

Larval nutrition affects life history traits in a capital breeding moth

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SUMMARY

Fitness depends not only on resource uptake but also on the allocation of these resources to various life history functions. This study explores the life-history consequences of larval diet in terms not only of larval performance but also of adult body composition and reproductive traits in the forest tent caterpillar (*Malacosoma disstria* H ubner). Caterpillars were reared on their preferred tree host, trembling aspen (*Populus tremuloides*), or on one of three artificial foods: high protein:low carbohydrate, equal protein-to-carbohydrate ratio or low protein:high carbohydrate. Survivorship, larval development rate and adult body size were lowest on the carbohydrate-biased diet and similar on the protein-biased and equal-ratio diets. Fecundity increased with body size but did not otherwise differ between diets. Moths reared on the carbohydrate-biased diet allocated a lower proportion of their mass to the ovaries and more to somatic growth whereas those on equal-ratio and protein-biased diets allocated more to reproductive tissue and less to somatic tissue. These differences in allocation to reproduction arose from differences in the size of eggs, an index of offspring quality. No differences were found in lipid and protein content of female ovaries, accessory glands or somatic tissue, or of the whole body of male moths. The findings show that physiological processes regulate the composition of the different components of the adult body. Diet effects occur as differences in overall body size and in relative allocation to these components. Although lepidopterans can, to a large extent, compensate post-ingestively for nutritionally deficient diets, investment in reproduction vs somatic growth depends on the nutrients available.

Key words: *Malacosoma disstria*, forest tent caterpillar, fecundity, resource allocation, life history, larval nutrition, post-ingestive processing, protein:carbohydrate ratio, reproduction-growth tradeoff.

INTRODUCTION

That resource availability influences life history traits is well understood, particularly for phytophagous insects (Awmack and Leather, 2002). For instance, the relative intake of the two main required nutritional resources, tissue building-blocks (i.e. protein) and an energy source (i.e. carbohydrate or lipid), has significant impact on an individual's growth, development, survival and fecundity (Joern and Behmer, 1997; Naya et al., 2007; van Huis et al., 2008). However, the role of post-ingestive resource processing and allocation is not clear (Boggs, 1992). Changes in resource processing can buffer life-history traits against environmental variability. For instance, post-ingestive processes that change the currency value of a nutrient (e.g. protein deamination for use as an energy source) can mitigate variations in diet and maintain homeostasis. On the other hand, post-ingestive processes can also underlie life-history trade-offs (Zhao and Zera, 2002) by varying allocation to different functions.

Resource allocation is obviously constrained by the variety and quantity of nutrients ingested. For instance, classical life-history theory predicates trade-offs between functions such as reproduction and survival (Boggs and Freeman, 2005), or reproduction and migration (Zera and Harshman, 2001), generally mediated by a single resource currency (e.g. energy). However, variations in diet nutrient composition and differences in nutrient requirements between life-history traits (e.g. protein for egg production and lipid for migration) can mediate allocation of resources to different life-history traits. A scarce nutrient (e.g. protein) may constrain allocation to certain functions (e.g. reproduction), leaving greater quantities of other nutrients (e.g. carbohydrates) available for other functions

(e.g. migration, longevity), thus leading to an apparent trade-off. Understanding physiological mechanisms of allocation in variable resource environments is, therefore, essential to the study of life history (Boggs, 2003).

Many Lepidoptera are capital breeders and feed very little, or not at all, as adults. Therefore, nutrients for adult survival, migration and reproduction must be acquired as larvae. These lepidopterans constitute a simple system to study how resource intake influences allocation to different functions because resource intake and allocation are separated developmentally; once these insects reach the pupal stage, the resource budget is closed and the only remaining flexibility is in the allocation of already acquired nutrients.

Many caterpillars have been shown to consistently select the appropriate ratio of nutrients from a range of different foods (Lee et al., 2002; Schiff et al., 1989; Simpson and Simpson, 1990). However, when confined to a single unbalanced (either excessively protein-biased or carbohydrate-biased) food, caterpillars can, to a certain extent, compensate post-ingestively by adjusting the efficiency of nutrient utilization (Friedman et al., 1991; Schiff et al., 1989; Simpson et al., 1988; Thompson and Redak, 2000). In this case, regulatory physiological processes alter resource processing and allocation patterns to correct for variation in larval diet and hence buffer life-history traits against environmental resource variability (Boggs, 2003).

Nonetheless, these mechanisms are not perfect and caterpillars on unbalanced diets generally exhibit lower survival (Despland and Noseworthy, 2006; Merckx-Jacques et al., 2008), delayed larval development (Despland and Noseworthy, 2006; Lee et al., 2002; Lee et al., 2003; Lee et al., 2004; Merckx-Jacques et al., 2008), lower

pupal mass (Lee et al., 2002) and altered biochemical composition of the pupa (Lee et al., 2002; Lee et al., 2003; Lee et al., 2004). However, it is not clear how variations in nutrient intake influence allocation to various fitness functions (Boggs, 2003). In particular, the implications of differences in both pupal mass and pupal composition are not understood. Could diet-induced differences in pupal composition be one physiological mechanism involved in mediating trade-offs between investment in reproduction and energy reserves for migration?

The forest tent caterpillar (*Malacosoma disstria* Hübner) is a defoliating gregarious insect native to temperate North America. In Canada, its preferred host is trembling aspen (*Populus tremuloides*). The caterpillars are nomadic foragers and move *en masse* in search of food and an aggregation site *via* networks of silk and pheromone trails (Fitzgerald, 1995). These caterpillars have 5–6 instars, after which they pupate. Forest tent moths do not feed, therefore it is essential that they receive all necessary nutrients during the larval stage. Female moths lay their whole egg complement at one time; the single egg mass may contain over 100 eggs that are protected by a frothy substance (spumaline) secreted by the accessory glands, allowing the eggs to survive diapause during the winter (Fitzgerald, 1995).

Previous work has established that forest tent caterpillars are not efficient at pre-ingestively regulating nutrient intake (Despland and Noseworthy, 2006). Food protein-to-carbohydrate ratio affects larval performance, which is best on an equal protein-to-carbohydrate ratio or on slightly protein-biased diets. Caterpillars on carbohydrate-biased diets show reduced survivorship, low growth and delayed development, whereas those on extremely protein-biased diets show reduced growth (Despland and Noseworthy, 2006). The question of interest here is how these diet effects extend to the adult stage; whether they underlie differential investment in various fitness functions or whether post-ingestive processing maintains resource allocation patterns despite differences in resource consumption.

The present study examines life-history consequences of caterpillar nutrition in terms of fecundity, individual egg mass and relative allocation to reproduction vs somatic growth in females. We also examine biochemical composition of male moths and of reproductive and somatic tissue in female moths. Artificial foods were used to allow the manipulation and alteration of nutrient content while excluding secondary chemicals. Natural foliage from aspen trees was used as a reference but not included in analyses.

MATERIALS AND METHODS

Experimental insects and diets

Laboratory colonies of the forest tent caterpillar were reared from egg masses supplied by the Great Lakes Forest Research Center in Sault Ste. Marie, Ontario, Canada. The egg bands were washed as described previously (Grisdale, 1985). Hatching caterpillars were maintained on a 16h:8h photoperiod at 22°C.

During their first instar, the caterpillars were reared on a meridic artificial diet (Addy, 1969) with approximately equal dry mass concentrations of protein and digestible carbohydrate (p23:c24). Once they moulted to the second instar, the larvae were split into four treatments, with five replicates of 12 larvae per treatment. Each group of 12 larvae was housed in a 8×5×15 cm plastic container. The first three treatments consisted of artificial diets with different protein to digestible carbohydrate dry mass concentrations (p21:c21, p14:c28 and p28:c14). Protein was given in the form of casein, while the carbohydrate consisted of dextrose; however, total content of each nutrient also included that contained in the wheat germ (see

below). Other components of the artificial diets were, in % dry mass, Wesson salts (5.7%), cholesterol (1%), Vanderzant vitamin mix (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%). Cellulose, indigestible by caterpillars, was used as a filling agent for the remainder of the food. The diets were a composite of 6:1 agar solution:dry ingredients ratio.

The fourth treatment was trembling aspen foliage collected from multiple trees in Brossard, Québec, Canada. Experiments began in early June 2005, such that caterpillars received appropriate age foliage throughout their development. Leaves were collected every two days and were washed in 0.06% sodium hypochlorite for 5 min and then rinsed in water (Hemming and Lindroth, 2000).

Developmental traits

Pupal mass and sex were determined approximately 24 h after pupation. Pupae were kept in individual Petri dishes, and the moths were frozen upon eclosion (−20°C, no defrost cycle). The developmental characteristics that were measured include pupal mass, adult mass, larval and pupal development time, and larval and pupal survivorship.

Adult female mass allocation

To determine the fecundity of the female moths, the ovaries were removed from the abdomen and the eggs were counted. In this species, the whole complement of mature oocytes is already formed when the moth ecloses.

We also examined mass allocation to accessory glands, ovaries and soma (the remaining non-reproductive tissue). Each component was placed in a drying oven at 35°C for approximately 48 h until the mass remained constant (Parry et al., 2001) and weighed on a Mettler-Toledo microbalance (range 1 µg–5.1 g).

Individual egg mass is an index of offspring quality (Braby, 1994; Sinervo, 1993; Stearns, 1992) and was determined by dividing the number of eggs by the mass of the ovary (Parry et al., 2001).

Lipid extraction

Dry-mass lipid content of male moths and of the various parts of the female (ovaries, accessory glands and soma) was determined by gravimetry following three chloroform extractions (Lee et al., 2002).

Nitrogen analysis

Elemental analysis was performed to determine the amount of nitrogen in a sub-sample of material, which was used as an index of protein concentration of the entire body part. For each sample, a 4 mg sub-sample of dried lipid-free material was crushed, weighed on the microbalance and sealed in a pressed 6×4 mm tin capsule. The tin capsules were dropped into a combustion chamber filled with catalytic material at 1000°C (Perkin-Elmer Series II CHNS/O Analyzer 2400, Shelton, CT, USA). The chromatographic responses were calibrated against pre-analyzed standards (cystine), and the CHN elemental contents were reported in mass percent (Gnaiger and Bitterlich, 1984).

Statistical analysis

Analyses compared between the three artificial diets only. Results on the aspen diet are nonetheless presented with the artificial diet results as a reference of performance on an optimal natural food.

Split-plot two-way analyses of variance (ANOVAs) with replicate containers nested within treatment were performed to determine the

Table 1. Larval performance on the different diets

Diet	Pupal mass (mg)		Larval development time (days)		Pupal development time (days)		Larval survivorship (%)	Pupal survivorship (%)
	Male	Female	Male	Female	Male	Female		
p14:c28	199±13 ^a	316±21 ^a	62.4±2.4 ^a	67.3±2.5 ^a	13.0±0.4	13.5±0.4	43.1	77.4
p21:c21	238±10 ^b	417±12 ^b	50.3±1.0 ^b	52.7±1.1 ^b	12.8±0.2	13.0±0.2	65.3	95.7
p28:c14	241±12 ^b	412±12 ^b	51.0±1.2 ^b	55.1±1.4 ^b	13.3±0.2	12.4±0.4	70.0	88.1
Aspen	307±8.4	543±76	36.6±0.6 ^c	38.7±0.9	12.9±0.2	12.8±0.2	88.3	92.4

Superscript letters indicate significant differences from the Tukey *post-hoc* tests between the three artificial diets (only shown for tests with overall significant differences). Values are means ± s.e.m.

effects of diet and sex on developmental characteristics, with Tukey's *post-hoc* tests used to determine differences between the diets. Survivorship of larvae and pupae were compared by two-way ANOVAs on proportion surviving per container.

Nested multiple analysis of covariance (MANCOVA) was performed to test the effects of the diets on female reproductive characteristics (Raubenheimer and Simpson, 1992). The dry mass of the total moth was used as a covariate, with the number of eggs, accessory gland dry mass, ovary dry mass and soma dry mass as dependent variables, diet as the independent variable, and container as the nested variable. Homogeneity of slopes was tested prior to MANCOVA (Sokal and Rohlf, 1995). In the case of significant differences, pairwise tests between diets were done using the Sidak approach to comparing estimated marginal means.

We next determined whether the diets had an effect on lipid content, using nested ANCOVAs with lipid mass of the body part in question (e.g. whole body for males; ovaries, accessory glands or soma for females) as the dependant variable, and mass before lipid extraction as a covariate.

A nested ANOVA was performed to determine whether diet influenced the percent nitrogen of the different female body parts and of the male moth.

All statistical tests were conducted with SPSS for Windows (v. 10-12, SPSS, Chicago, IL, USA).

RESULTS

Developmental traits

The split-plot ANOVA showed that females were heavier than males at pupation ($F_{1,10}=150$, $P<0.001$). Diet also affected pupal mass ($F_{2,11}=21.8$, $P<0.001$). Tukey *post-hoc* tests show that caterpillars reared on the low protein:high carbohydrate diet (p14:c28) were the lightest, but that those reared on p21:c21 and p28:c14 did not differ significantly from each other (Table 1). The interaction between diet and sex was not significant ($F_{2,10}=2.4$, $P=0.14$).

Female larvae took longer to become pupae ($F_{1,10}=8.74$, $P=0.009$). Tukey *post-hoc* tests show that, for both sexes, the larvae reared on the p14:c28 diet developed the slowest whereas those on the p28:c14 and p21:c21 diet did not differ significantly from each other ($F_{2,11}=65.0$, $P<0.001$) (Table 1). No interaction between diet and sex was seen ($F_{2,10}=0.59$, $P=0.63$). Looking at the time that it took the pupae to eclose to moths, no significant differences were observed (effects of diet, $F_{2,11}=0.82$, $P=0.50$; effects of sex, $F_{1,10}=0.001$, $P=0.90$; effects of interaction, $F_{2,11}=0.14$, $P=0.14$) (Table 1).

No significant differences were detected in either larval or pupal survival rate between the diets (larval, $F_{2,14}=1.7$; $P=0.22$; pupal, $F_{2,12}=1.5$; $P=0.27$) (Table 1).

Adult female mass allocation

Diet did not affect the shape of the relationship between body mass and any of the dependent variables (accessory glands, $F_{2,48}=1.146$, $P=0.340$; soma, $F_{2,48}=0.597$, $P=0.620$; ovaries, $F_{2,48}=0.299$,

$P=0.826$; individual egg mass, $F_{2,48}=1.175$, $P=0.174$; number of eggs, $F_{2,48}=0.517$, $P=0.672$). MANCOVA can therefore be used to test for diet effects on these variables.

Accessory gland mass increased with total mass ($F_{1,38}=121$, $P<0.001$, slope=0.13, $R^2=0.71$), but the relative mass allocation to accessory glands was not affected by diet ($F_{2,36}=1.41$, $P=0.26$) (Fig. 1; Fig. 2; Table 2). For the soma, not only did absolute mass increase with moth mass ($F_{1,39}=117$, $P<0.001$, slope=0.35, $R^2=0.70$) but relative mass allocation also varied between diets ($F_{2,35}=5.37$, $P=0.009$). Moths reared on the p14:c28 diet allocated a relatively larger amount to the soma than those on the p21:c21 and p28:c14 diets (Fig. 1; Fig. 2; Table 2). Conversely, although ovary mass also showed an increase with total body mass ($F_{1,39}=58.9$, $P<0.001$, slope=0.51, $R^2=0.82$), moths that had been reared on p14:c28 diet allocated relatively less mass to the ovaries than those reared on p21:c21 or p28:c14 diets ($F_{2,37}=3.89$, $P=0.017$) (Fig. 1; Fig. 2; Table 2).

The number of eggs increased with body mass ($F_{1,39}=46.1$, $P<0.001$, slope=2.04, $R^2=0.74$) but was not otherwise influenced by diet ($F_{2,21}=1.0$, $P=0.38$) (Fig. 2; Table 2). However, the mass of individual eggs was not only higher in heavier individuals ($F_{1,39}=11.790$, $P=0.001$, slope=0.001, $R^2=0.20$) but was also relatively higher in insects raised on p21:c21 and p28:c14 diets than in those raised on p14:c28 diet ($F_{3,30}=3.203$, $P=0.037$) (Fig. 2; Table 2).

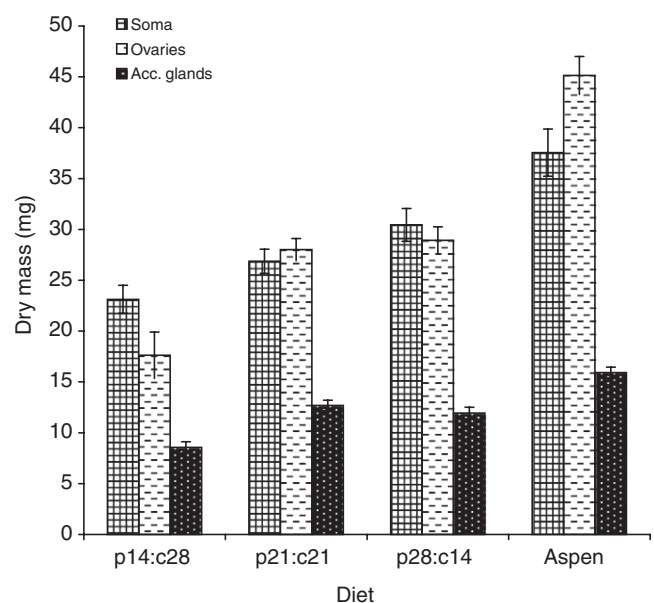


Fig. 1. Effect of diet composition on dry mass of the soma, ovaries and accessory glands. The sum of the three bar heights for each treatment is the total body dry mass. Values are means ± s.e.m.

In summary, the pupae reared on the p28:c14 and p21:c21 diets were heavier than those fed the p14:c28 diet (Table 1). The number of eggs depended only on body mass, and this relationship did not vary between diets. However, individual egg mass did change with diet (heavier on p21:c21 and p28:c14 and lighter on p14:c28), such that the relative mass allocated to the ovaries was higher on p21:c21 and p28:c14 and lower on p14:c28. In compensation, the relative mass allocated to the soma was lower on p21:c21 and p28:c14 and higher on p14:c28. Allocation to the accessory glands did not vary with diet (Fig. 1; Table 2). Moths reared on aspen were heavier, had relatively heavier eggs and allocated relatively more mass to the ovaries and less to the soma than those reared on any of the artificial diets.

Adult lipid content

Diet did not affect the relationship between soma mass and lipid content ($F_{2,47}=0.142$, $P=0.934$). The soma lipid mass increased with total somatic mass ($F_{1,37}=5.33$, $P=0.003$, slope=0.29, $R^2=0.37$) but did not change with diet ($F_{2,20}=0.90$, $P=0.42$) (Fig. 3A; Table 3). For the mass of lipid in the accessory glands, the covariate had no significant effect ($R^2=0.005$). A simple ANOVA, rather than the ANCOVA, was therefore used and showed no difference between the diets ($F_{2,48}=0.195$, $P=0.899$) (Fig. 3B; Table 3). For the ovary lipid content, there was no difference between the slopes of the three diets ($F_{2,44}=0.142$, $P=0.934$). The mass of lipid in the ovaries increased with total ovary mass ($F_{1,35}=13.8$, $P=0.001$, slope=0.20, $R^2=0.51$) but was not affected by diet ($F_{2,24}=0.53$, $P=0.60$) (Fig. 3C; Table 3).

For the total body lipid content of male moths, there was no difference between the ANCOVA slopes of the three diets ($F_{2,51}=2.624$, $P=0.060$). The mass of lipid in the body of the male moths increased with total body mass ($F_{1,54}=261.180$, $P<0.001$, slope=0.41, $R^2=0.81$) (Fig. 3D; Table 3) but was not affected by diet ($F_{2,35}=2.705$, $P=0.06$).

Adult nitrogen content

There was no significant effect of diet on the proportion of nitrogen in the female soma ($F_{2,38}=2.99$, $P=0.06$), ovaries ($F_{2,39}=2.62$, $P=0.08$) or accessory glands ($F_{2,35}=0.96$, $P=0.39$) (Table 3). Additionally, there was no significant effect of diet on the proportion of nitrogen in the male moths ($F_{2,40}=1.30$, $P=0.28$) (Table 3).

Nitrogen content is a standard index of protein content, which can be calculated by dividing nitrogen content by 16%. Thus, on average, male moths contained 41% per dry mass lipid and 54%

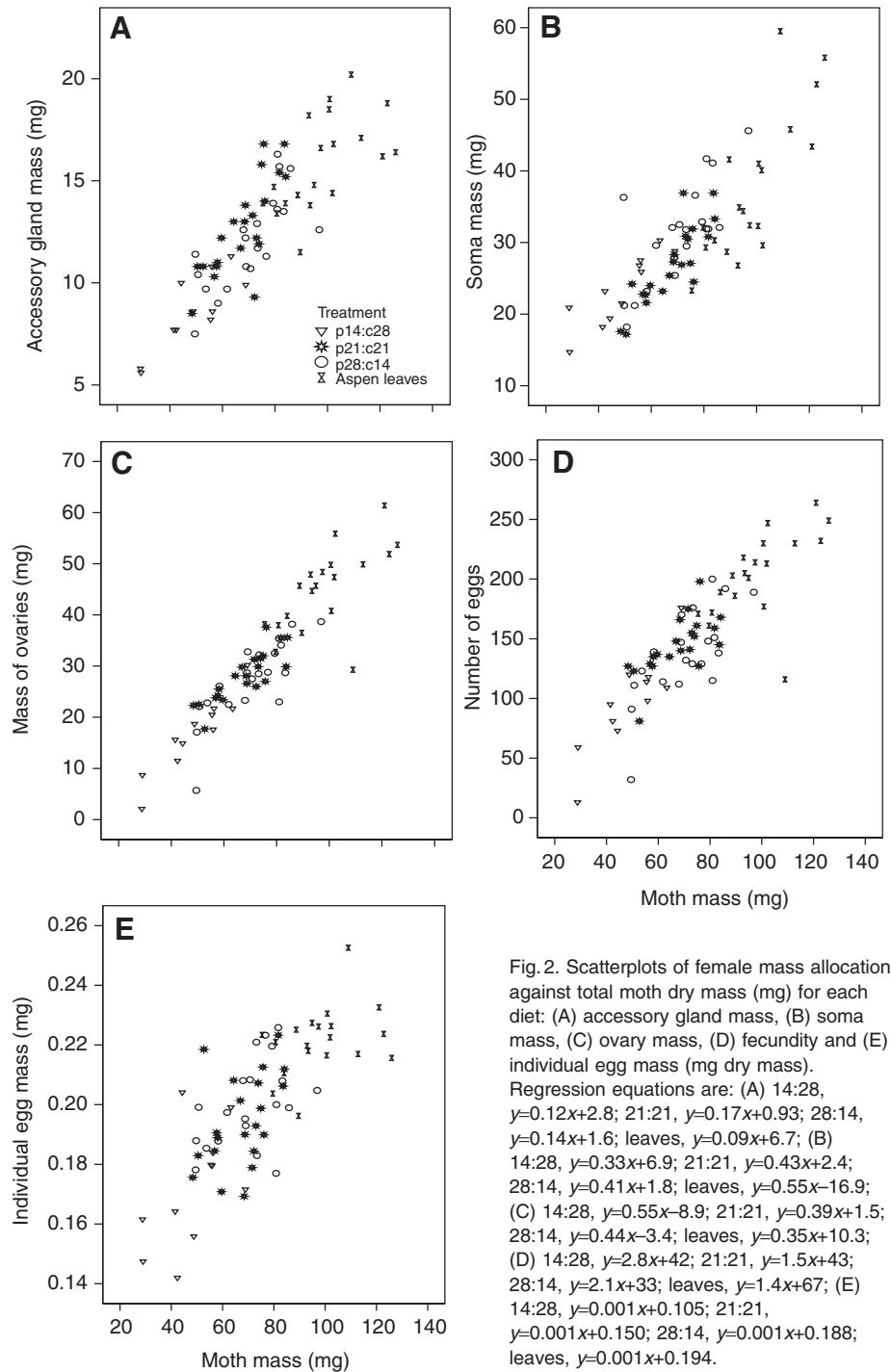


Fig. 2. Scatterplots of female mass allocation against total moth dry mass (mg) for each diet: (A) accessory gland mass, (B) soma mass, (C) ovary mass, (D) fecundity and (E) individual egg mass (mg dry mass). Regression equations are: (A) 14:28, $y=0.12x+2.8$; 21:21, $y=0.17x+0.93$; 28:14, $y=0.14x+1.6$; leaves, $y=0.09x+6.7$; (B) 14:28, $y=0.33x+6.9$; 21:21, $y=0.43x+2.4$; 28:14, $y=0.41x+1.8$; leaves, $y=0.55x-16.9$; (C) 14:28, $y=0.55x-8.9$; 21:21, $y=0.39x+1.5$; 28:14, $y=0.44x-3.4$; leaves, $y=0.35x+10.3$; (D) 14:28, $y=2.8x+42$; 21:21, $y=1.5x+43$; 28:14, $y=2.1x+33$; leaves, $y=1.4x+67$; (E) 14:28, $y=0.001x+0.105$; 21:21, $y=0.001x+0.150$; 28:14, $y=0.001x+0.188$; leaves, $y=0.001x+0.194$.

protein. For females, the soma was 29% lipid and 64% protein, the accessory glands were 5% lipid and 95% protein and the ovaries were 20% lipid and 62% protein.

DISCUSSION

Among the artificial diets, we observed higher survivorship, faster development and greater growth on the protein-biased (p28:c14) and equal-ratio (p21:c21) diets than on the carbohydrate-biased (p14:c28) diet. These findings confirm those of a previous study examining forest tent caterpillar growth, development and nutrient

Table 2. The estimated marginal means for the mass allocated to the different female body parts, and the number and individual mass of eggs

Diet	Soma dry mass (mg)	Ovary dry mass (mg)	Access. gland dry mass (mg)	Number of eggs	Dry mass of one egg (mg)
p14:c28	36.4±1.8 ^a	25.8±2.0 ^a	11.6±0.7	139±9	0.18±0.01 ^a
p21:c21	29.6±1.3 ^b	31.0±1.4 ^b	13.3±0.51	155±5	0.20±0.004 ^b
p28:c14	31.2±1.2 ^b	30.5±1.3 ^b	12.2±0.41	145±5	0.20±0.003 ^b
aspen	24.9±1.4	36.1±1.5	13.0±0.56	162±8	0.21±0.005

The estimated marginal mean from the MANCOVA is the mean corrected for the effect of the covariate and shows differences between diets for individuals with the same value of the covariate (total moth mass=73.9 mg). Values are means ± s.e.m. Superscript letters indicate significant differences using the Sidak approach to comparing estimated marginal means (only shown for tests with overall significant differences).

utilization over the fourth larval instar (Despland and Noseworthy, 2006). Examination of adult traits showed no difference between diets in body biochemical composition but some variation in allocation to different life-history functions. However, this variation was minor compared with the difference in nutrient intake between the diets. Thus, post-ingestive processes regulate body composition despite differences in nutrient intake but do not completely maintain patterns of resource allocation. Investment in reproduction decreased under carbohydrate bias, and this occurred *via* a decrease in egg size rather than a drop in fecundity.

We observed better performance on aspen than on Addy's diet, the standard diet that has been developed specifically for the forest tent caterpillar (Addy, 1969; Grisdale, 1985). The protein:carbohydrate ratio of aspen foliage is variable but tends to fluctuate within the range encompassed by our artificial diets (Hwang and Lindroth, 1997), and the high performance we observed on foliage is more likely to be linked to other factors such as micronutrients, moisture, mechanical properties or secondary chemistry. However, general conclusions about natural and artificial foods cannot be drawn from these results because other types of

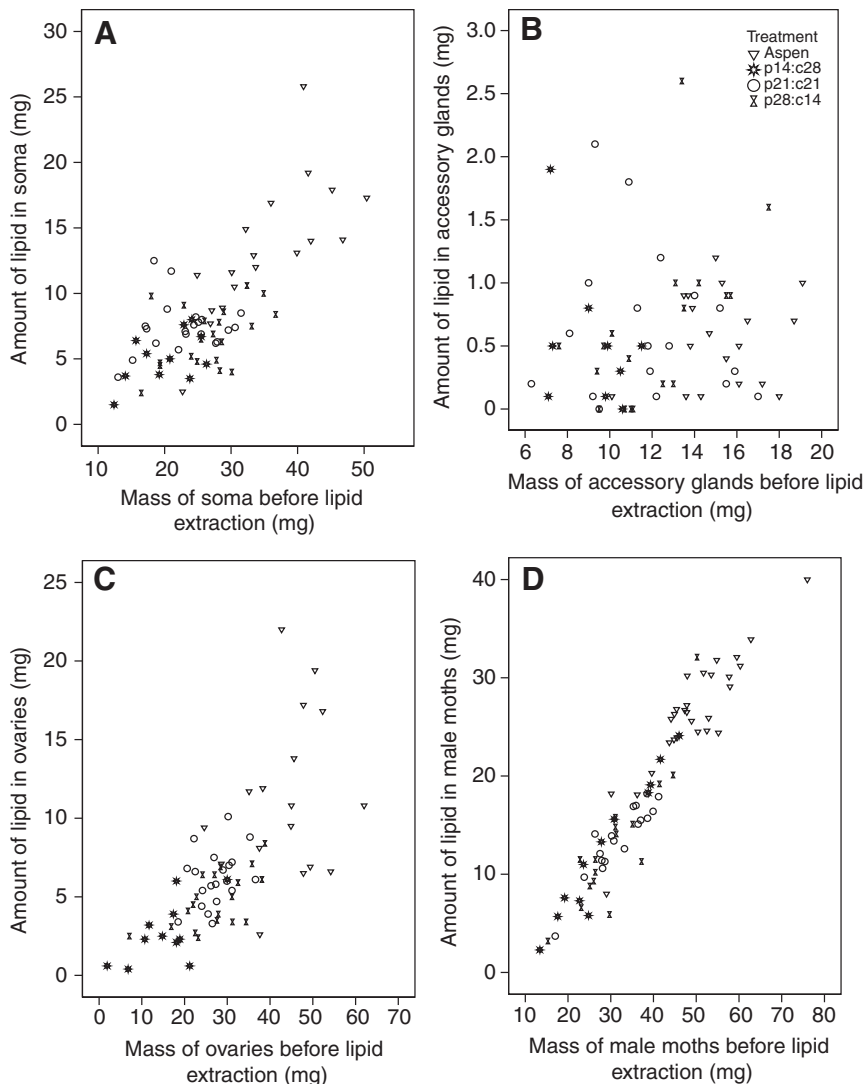


Fig. 3. Scatterplots of lipid content (mg) versus total dry mass (mg) for the four diets: (A) female soma, (B) accessory glands, (C) ovary, (D) male moth. Regression equations are: (A) 14:28, $y = 0.21x + 0.80$; 21:21, $y = 0.15x + 0.62$; 28:14, $y = 0.18x + 0.80$; leaves, $y = 0.23x + 3.13$; (B) equations not given because regressions not significant; (C) 14:28, $y = 0.16x + 0.18$; 21:21, $y = 0.14x + 2.44$; 28:14, $y = 0.14x + 1.01$; leaves, $y = 0.13x + 5.6$; (D) 14:28, $y = 0.66x - 6.3$; 21:21, $y = 0.51x - 2.7$; 28:14, $y = 0.68x - 8.1$; leaves, $y = 0.54x - 0.65$.

Table 3. Relative content of lipid and nitrogen in the female and male moths (in mg)

	Soma lipid (mg)	Ovary lipid (mg)	Access. gland lipid ^a (mg)	Male lipid (mg)	Soma % N	Ovary % N	Acc. gland % N	Male % N
p14:c28	6.98±0.59	5.38±0.67	0.52±0.19	18.2±0.8	14.2±0.16	11.8±0.45	16.4±0.10	14.8±0.21
p21:c21	8.52±0.4	6.17±0.838	0.64±0.14	17.1±0.67	14.2±0.09	12.6±0.18	16.7±0.30	14.5±0.15
p28:c14	6.72±0.52	5.39±1.18	0.64±0.16	17.0±0.70	15.0±0.18	12.6±0.25	17.0±0.22	14.8±0.12
Aspen	9.87±1.21	8.12±1.0	0.56±0.09	19.3±0.67	14.3±0.14	12.3±0.25	16.7±0.08	14.7±0.12
Covariate	26.7	29.42	n/a	37.9	n/a	n/a	n/a	n/a

The relative lipid content is given as the estimated marginal mean from the ANCOVA, i.e. the mean corrected for the effect of the covariate, showing differences between diets for individuals with the same value of the covariate. ^aThe uncorrected means, instead of estimated marginal means, are given for the amount of lipid content in the accessory glands because the covariate (mass before lipid extraction) did not influence the outcome. The final row of the table indicates the covariate value (in mg) for which the estimates were calculated. The relative N content is given as the % N obtained from the elemental analysis. Values are means ± s.e.m. No significant differences were found between treatments.

foliage that are used by forest tent caterpillars in the field, such as sugar maple (*Acer saccharum*), support performance that is similar to or poorer than what we observed on the artificial diets (Lorenzetti, 1993; Nicol et al., 1997; Parry and Goyer, 2004). The formulation of optimal artificial diets is a complex, multidimensional problem; it is a field that is still poorly understood for any animal and especially so for insects (Cohen, 2003).

Despite huge differences in carbohydrate and protein consumption [insects on the p28:c14 diet consumed close to twice as much protein and less than half as much carbohydrate as those on p14:c28 (Despland and Noseworthy, 2006)], no differences were observed in adult-body lipid and nitrogen content and only a slight difference was seen in relative allocation to different body parts. This suggests that ingested nutrients are differentially processed such that the eggs, accessory glands and soma attain the appropriate composition. Thus, physiological processes maintain homeostasis in adult body composition despite variations in larval nutrient intake, thus buffering critical traits against environmental variability.

Such post-ingestive compensatory responses have also been reported in other caterpillars. For example, Lee et al. found that *Spodoptera littoralis*, despite having eaten different amounts of protein as larvae, arrived at similar pupal nitrogen content (Lee et al., 2002). Pupal lipid content was also regulated to a certain extent, since the efficiency with which ingested carbohydrate was converted to lipid decreased with increasing diet carbohydrate, but this was not sufficient to fully compensate for differences in intake, and pupal lipid content still increased on carbohydrate-biased diets (Lee et al., 2002). However, lepidopteran pupae undergo further biochemical changes during pupation and metamorphosis as energy stores are used up and resources are partitioned between the adult somatic tissues and the developing ovaries (Rudolfs, 1926a; Rudolfs, 1926b; Wellington and Maelzer, 1967). Our results suggest that such changes during the pupal stage further buffer the composition of adult tissues, accessory glands and eggs against dietary variability. Several possible mechanisms exist, including differential efficiency of nutrient use depending on availability and deamination of protein for lipid biosynthesis (Merckx-Jacques et al., 2008).

There was nonetheless an influence of the three artificial diets on resource allocation between life-history functions. Moths reared on the carbohydrate-biased diet allocated a lower proportion of their (already smaller!) mass to egg production. Indeed, eggs have a higher protein-to-carbohydrate ratio than does somatic tissue (0.68:1 vs 0.54:1), and hence it is not surprising that protein limitation leads to a shift towards lower investment in eggs. However, no direct effect of diet on fecundity was observed. Instead, lower reproductive investment occurred through a drop in egg size. Previous studies suggest that fecundity is generally tightly coupled to body size in

Lepidoptera (Honek, 1993) but that increased dietary protein is often allocated to eggs. In *Speyeria mormonia* (which does feed as an adult), adult size decreased when larval feeding was restricted, but the relationship between size and fecundity did not change (Boggs and Freeman, 2005). In *Heliothis virescens*, increased protein in the larval diet increased the relative proportion of storage proteins in the adult prior to gametogenesis (Telang et al., 2002), and in *Lymantria dispar* vitellogenin supply to individual eggs increased with increasing nitrogen in the maternal diet (Rossiter et al., 1993). In the present study, ovary mass is more variable between diets than somatic or accessory gland mass (ovary mass varied by a factor of 2.8 between diets, compared with 1.6 and 1.8 for the soma and accessory glands, respectively) (Fig. 1), suggesting that it is a more labile trait.

Large eggs generally have fitness advantages in Lepidoptera (Braby, 1994; Sinervo, 1993; Stearns, 1992), as they lead to greater over-winter survival [*Choristoneura fumiferana* (Carisey and Bauce, 2002)], higher probability of hatching [*M. disstria* (McCowan, 1952)] and larger, more active, caterpillars [*Malacosoma pluviale* (Wellington, 1965); *Lymantria dispar* (Diss et al., 1996)] with longer resistance to starvation [*Lobesia botrana* (Torres-Villa and Rodriguez-Molina, 2002)] in the next generation. In the forest tent caterpillar, larger eggs are thought to confer an advantage in cold climates, where hatchlings sometimes emerge into harsh conditions where food is not immediately available (Cooke and Roland, 2003; Parry et al., 2001).

In traditional plant-insect studies, the effects of diet are usually measured in terms of larval survival, development rate and growth, sometimes over the entire larval stage or sometimes only over a single instar. Pupal mass is often taken as a proxy for reproductive potential. In the present study, pupal mass was indeed a good index of fecundity, but not of investment in reproduction: the carbohydrate-biased diet decreased relative allocation to reproduction via a decrease in the size of eggs. Thus, life-history effects of diet cannot entirely be predicted from traits that can be measured in the larval or the pupal stage.

Our findings show that changes in resource processing buffer variation in certain life-history traits, suggesting strong selection pressures on these traits (i.e. biochemical composition of body parts and relative allocation to accessory glands), while other traits are more plastic (total body size and hence fecundity, and individual egg size). These selection pressures will clearly vary between species, and hence buffering of life-history traits will also vary: see Hahn (Hahn, 2005) for an example of a long-lived migratory income-breeding insect. In the forest tent caterpillar, it seems likely that appropriate biochemical composition of eggs and accessory glands is necessary for functioning of these organs

(Boggs, 2003). Similarly, accessory glands did not change in relative size under protein limitation, despite their high protein content (95%). Indeed, sufficient spumaline is critical for winter egg survival (Cooke and Roland, 2003) and, in a Canadian climate, limiting investment in spumaline devalues investment in eggs. Additional protein is invested in increasing overall body mass (and hence fecundity) and in increasing individual egg size. These traits seem to be labile, not only to environmental but also to evolutionary change, and to underlie a trade-off between fecundity and egg size. Forest tent caterpillars from the southern part of the species distribution range allocate a greater proportion of adult female body mass to eggs and less to accessory glands and show higher fecundity and smaller egg size than insects from more northern populations (Parry et al., 2001).

In conclusion, this study shows that changes in resource processing do, in large part, buffer life-history traits against environmental dietary variability, but not completely. Resource allocation remained constrained by the variety of nutrients ingested; in particular, a carbohydrate-biased diet limited allocation to reproduction and led to a greater relative allocation to somatic growth. The balance point between investment in reproduction and somatic growth thus depends not only on the fitness functions associated with these two traits but on the nutrient budget available. Diet-based nutrient constraints thus appear to be an important factor to take into consideration in investigating trade-offs between investment in different life-history functions.

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