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Do food protein and carbohydrate content influence the pattern of feeding and the tendency to explore of forest tent caterpillars?

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Abstract

This study examines whether the ratio of protein to carbohydrate affects the timing of meals and the propensity to explore of forest tent caterpillars (*Malacosoma disstria*). The behavior of fourth instar caterpillars was observed on three semi-defined artificial diets varying in protein (p)–carbohydrate (c) ratio. These diets were (a) p14:c28, (b) p28:c14, and (c) p35:c7. The probability of initiating feeding at first contact with the food and the duration of the first feeding event did not vary across diets, suggesting not much difference in phagostimulatory power. There was also no difference in the total time spent eating, at rest and in motion between diets. However, the timing and duration of meals varied significantly; more short meals were observed on the carbohydrate-biased diet. The duration of pauses between meals also increased with food protein content. Furthermore, caterpillars on the carbohydrate-biased diet were more likely to leave the trail leading to the known food source and to discover a second food source, suggesting that protein deprivation promotes exploration. These findings shed insight into the physiological responses to protein and carbohydrate ingestion and demonstrate how post-ingestive effects can favor consumption of foods containing protein without invoking an explicit mechanism of independent nutrient regulation, but simply by influencing the pattern of feeding and the propensity to explore.

Keywords: *Malacosoma disstria*; Lepidoptera; Protein; Carbohydrate; Nutrition

1. Introduction

Forest tent caterpillars (*Malacosoma disstria*) are an oligophagous outbreaking forest pest native to North America; their primary host in Canada is trembling aspen (*Populus tremuloides*). They are social nomadic foragers that move en masse in search of food and aggregation sites. Foraging caterpillars deposit a trail of silk and pheromone and preferentially follow trails rather than advance over bare ground (Fitzgerald, 1995).

Forest tent caterpillars exhibit more growth, faster development and higher survivorship on diets with more protein than digestible carbohydrate (28% protein and 14% carbohydrate per dry weight). On a carbohydrate-biased diet (p14:c28, % protein and carbohydrate, respectively), food consumption rate is similar, but development is retarded. On an extremely protein-biased diet (p35:c7), consumption rate, growth rate and survival decrease (Despland and Noseworthy, 2006). However, unlike previously studied generalist caterpillars (Lee et al., 2002, 2006; Thompson and Redak, 2000; Simpson et al., 1988; Friedman et al., 1991), forest tent caterpillars do not defend a target of nutrient intake (Despland and Noseworthy, 2006). Instead, they feed equally on carbohydrate-biased to slightly protein-biased foods, but consume less of highly unbalanced foods (Despland and Noseworthy, 2006). To further explore the mechanisms by which nutrients influence food consumption, this study tests if food protein-to-carbohydrate ratio affects (1) the timing and duration of forest tent caterpillar meals, and (2) the propensity to leave a known food source and the trail leading to it, and to discover a novel food source.

Most of the temporal analysis of feeding behavior in insects has concentrated on *Locusta migratoria* (Simpson,

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1982, 1995), whose feeding occurs in bouts that are clearly separated by periods of resting on a perch away from the food (Simpson, 1982). In caterpillars, the pattern of feeding has been quantified in *Manduca sexta* (Reynolds et al., 1986; Bowdan, 1988a b; Bernays and Woods, 2000), *Helicoverpa armigera* (Raubenheimer and Barton Browne, 2000) and *Bombyx mori* (Nagata and Nagasawa, 2006).

Previous research with other caterpillars shows that the pattern of feeding (i.e., the frequency and duration of meals) varies across foods (Reynolds et al., 1986; Timmins et al., 1988; Bernays and Singer, 1998) and can provide information about the insect's physiological responses to those foods. For instance, the phagostimulatory power (i.e., taste) of a food is detected immediately on contact with that food and influences both the probability of initiating feeding and the duration of the meal (Simpson and Raubenheimer, 2000). Once the insect has begun feeding, post-ingestive feedback about the food's nutrient content becomes available and can influence the duration of the meal and future feeding. The duration of the interval until the next meal reflects the size and nutrient content of the previous meal (Simpson and Raubenheimer, 2000). These mechanisms can lead to different schedules of feeding on foods of different quality: for instance, *M. sexta* showed a compensatory increase in time spent eating when artificial diet was diluted (Timmins et al., 1988). On diets diluted with water, compensation occurred via an increase in meal duration, whereas on diets diluted with cellulose, both the frequency and duration of meals increased. *Spodoptera littoralis* took frequent small meals on diets lacking a major nutrient (either protein or carbohydrate) and longer ones on a diet containing both nutrients, resulting in higher consumption on the balanced diet (Simpson et al., 1988).

Forest tent caterpillars exposed to a diet completely lacking in carbohydrate have been shown not only to take shorter meals, but also to be more active than caterpillars eating a nutritionally adequate diet (Dussutour et al., 2007). Indeed, increased activity is a mechanism by which insects on an inadequate food source can increase the probability of discovering a better or complementary food (Barton Browne, 1993; Nagata and Nagasawa, 2006). In a gregarious species that uses trail-based foraging, discovering novel food sources would require not only increased activity but also increased propensity to leave the trail leading to the known source.

The present study further builds on this work to examine the role of varying food protein to carbohydrate ratio and tests the hypotheses that patterns of feeding and the propensity to leave the trail differ between nutritionally adequate and inadequate diets. Specifically, we hypothesize that meals and interfeed intervals will be longest and that the tendency to leave the trail to the known food source and to discover a new source will be lowest on the food that supports good growth (i.e., p28:c14), relative to two unbalanced foods (p35:c7 and p14:c28). Together, these mechanisms would tend to decrease consumption of unbalanced foods and promote ingestion of the appropriate nutrient ratio.

2. Materials and methods

2.1. Experimental insects and diets

Laboratory colonies of the forest tent caterpillar were reared from egg masses collected in March 2005, 40 km North East of Wabasca, Alberta. The egg bands were washed as per Grisdale (1985). They were maintained on a 16:8 h photoperiod in growth chambers.

During the first three instars the caterpillars were reared on a meridic artificial diet (Addy, 1969; Grisdale, 1985), containing approximately equal levels of protein and carbohydrate. Fourth instar caterpillars were subjected to one of three treatments. The treatments consisted of artificial diets, based on Addy's (1969) formulation, with different protein to carbohydrate dry weight concentrations: 14% protein: 28% carbohydrate, 28% protein: 14% carbohydrate, and 35% protein: 7% carbohydrate. Food nutrient content includes protein (casein) and sugar (dextrose) added to the diet, as well as that present in the wheat germ (see below). Other components of the artificial diets were salt (5.7%), cholesterol (1%), vitamins (8.2%), raw linseed oil (1.9%), sorbic acid (0.7%), methyl paraben (0.4%), choline chloride (0.6%), ascorbic acid (2.9%), sodium alginate (2.9%), and wheat germ (27% of total dry mass, contains 29% protein and 16% sugar). Cellulose was used as a filling agent for the remainder of the diet. The diets were a composite of 6:1 ratio of 3% agar solution:dry ingredients.

2.2. Feeding pattern

Individual mid-fourth instar caterpillars were each placed in one quarter of a Petri dish (14 cm) that was divided into four using cardboard separators. Each Petri dish contained two insects and two empty compartments used for the test of exploratory behavior. A piece of food was placed in the same compartment as the caterpillar. Humidity was kept constant, with room temperature at 22 °C. The experiments were filmed for 48 h at a rate of one image per second (N for p14:c28 = 17, N for p28:c14 = 19, N for p35:c7 = 17).

Eight-hour observations were conducted on all caterpillars to evaluate the latency between contact and feeding, and the duration of the first feeding event. Forty-eight hour observations were conducted for a random subset of caterpillars to compare the pattern of feeding between treatments (N for p14:c28 = 7, N for p28:c14 = 7, N for p35:c7 = 6).

Log-survivorship analyses were performed to calculate parameters describing the pattern of feeding from the raw data (Simpson, 1995; Sibly et al., 1990; Zorrilla et al., 2005). The first step is to determine the bout criterion used to classify non-feeding gaps as either intrafeed pauses (gaps shorter than the bout criterion) or interfeed intervals (gaps longer that the bout criterion). According to this two-process model, a plot of # gaps longer than duration *d* vs. *d* follows the sum of two exponentials. Log transformation of the *y*-axis gives a broken-stick plot and the bout criterion is given by the break in the stick. Linear regression was used to find the equations that best fit the two parts of the broken stick (Jeanson et al., 2003). Meal durations are then merged over intrafeed pauses (see Fig. 2 for a visual representation of definitions of terms). The next step is to use the same approach to check for a meal criterion used to classify feeding bouts as either meals or sampling events (Simpson, 1995) by examining the log-survivorship curve of feeding bout durations.

The data from the log-survivorship analysis was then used to compare meal duration, interfeed interval duration, and number of meals per hour between treatments. The proportion of the feeding period spent eating (rather than in intrafeed pauses) was also compared between diets, as was the proportion of the total time spent feeding, resting or moving. Each variable was tested for normality and then analyzed using Kruskal–Wallis tests for the non-parametric data, or one-way ANOVAs for the parametric data.

The effect of the previous interfeed interval on meal duration is represented by the pre-prandial regression, and the effect of the previous meal on interfeed interval duration is given by the post-prandial regression (Raubenheimer and Barton Browne, 2000). Analyses of co-variance were used to compare pre- and post-prandial regressions between the diets, using diet as a factor and duration of the previous interval (or meal) as a covariate. To determine whether the regression slopes for the treatments are homogeneous, a full-model ANCOVA examines the interaction term (treatment × preceding duration). If it is not significant, the slopes are homogeneous, and the ANCOVA model without the interaction term can be used to test for differences in intercepts, representing diet effects (Sokal and Rohlf, 1995).

2.3. Exploratory behavior

After the 48 h, the cardboard barrier separating the caterpillar from the empty quarter of the Petri dish was removed, and a piece of artificial diet (food 2: 21% protein and 21% carbohydrate per dry weight) was placed in the heretofore empty compartment. This food was chosen because it is nutritionally adequate, yet novel to all test insects. The caterpillars were filmed using one image per second for 24 h. This part of the experiment was done in order to determine how long it takes the caterpillars to leave the pre-established pheromone trail and move to the new source (food 2).

The rate at which insects contacted the second food source was compared between treatments using life table-based survival analysis with the Wilcoxon test for differences between groups. This test considers both the latency until the insects contact the food and the proportion of insects that do not reach the food within the 12 h trial period.

3. Results

3.1. Description of feeding pattern

The bout criterion was found to be approximately 16 min (see breaks for each treatment in Fig. 1). The log-survivorship analysis examining meals showed no evidence of a meal criterion (see Fig. 3): the one-process model fit the data well ($R^2 = 0.98$, 0.92, and 0.97 for the p14:c28, p28:c14, and p35:c7 diets, respectively) and the two-process model did not significantly improve the fit. Previous studies on caterpillar feeding patterns also showed a bout criterion but no meal criterion (Nagata and Nagasawa, 2006; Raubenheimer and Barton Browne, 2000; Reynolds et al., 1986). Hence, a meal is the sum of feeding events separated by intrafeed pauses shorter than 16 min. A feeding period is the total duration of the meal plus that of the intrafeed pauses. Feeding periods are separated from other feeding periods by interfeed intervals greater than 16 min (see Fig. 2 for a graphical representation of definitions).

3.2. Responses to food at first contact

The proportion of caterpillars that ate immediately upon first contacting the food did not differ between treatments ($\chi^2 = 3.824$, d.f. $= 2$, $p = 0.148$, see Table 1). Furthermore, there was no difference in the duration of the first feeding event between the different treatments (Kruskal–Wallis, $\chi^2 = 0.806$, d.f. $= 2$, $p = 0.668$). These findings suggest that, at the beginning of the experiment, there is no significant difference in phagostimulatory power between the three foods.

3.3. Meals and interfeed intervals

The proportion of time spent feeding within a feeding period (i.e., meal duration/feeding period duration) varied significantly across the treatments ($F_{2,354} = 3.45$, $p = 0.03$) and increased with increasing protein content (p14:c28: 0.60 ± 0.029 S.E.; p28:c14: 0.68 ± 0.031 S.E.; p35:c7: 0.72 ± 0.018 S.E.). The duration of meals was significantly different across treatments (Kruskal–Wallis, $\chi^2 = 27.021$, d.f. $= 2$, $p < 0.001$) and increased on more protein-rich foods (Fig. 3). On p14:c28, there were many very short (<5 min) meals and most meals lasted under 10 min. The number of very short meals decreased with increasing food protein content, and there were also more long meals (10–20 min) on p28:c14 and p35:c7. The duration of interfeed intervals also increased on more protein-rich diets (Kruskal–Wallis, $\chi^2 = 17.605$, d.f. $= 2$, $p < 0.001$, Fig. 4a). Consistent with this, a one-way ANOVA comparing the number of feeding periods across treatments approached significance ($F_{2,17} = 3.363$, $p = 0.059$), showing

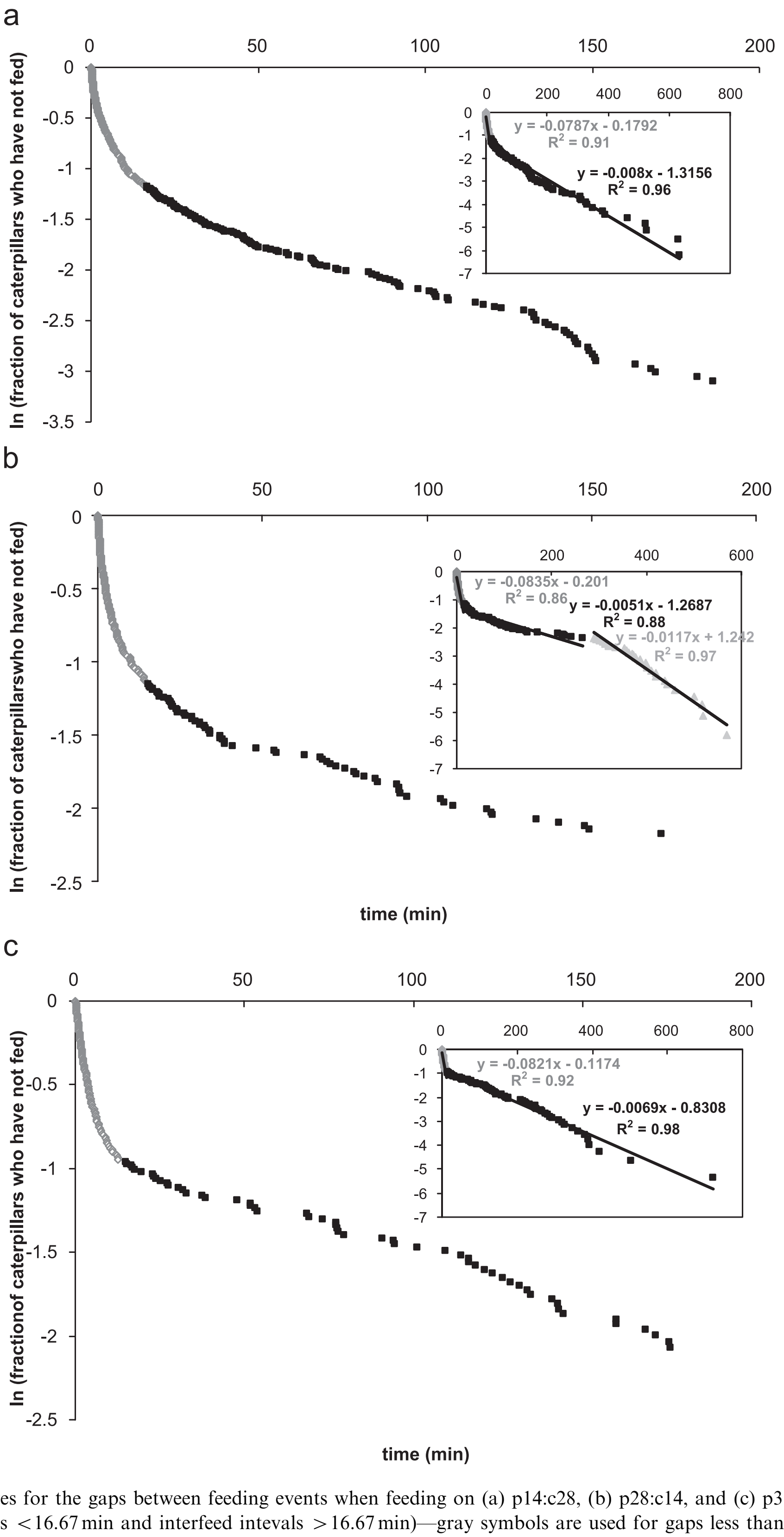

Fig. 1. Log-survivorship curves for the gaps between feeding events when feeding on (a) p14:c28, (b) p28:c14, and (c) p35:c7. Two types of gaps were distinguished (intrafeed pauses <16.67 min and interfeed intevals >16.67 min)—gray symbols are used for gaps less than the bout criterion and black symbols represent gaps longer than the bout criterion. The probability of the caterpillars to start feeding during the first 16.67 min were (a) 7.87×10^{-2}/min $\pm3.0\times10^{-3}$ C.I.$_{95}$ ($R^2=0.91$, $F_{1,351}=3612.70$, $p<0.001$); (b) 8.35×10^{-2}/min$\pm4.5\times10^{-3}$ C.I.$_{95}$ ($R^2=0.862$, $F_{1,228}=1424.89$, $p<0.001$); and (c) 8.21×10^{-2}/min$\pm3.9\times10^{-3}$ C.I.$_{95}$ ($R^2=0.92$, $F_{1,131}=1508.84$, $p<0.001$). These probabilities decrease after 16.67 min: (a) 8.0×10^{-3}/min$\pm2.5\times10^{-4}$ C.I.$_{95}$ ($R^2=0.96$, $F_{1,156}=4070.88$, $p<0.001$); (b) 5.1×10^{-3}/min$\pm9.0\times10^{-4}$ C.I.$_{95}$ ($R^2=0.88$, $F_{1,73}=548.62$, $p<0.001$); and (c) 6.9×10^{-3}/min $\pm1.0\times10^{-4}$ C.I.$_{95}$ ($R^2=0.98$, $F_{1,81}=4285.20$, $p<0.001$). After 200 min the probability to start feeding increases again for (b) 1.17×10^{-2}/min $\pm1.3\times10^{-3}$ C.I.$_{95}$ ($R^2=0.97$, $F_{1,30}=838.21$, $p<0.001$).

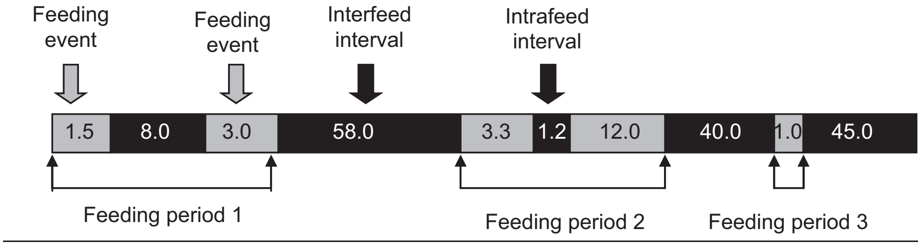

Fig. 2. An example to show how pattern of feeding was described. The numbers indicate the duration of the event in minutes. A meal is the sum of feeding events that are separated by intervals less than 16.67 min. For example, the duration of the first meal would be 1.5 + 3.0 min. A feeding period is the sum of feeding events and the intrafeed pauses. The first feeding period duration would be 1.5 + 8.0 + 3.0 min.

Table 1
The number of caterpillars that contacted and that ate the food source provided to them within 48 h, as well as the number that commenced feeding upon first contact with the food

Treatment	# Caterpillars that contacted food	# Caterpillars that ate food	# Caterpillars that ate at first contact
p14:c28	17	11	6
p28:c14	19	16	3
p35:c7	17	16	5

a trend toward slower alternation between meals and interfeed intervals on the more protein-rich diets (Fig. 4b).

Pre-prandial regressions demonstrated that the slope of the relationship between meal duration and that of the preceding interfeed interval did not differ across treatments ($F_{2,327} = 2.197$, $p = 0.113$); however the intercept did ($F_{3,326} = 15.846$, $p < 0.001$). The estimated marginal means (EMM), which correct for the effects of the covariate, demonstrated that the caterpillars took longer meals relative to the duration of the preceding interfeed interval with increasing food protein content (EMM—p14:c28: 312.4±35.3 s; p28:c14: 420.5±45.4 s; p35:c7: 515.4±49.5 s). Post-prandial regressions demonstrated that the slope of the relationship between the duration of an interfeed interval and that of the preceding meal differed significantly between treatments ($F_{2,334} = 9.6$, $p < 0.001$). Regression equations were: 6.3 (±1.17) × meal duration + 4623 (±606) for p14:c28; 11.9 (±1.62) × meal duration + 5288 (±1014) for p28:c14; and 12.5 (±1.76) × meal duration + 3552 (±1117) for p35:c7. Thus, the duration of the interfeed interval increased faster with the length of the preceding meal when the caterpillars were on a more protein-rich diet.

There were no significant differences in the total time spent eating, resting and in motion between the artificial diets (one-way ANOVA, eating: $F_{2,21} = 1.739$, $p = 0.200$; resting: $F_{2,20} = 1.150$, $p = 0.336$; in motion: $F_{2,21} = 1.448$, $p = 0.258$).

3.4. *Exploratory behavior*

Exploratory behavior involves leaving the pheromone trail that leads to the known food source and covering the new territory where food 2 is located. The rate at which caterpillars contact the second food source was highest for those individuals that were initially on the p14:c28 diet, and did not differ significantly between the p28:c14 and p35:c7 treatments (Wilcoxon statistic with 1 d.f., p14:c28 vs. p28:c14: 6.23 ($p = 0.013$); p14:c28 vs. p35:c7: 5.30 ($p = 0.021$); p28:c14 vs. p35:c7: 0.12 ($p = 0.73$)). Overall, 81% of insects treated on the p14:c28 diet contacted food 2, compared with 53% from the p28:c14, and 43% from the p35:c7 treatments (see Fig. 5). No evidence of selective feeding on the second, nutritionally adequate food source, was observed confirming previous work showing that forest tent caterpillars are not very adept at regulation of nutrient intake (Despland and Noseworthy, 2006; Noseworthy and Despland, 2006).

4. Discussion

This study demonstrated that the range of protein and carbohydrate concentrations tested did not influence the caterpillars' probability to initiate feeding upon contact with the food or the duration of the first feeding event, suggesting no, or only minor, differences in phagostimulatory responses of naïve insects to the foods (Simpson and Raubenheimer, 2000). In addition, the overall total time spent eating in this experiment was the same on all diets. However, the temporal pattern of food ingestion varied significantly; the shorter meals and interfeed intervals, and increased exploration expected on the two unbalanced foods (p35:c7 and p14:c28) were only observed on the carbohydrate-biased food (p14:c28). Indeed, there were more short meals on the carbohydrate-biased food. The duration of interfeed intervals increased with increasing protein content of the food, such that the frequency of feeding periods decreased and the overall rhythm of

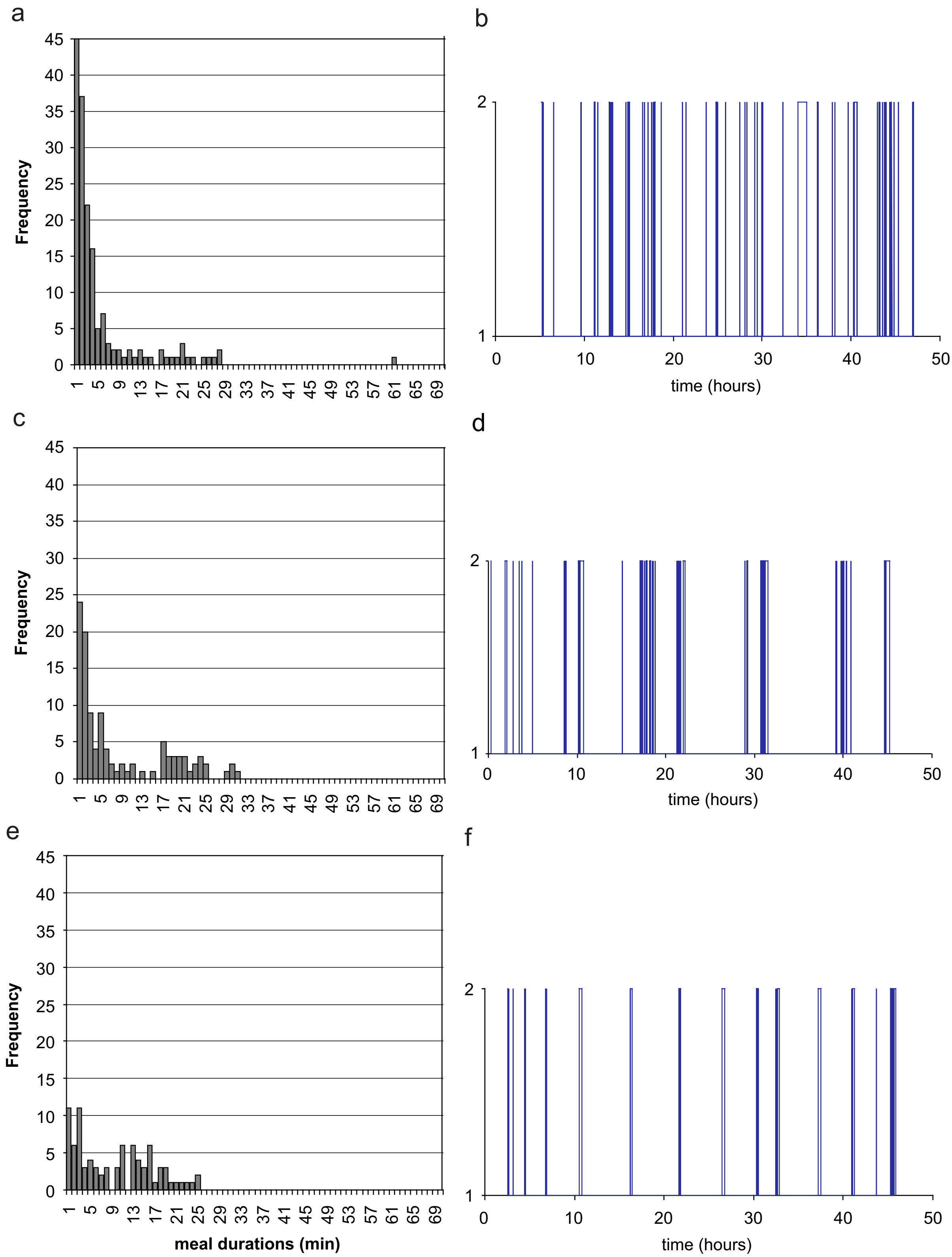

Fig. 3. Left panel: frequency distributions for the duration of meals (in minute) and right panel: raw data for one representative insect. In the right panel, the vertical bars represent feeding events. From top to bottom: (a) and (b) p14:c28; (c) and (d) p28:c14; (e) and (f) p35:c7.

alternation between feeding and food processing slowed down. Pre- and post-prandial regressions indicated that longer meals on protein-rich foods are not fully explained by the longer interval since the previous meal, and longer interfeed intervals on protein-rich diets are not entirely due to the larger size of the preceding meal. Instead, these

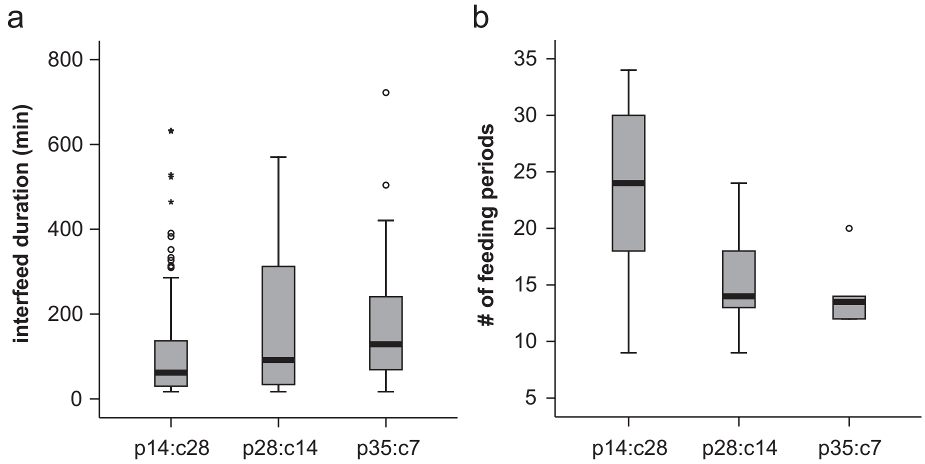

Fig. 4. (a) The duration of interfeed intervals for each treatment and (b) the number of feeding periods. The dark bar within the box represents the median, while the box is the first quartile, the whiskers are the second quartile, the circles are outliers (cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box) and the asterisks are extreme cases (cases with values more than 3 box lengths from the upper or lower edge of the box).

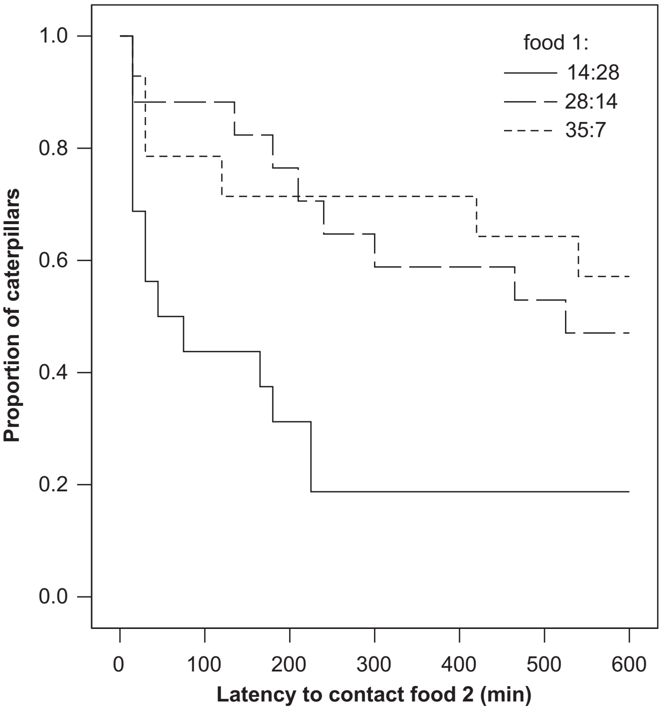

Fig. 5. The latency to contact the second food source (p21:c21) once the barrier was removed. The caterpillars were initially eating one of the three experimental diets (p14:c28, p28:c14, p35:c7). The y-axis represents the proportion of caterpillars that have not yet reached the food source at the time indicated on the x-axis. The height at which the curve terminates shows the proportion of caterpillars that did not reach the food within the 12 h trial period.

differences in the duration of meals and of interfeed intervals also reflect physiological responses to the nutrients ingested. These differences in feeding pattern, combined with differences in propensity to exploration, could contribute to regulating nutrient consumption.

4.1. Physiological feedbacks underlying the pattern of feeding

In caterpillars, durations of both interfeed intervals and meals appear to be regulated to a large extent by volumetric feedback from gut stretch receptors and by nutritional feedback from haemolymph metabolite content (Reynolds et al., 1986; Timmins and Reynolds, 1992; Bernays, 1985; Simpson, 1995). Haemolymph provides internal information on the nutritional status of the insect because its composition fluctuates dramatically as nutrients are absorbed from the gut and processed (Thompson, 2003). Both of these mechanisms could play a role in our findings on durations of interfeed intervals and of meals.

For the longer interfeed intervals observed on the higher protein foods, the simplest explanation would be a longer time required to process these foods. Indeed, previous work has shown that protein can slow the passage of food through the midgut (Bernays, 1985). In this case, signals from gut stretch receptors would inhibit feeding for a longer time on higher protein foods. However, in locusts, longer interfeed intervals on high protein diets are not related to the rate of passage of food through the gut, but instead are explained by an increase in levels of ten free amino acids in the haemolymph (Simpson and Abisgold, 1985; Abisgold and Simpson, 1987). These free amino acids decrease with time since the last protein-containing meal. As haemolymph amino acid levels fall, activity increases, along with the sensitivity of taste receptors to amino acids. Thus, the locust is more prone to find and consume food-containing amino acids (Abisgold and Simpson, 1987). For the forest tent caterpillar, further work would be needed to determine the relative roles of volumetric and nutritional feedbacks on interfeed interval lengths.

For the fact that more short meals were observed on the carbohydrate-biased diet, the explanation likely involves nutritional feedbacks from haemolymph trehalose concentration. In *M. sexta*, haemolymph trehalose increased with diet carbohydrate bias but decreased with diet protein bias (Thompson and Redak, 2000; Thompson et al., 2003). Thus, in the present study, the haemolymph trehalose concentration of the insects on the carbohydrate-biased diet was likely elevated; therefore, these insects probably stopped feeding sooner because haemolymph trehalose had exceeded the threshold at which feeding on carbohydrate is suppressed. This suppression is thought to occur via a central nervous system effect decreasing taste sensitivity to sugar (Thompson, 2003). Similarly, when *M. sexta* caterpillars were given equal ratio protein:carbohydrate diets, they had lower haemolymph trehalose levels than those fed carbohydrate-biased diets, and therefore they consumed more food during the 4 h observation period (Thompson and Redak, 2000). Differences in haemolymph trehalose levels could explain why, in our study, short meals were frequent on the carbohydrate-biased diet and rarer on more protein-rich diets.

These nutritional feedback mechanisms lead to compensatory feeding in response to nutrient deficiency in other generalist caterpillars (Friedman et al., 1991; Thompson and Redak, 2000). However, previous work has shown that nutrient compensation does not occur in the forest tent caterpillar (Despland and Noseworthy, 2006). Nonetheless, the present study shows more subtle post-ingestive responses to nutrient consumption, reflected in differences in the pattern of feeding and in the tendency to explore.

4.2. Implications for nutrient consumption

On the carbohydrate-biased diet, most meals and intermeal intervals were short, and the propensity to leave the trail to the known food source and to discover a novel source increased. In a choice situation, both these mechanisms would tend to increase the consumption of protein-biased foods.

The propensity to explore is expected to be higher on inadequate diets since increasing exploration is a way in which insects can locate a new, possibly nutritionally superior, food (Barton Browne, 1993; Nagata and Nagasawa, 2006). For instance, locusts show increased locomotion in response to falling levels of amino acids in the haemolymph (Abisgold and Simpson, 1987). Forest tent caterpillars use trail-based foraging and hence exploration can be evaluated as the propensity to leave the trail to the known source and to discover a novel source located on unmarked territory. In the present study, increased exploration in response to poor food quality was only observed on the carbohydrate-biased p14:c28 food, and not on the extremely protein-biased p35:c7 food, despite the fact that p35:c7 also only supports poor growth (Despland and Noseworthy, 2006). This suggests that protein deficiency can stimulate increased exploration but that carbohydrate deficiency relative to protein does not. Dussutour et al. (2007) showed that groups of second instar forest tent caterpillars that had established a trail to a protein-only food source (p20:c0) were no more likely to leave the trail and discover a new food source than were groups established on a nutritionally adequate food (p20:c20).

If caterpillars were put in a choice situation, the pattern of feeding documented in this study would lead those individuals that first contact a carbohydrate-biased food to take only a small meal, followed by a short interfeed interval. The increased exploration would increase the probability that the rapidly following next meal would be taken on a different, possibly more protein-rich food.

One of the main mechanisms by which haemolymph composition, reflecting past nutrient intake, influences future feeding behavior is via modulation of taste responses to nutrients in foods. Some insects, including some caterpillars, show changes in taste responses to both sugars and amino acids in response to haemolymph composition (Simpson and Raubenheimer, 2000; Simmonds et al., 1992); however, in other species this is not the case. For instance, *Grammia geneura* shows modulation of taste responses to sugars but not to amino acids in response to past nutrient deprivation (Bernays et al., 2004). Some caterpillars, including the forest tent caterpillar, do not appear to even be able to taste protein in food (Schoonhoven and van Loon, 2002). In this case, post-ingestive effects appear to regulate protein consumption by some other mechanism bypassing taste responses (Bernays et al., 2004). This study demonstrates two possible simple behavioral mechanisms, namely increased meal duration and decreased exploration, by which post-ingestive effects can promote consumption of protein-containing foods.

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References

Abisgold, J.D., Simpson, S.J., 1987. The physiology of compensation by locusts for changes in dietary protein. Journal of Experimental Biology 129, 329–346.

Addy, N.D., 1969. Rearing the forest tent caterpillar on an artificial diet. Journal of Economic Entomology 62, 270–271.

Barton Browne, L., 1993. Physiologically induced changes in resource-oriented behavior. Annual Review of Entomology 38, 1–25.

Bernays, E.A., 1985. Regulation of feeding behaviour. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 4. Pergamon Press, Oxford, pp. 1–32.

Bernays, E.A., Singer, S., 1998. A rhythm underlying feeding behaviour in a highly polyphagous caterpillar. Physiological Entomology 23, 295–302.

Bernays, E.A., Woods, H.A., 2000. Foraging in nature by larvae of *Manduca sexta* influenced by an endogenous oscillation. Journal of Insect Physiology 46, 825–836.

Bernays, E.A., Chapman, R.F., Singer, M.S., 2004. Changes in taste receptor sensitivity in a polyphagous caterpillar reflect carbohydrate but not protein imbalance. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 190, 39–48.

Bowdan, E., 1988a. Microstructure of feeding by tobacco hornworm caterpillars, *Manduca sexta*. Entomologia Experimentalis et Applicata 47, 127–136.

Bowdan, E., 1988b. The effect of deprivation on the microstructure of feeding by the tobacco hornworm caterpillar. Journal of Insect Behavior 1, 31–50.

Despland, E., Noseworthy, M., 2006. How well do specialist feeders regulate nutrient intake? Evidence from a gregarious tree-feeding caterpillar. Journal of Experimental Biology 209, 1301–1309.

Dussutour, A., Simpson, S.J., Despland, E., Colasurdo, N., 2007. When the group denies individual nutritional wisdom. Animal Behavior, in press.

Fitzgerald, T.D., 1995. The Tent Caterpillars. Cornell University Press, Ithaca, USA.

Friedman, S., Waldbauer, G.P., Eertmoed, J.E., Naeem, M., Ghent, A.W., 1991. Blood trehalose levels have a role in the control of dietary self-selection by *Heliothis zea* larvae. Journal of Insect Physiology 37, 919–928.

Grisdale, D., 1985. *Malacosoma disstria*. In: Singh, P., Moore, R.F. (Eds.), Handbook of Insect Rearing, vol. 2. Elsevier, Amsterdam, pp. 369–379.

Jeanson, R., Blanco, S., Fournier, R., Deneubourg, J.L., Fourcassié, V., Theraulaz, G., 2003. A model of animal movements in a bounded space. Journal of Theoretical Biology 4, 443–451.

Lee, K.P., Behmer, S.T., Simpson, S.J., Raubenheimer, D., 2002. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). Journal of Insect Physiology 48, 655–665.

Lee, K.P., Behmer, S.T., Simpson, S.J., 2006. Nutrient regulation in relation to diet breadth: a comparison of Heliothis sister species and a hybrid. Journal of Experimental Biology 209, 2076–2084.

Nagata, S., Nagasawa, H., 2006. Effects of diet-deprivation and physical stimulation on the feeding behaviour of the larvae of the silkworm, *Bombyx mori*. Journal of Insect Physiology 52, 807–815.

Noseworthy, M.K., Despland, E., 2006. How do primary nutrients affect the performance and preference of forest tent caterpillars on trembling aspen? The Canadian Entomologist 138, 367–375.

Raubenheimer, D., Barton Browne, L., 2000. Developmental changes in the patterns of feeding in fourth- and fifth-instar *Helicoverpa armigera* caterpillars. Physiological Entomology 25, 390–399.

Reynolds, S.E., Yeomans, M.R., Timmins, W.A., 1986. The feeding behavior of caterpillars (*Manduca sexta*) on tobacco and on artificial diet. Physiological Entomology 11, 39–51.

Schoonhoven, L.M., van Loon, J.J.A., 2002. An inventory of taste in caterpillars: each species its own key. Acta Zoologica Academiae Scientiarum Hungaricae 48, 215–263.

Sibly, R.M., Nott, H.M.R., Fletcher, D.J., 1990. Splitting behaviour into bouts. Animal Behaviour 39, 63–69.

Simmonds, M.S.J., Simpson, S.J., Blaney, W.M., 1992. Dietary selection behaviour in *Spodoptera littoralis*: the effects of conditioning diet and conditioning period on neural responsiveness and selection behaviour. Journal of Experimental Biology 162, 73–90.

Simpson, S.J., 1982. Changes in the efficiency of utilisation of food throughout the fifth instar nymphs of *Locusta migratoria*. Entomologia Experimentalis et Applicata 31, 265–275.

Simpson, S.J., 1995. Regulation of a meal: chewing Insects. In: Chapman, R., de Boer, G. (Eds.), Regulatory Mechanisms in Insect Feeding. Chapman & Hall, New York, pp. 137–156.

Simpson, S.J., Abisgold, J., 1985. Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. Physiological Entomology 10, 443–452.

Simpson, S.J., Raubenheimer, D., 2000. The hungry locust. Advances in the Study of Behavior 29, 1–44.

Simpson, S.J., Simmonds, M.S.J., Blaney, W.M., 1988. A comparison of dietary selection behaviour in larval *Locusta migratoria* and *Spodoptera littoralis*. Physiological Entomology 13, 228–238.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The principles and Practice of Statistics in Biological Research, third ed. Freeman, New York.

Thompson, S.N., 2003. Trehalose—the insect 'blood' sugar. In: Simpson, S.J. (Ed.), Advances in Insect Physiology, vol. 31. Elsevier Academic Press, London, pp. 205–285.

Thompson, S.N., Redak, R.A., 2000. Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect *Manduca sexta* L. Biochimica et Biophysica 1573, 91–102 (general subjects).

Thompson, S.N., Borchardt, D.B., Wang, L.-W., 2003. Dietary nutrient levels regulate protein and carbohydrate intake, gluconeogenic/glycolytic flux and blood trehalose level in the insect *Manduca sexta* L. Journal of Comparative Physiology B 173, 149–163.

Timmins, W.A., Reynolds, S.E., 1992. Physiological mechanisms underlying the control of meal size in *Manduca sexta* larvae. Physiological Entomology 17, 81–89.

Timmins, W.A., Bellwards, K., Stamp, A.J., Reynolds, S.E., 1988. Food intake, conversion efficiency, and feeding behaviour of tobacco hornworm caterpillars given artificial diet of varying nutrient and water content. Physiological Entomology 13, 303–314.

Zorrilla, E.P., Inoue, K., Fekete, E.M., Tabarin, A., Valdez, G.R., Koob, G.F., 2005. Measuring meals: structure and water intake of rats. American Journal of Physiology—Regulatory, Integrative and Comparative Physiology 288, 1450–1467.