Despland, 2005). In addition, they can reach very high local densities, particularly during outbreaks. In this context, excess choosiness might lead to a loss of feeding opportunities during scramble competition (Despland and Simpson, 2005).

Individual fourth-instar forest tent caterpillars have a narrow diet breadth and often experience high intraspecific competition. Moreover, they perform well on a range of protein to carbohydrate ratios (experiment 1; Fig. 2). In this context, independent regulation of protein and carbohydrate intake might not be advantageous (Simpson and Raubenheimer, 2000). Our findings are consistent with studies on forest tent caterpillar feeding behaviour on natural foods (Fortin, 1994; Lévesque et al., 2002; Lorenzetti, 1993; Panzuto et al., 2001) and with previous research on mechanisms of nutritional regulation in lepidopteran larvae (Bernays et al., 2004; Thompson, 2003). Our results also concur with previous studies on diet breadth and nutritional regulation in locusts (Simpson and Raubenheimer, 2000; Simpson et al., 2002) and armyworms (Lee et al., 2003; Lee et al., 2005). Our findings thus support the hypothesis that nutritional regulation mechanisms vary according to feeding ecology and that compensation for dietary imbalances is better developed in animals with a wider dietary breadth.

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