Larval Performance, Adult Reproductive Traits and Pattern of Feeding of the Forest Tent Caterpillar (*Malacosoma disstria*) on Artificial and Natural Diets

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Abstract

Larval Performance, Adult Reproductive Traits and Pattern of Feeding of the Forest Tent Caterpillar (*Malacosoma disstria*) on Artificial and Natural Diets

Nadia Colasurdo

Forest tent caterpillars are an outbreaking forest pest native to North America, whose population cycles have been linked to variations in larval survivorship, adult fecundity and nutrition. These insects only feed as larvae, therefore the acquisition of appropriate nutrients during the larval stage for survival and reproduction of adults is essential.

The present work focuses on the nutritional performance and behaviour of this caterpillar based on diets of varying nutritional quality: their preferred tree host (aspen), or artificial food. Caterpillars performed best on their preferred food source. Protein deficiency did have negative fitness consequences, both in larval survivorship, and quality of offspring. Adult body composition was regulated despite variation in food nutrient ratios.

Feeding patterns demonstrated that on diets high in protein content, feeding bouts and pauses between feedings were longer than on low protein diets, on which the caterpillars were more active. This difference might be related to post-ingestive effects *via* haemolymph trehalose levels. There was no difference in the total time spent feeding or in exploratory behaviour between the artificial diets. When faced with aspen, the caterpillars were also active, had short feeding durations and interfeed pauses.

Caterpillars on aspen were more likely to leave the trail and discover a new food source, and they preferred balanced artificial diet to aspen foliage.

It is suggested that forest tent caterpillars are inefficient at making initial choices, but in the long run they are capable of post-ingestively regulating their body content.

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Chapter 1
General Introduction

Nutrition greatly influences all organisms because, in essence, we are the products of what we eat (Mattson and Scriber 1987). This reality is of utmost importance for caterpillars that spend most of their time eating and growing. When food is available larvae generally feed continuously; in fact it is during these stages that larvae ingest more than their body weight daily (Nation 2002). A constraint for insects in terms of growth and fecundity is the availability and acquisition of proper nutrients necessary to grow, moult and metamorphose into adults (Nation 2002).

Many insects have evolved closely with plants, their sources of food in nature (Bernays and Chapman 1994, Schoonhoven 1998). They acquire their nutritional requirements directly from their food or indirectly through the formation of nutrients *via* biochemical processes (Nation 2002). The quality of this food is essential for herbivores; it generally contains all nutrients required by insects, but the quantity and concentrations of nutrients can vary greatly within and among plant species (Slansky and Scriber 1985). The main foliar constituents that are responsible for the survival and proper performance of phytophagous insects are sugars, proteins and secondary metabolites. Consequently, a diet balanced in essential nutrients is necessary for the maintenance of homeostasis, to supply energy for the cellular work in the insect body, as well as to influence the allocation of nutrients for energy reserves, metabolism and development (Campbell and Reece 2002).

In nature, variability in nutritional quality of food is encountered by all phytophagous insects, making successful foraging and nutrient allocation difficult

(Slansky and Scriber 1985). Diets unbalanced in essential nutrients can affect many aspects of an insect's life. Growth rate influences the timing of events in the insect's life cycle, which subsequently influences the exposure to predators and access to the appropriate food and mates. Food quality can also influence larval growth rate, timing of moults, metamorphosis, and the resulting adult body size. Furthermore, nutrient quality also affects insect reproductive strategies, such as mating success, egg size and number, the allocation of resources to eggs and the choice of oviposition sites (Awmack and Leather 2002, Harvey 1983, Slansky and Rodriguez 1987).

Protein and carbohydrate have been the focus of most of the nutrition-based literature. In caterpillars, sugars are shown to be phagostimulants (Panzuto *et al.* 2001), and when faced with diets that are deficient in sugars, caterpillars do not perform as well as those on balanced diets, in terms of lower pupal weights (Lee *et al.* 2002, 2003), longer development times with extended stadium durations (Lee *et al.* 2002, 2004, Despland and Noseworthy 2006), and poor survival (Harvey 1974, Hemming and Lindroth 1999, Despland and Noseworthy 2006).

Protein content is also vital for insect development. In grasshoppers, protein is the limiting factor for growth, survival and egg production (Joern and Behmer 1997, McCaffery 1975). For caterpillars, first instar mortality is higher and development times longer on diets low in protein, which was also seen for fourth instar caterpillars (Lindroth and Bloomer 1991). Furthermore, caterpillar survivorship is lowest on extreme protein-biased diets (Despland and Noseworthy 2006). Thus, there are strong selection pressures on animals to find efficient ways to regulate the balance of nutrients that they obtain from their foods (Simpson and Raubenheimer 1993).

Many animals regulate food intake pre-ingestively and post-ingestively to meet nutrient needs. They can adjust their intake of multiple nutrients and may use these nutrients differently to arrive at similar growth targets no matter what diet they consume (Simpson 1995). Pre-ingestive methods include dietary self-selection, and compensatory feeding (Slansky and Rodriguez 1987). Dietary self-selection is the ability to distinguish the nutrient composition of a food source and select the nutrients required by consuming the appropriate source. Independent regulation of nutrients is thought to be more important for polyphagous species that feed on multiple food sources differing in amounts of nutrients, than for species that have more restricted diet ranges (Simpson and Raubenheimer 2001, Slansky and Scriber 1985). These species need to regulate their nutritional intake in order to obtain the balance of nutrients they require from nutritionally varied foods. In general, locusts have been the focus of nutritional regulation studies (Simpson and Raubenheimer 2000; *Locusta migratoria*: Simpson *et al.* 1988, *Schistocerca gregaria*: Raubenheimer and Simpson 2003). However, independent regulation of protein and carbohydrate has also been shown in other Lepidopteran species (*Manduca sexta*: Thompson and Redak 2000; *Spodoptera littoralis*: Simpson *et al.* 1988; *Heliothis zea*: Friedman *et al.* 1991). Lee *et al.* (2002, 2003) demonstrated that caterpillars independently regulated their intake of protein and carbohydrate by differentially selecting between two complementary diets, as has been previously demonstrated for locusts. However, not all caterpillars can independently regulate protein and carbohydrate; *Grammia geneura* demonstrates short-term compensatory feeding to carbohydrate deficiency but not to protein deficiency (Bernays *et al.* 2004). Furthermore, forest tent caterpillars are not very good regulators (Despland and Noseworthy 2006),

which is expected since although the species is relatively generalist, the individuals are specialist feeders.

Animals that have not been able to obtain an optimal diet through pre- ingestive methods may potentially post-ingestively regulate their food in order to reach an appropriate growth target. Some insects have the capacity to deaminate protein and remove excess nitrogen through fecal uric acid (Simpson and Raubenheimer 2001, Thompson 1998, Thompson and Redak 2000), or release excess carbohydrate through increasing their rate of respiration (Zanotto *et al.* 1997).

The mechanisms by which feeding regulation occur are not completely known; however, the understanding of an insect's internal nutritional status may hold the answer. For caterpillars this may occur through trehalose (the main storage sugar in insects) concentrations in the haemolymph (insect blood) (Thompson 2003). For example, when *Heliothis zea* caterpillars are fed a diet composed of 80% protein: 20% carbohydrate, they have low levels of haemolymph trehalose. Trehalose levels increase when the insects were fed diets with greater sugar concentrations, as well as during self-selection. Larvae that have been feeding on sugar diets get satiated with the sugar content in the diet, and then proceed to start feeding on foods rich in protein (Friedman *et al.* 1991).

In the present study, we will test first the effect of different nutrients on forest tent caterpillar *Malacosoma disstria* (Lepidoptera: Lasiocampidae) larval performance, and on adult traits that may have implications for fitness, and second, the pattern of feeding on these different foods. Feeding patterns, i.e. feeding durations and the pauses between feeding, vary greatly depending on diet, and this variation in feeding rhythms depends mostly on factors that determine when a meal is initiated. Once a meal has started the

amount of food eaten, the duration of the meal, and the rate of ingestion during the meal are what define it. Meal initiation and termination are influenced by environmental conditions, but also reflect the quality of the food through taste responses and post-ingestive effects (Simpson 1995).

The forest tent caterpillar is a defoliating social insect native to temperate regions of North America. In Québec its preferred host is trembling aspen (*Populus tremuloides*) with sugar maple (*Acer saccharum*) as its secondary host (Panzuto *et al.* 2001). Forest tent caterpillars are early spring feeders (Robison and Raffa 1997), whose egg hatching is linked to the phenology of their host trees. Their eggs hatch in early spring in synchrony with the budding of leaves, when nutrients in these leaves are elevated (Fitzgerald 1995). 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 and Edgerly 1979, Fitzgerald 1995). Tent caterpillars have chemoreceptors on the maxillary palpi that they utilize to sense pheromone molecules on the trail (Roessingh *et al.* 1988). The long-standing nature of their trails and their gregarious behaviour leads to the eventual regrouping of the caterpillars at new locations (Fitzgerald and Costa 1999). In total they have 5 or 6 instars, and at these later stages they consume much food and cause the most damage to the trees. As they enter their last instar they stop eating in preparation for pupation. The caterpillars form pupae, spin a cocoon, and emerge as adult moths within two weeks, allowing for females and males to mate. As adults, they do not forage. Females disperse to find suitable egg-laying sites and oviposition occurs soon after. Female moths lay their whole egg complement at one time; the single egg mass may

contain 150-450 eggs that are protected by a frothy substance, allowing the eggs to survive a diapause during the winter (Fitzgerald 1995).

In general, forest tent caterpillars are restricted to a sole host tree and are not subjected to much diversity in nutrients. Nonetheless, it has been shown that they can distinguish between the variations of nutrients in leaves (Panzuto *et al.* 2001) and develop better on preferred foliage (Lorenzetti 1993). Levels of primary nutrients (primarily protein and carbohydrate) and secondary metabolites (tannins and phenolic glycosides) vary both within and between trembling aspen trees, and affect caterpillar feeding behaviour and performance. During forest tent caterpillar outbreaks, specific trees and specific tree parts are defoliated more than others, possibly because of the difference in nutrients in various parts of the tree (Lévesque *et al.* 2002). This discrimination is possible as a result of chemosensory inputs. Insect taste neurons are located within sensory sensilla on the mouthparts; these sensilla contain several chemoreceptor cells that respond to chemical stimuli, such as sugar or salt. Insects use their taste senses to select appropriate food (Campbell and Reece 2002).

Interest in the forest tent caterpillar stems from the fact that they defoliate economically important trees (Britt 1970), and that their defoliation cycles are not well known. The defoliation of aspen by forest tent caterpillars occurs at 6-16 year intervals and may continue for two to six years (Batzer *et al.* 1995). During this time the caterpillars can defoliate acres of trees (Lindroth and Bloomer 1991) leading to reduced growth and possible death (Duncan and Hodson 1958). The successful management of this pest species entails the knowledge of the aspects that influence

food consumption, growth, reproduction, and their activity (Slansky and Rodriguez 1987).

Previous work on the forest tent caterpillar has established that they are not efficient at pre-ingestively regulating nutrient intake (Despland and Noseworthy 2006). Forest tent caterpillars are oligophagous insects; therefore they encounter a limited range of food. Thus, independent regulation of protein and carbohydrate is thought to be less important for them than it is for generalist feeders.

The objective of this thesis was to determine the effect of nutrient ratios on the forest tent caterpillar, concentrating on protein and digestible carbohydrate, in the form of sugar. The first part of this study centered on the performance of the forest tent caterpillar on foods varying in protein to sugar ratio, focusing on larval development and adult reproductive characteristics. Here, body composition and fecundity were also measured to allow for more direct fitness indices. Artificial foods were used to allow the manipulation and alteration of nutrient content while excluding secondary chemicals, and may determine the impact of nutrient level and requirements (Slansky and Rodriguez 1987). Natural foliage from aspen trees was used as a control. The second part of this study was to evaluate the caterpillars' feeding behaviour on diets with various protein: sugar ratios, focusing on both short-term and long-term effects, as well as on exploratory behaviour. On the whole, we would like to determine if forest tent caterpillars demonstrate behavioural responses to food sources differing in nutrient content that are consistent with their performance differences.

Chapter 2
Nutritional effects on survival, fecundity and offspring quality in an outbreaking caterpillar

2.1 Abstract

The performance of the forest tent caterpillar on its preferred host plant, trembling aspen, and on artificial foods varying in protein to sugar ratio was evaluated. The focus was on larval development, adult reproductive characteristics, and adult lipid and nitrogen content. The three artificial diets used were a) high protein-low sugar, b) balanced diet of equal protein and sugar, and c) low protein-high sugar. Survivorship, development rate and growth were highest on aspen, lowest on the carbohydrate-biased diet, and similar on the protein-biased and balanced diets.

For the adult stage, individuals fed low protein were lighter, but allocated relatively more resources to their soma and had lighter eggs. Larvae reared on aspen gave rise to heavier moths and allocated more to reproduction *via* heavier eggs. Lipid content in the female soma was higher in individuals reared on aspen. A similar trend, although not significant, was observed for males. However, no differences in the amount of lipid in the adults' eggs or accessory glands were found between treatments. No differences in nitrogen levels were found among the treatments for both males and females.

These findings confirm that when looking at artificial diets, these caterpillars perform better on protein-biased diets, but perform optimally on aspen foliage. Protein deficiency had negative fitness consequences, as reflected in larval survivorship, and quality of offspring (as predicted by egg weight). We established that forest tent

caterpillars regulate their body composition despite variation in food nutrient ratios, and that fitness implications can be suggested based on weight, and not body composition.

2.2 Introduction

Arthropods have two life stages: the immature (larvae or nymphs) and mature (adult) stage. The performance of each life stage depends on the success of preceding stages in obtaining, synthesizing and collecting the appropriate nutrients in the right proportions (Slansky and Rodriguez 1987). Lepidoptera, that go through a metamorphic phase, need to acquire all the necessary nutrients during their larval stages in order to achieve proper adult reproduction and fitness. More specifically, forest tent moths do not feed and therefore, it is essential that they receive all nutrients required when in their larval stage.

Carbohydrates are essential for tissue building, especially in the formation of chitin in the insects' cuticle (Campbell and Reece 2002). Glucose is the most common simple sugar (monosaccharide) in animals and is used as a source of energy during metamorphosis and for cellular building materials (Campbell and Reece 2002). Furthermore, many insects use digestible sugars to determine an appropriate food source (Panzuto *et al.* 2001). For example, lepidopteran haemolymph (insect blood) sugar concentrations may be used to regulate intake of food (Thompson and Redak 2000; Thompson *et al.* 2001, Thompson 2003).

Carbohydrates are also used to build lipid reserves (Thompson 2003). Lipids are usually stored and may be used either as energy for metamorphic processes or for the maintenance of life processes (Rudolfs 1927) during pupal and adult stages when little or no foraging occurs in most caterpillars. Lipids in the pupa are only partly used, while the remainder seems to be retained for energy and for the formation of

eggs. Upon eclosion, male moths contain an abundance of fat tissue for energy-demanding flights in search of mates, while female moths do not contain much fat tissue but have ovaries full of mature eggs ready to be laid (Snodgrass 1935). Rudolfs (1926a) suggested that lipids in the adult female are used for energy in adult, flight and the deposition of the eggs.

Proteins are essential for the growth and maintenance of tissues, as well as for structural support, storage, transport, movement, and in the maintenance of cellular metabolism (Campbell and Reece 2002). Protein content is usually measured through nitrogen content. Nitrogen is a building block of proteins and is regularly considered a limiting nutrient, limiting insect performance because in general there are low levels of nitrogen in plant tissue relative to nutritional requirements of the insect. Nitrogen is often bound up in plant proteins, therefore, it is difficult to access; insects need to find ways to utilize nitrogen in an efficient and productive manner (Campbell and Reece 2002). Nitrogen appears to play an important role at three critical stages of an insect's life, when the larvae are formed, for pupation and for pupal metamorphosis (Rudolfs 1927).

The different roles of adult females and males are generally reflected in differences in larval performance. Adult females have greater investments in reproduction than males, and are thus usually larger and heavier (Fitzgerald 1995). This is often accomplished by longer development times in female larvae, or by the addition of another instar (Slansky and Scriber 1985). Greater differences among female and male larvae are expected in species with non-feeding adults (Slansky and Scriber 1985).

Many insects display an interaction between reproduction and flight. Both flight capability and reproduction are metabolically expensive and need to compete with the internal allocation of nutrients, thus resulting in a trade-off between fecundity and dispersal. This effect is termed the "oogenesis-flight syndrome." Such fitness trade-offs resulting from the allocation of nutrients can be accentuated with altered nutrient intake. Flight ability is very energetically expensive and requires lipid reserves, while egg production needs supplies of nitrogen for proper development (Slansky and Rodriguez 1987, Zera and Denno 1997).

Previous work on the forest tent caterpillar has demonstrated that they do not perform well on diets low in carbohydrates; they have greater pupal mass on balanced and slightly protein-biased diets. The timing of moulting appears linked to protein intake; insects reached the fifth instar more quickly on balanced and protein-biased diets compared to carbohydrate-biased diets. Survivorship is highest on carbohydrate- biased diets, but lowest on the extreme protein-biased diet (Despland and Noseworthy 2006).

This study focuses on the effects of proteins and digestible sugars on the performance, growth and development of forest tent caterpillars throughout the whole larval stage, and whether this is reflected in adult performance. The larval development characteristics that were tested included final pupal weight, the duration of the larval stage and the duration of the pupal stage. Adult reproductive parameters explored were weight allocation to different female body parts: the ovaries to investigate the investment in reproduction, accessory glands, that produce a frothy substance that protects the over-wintering eggs from desiccation ensuring winter survival (Fitzgerald 1995), and the soma (the remaining carcass of the adult) to investigate investment in adult flight. Fecundity

and individual egg weight, which were also measured, are generally regarded as good predictors of egg quality, to potentially understand the effects of nutrition on fitness of the next generation. Female forest tent caterpillars deposit all their eggs in a single clutch (Fitzgerald 1995). This egg-laying habit allows accurate estimation of fecundity because each clutch is the full egg complement of an individual female. In addition, for males and females we looked at the distribution of lipid (carbohydrate-derived) and nitrogen (protein-derived) within the moths and determined whether these were affected by the different diets. As well, we explored whether the variation in diets might mediate the oogenesis- flight syndrome.

It is expected that larvae will perform best on aspen foliage, as well as on balanced diets in faster development times, better survivorship and heavier larvae, and will also allow females to be more fecund. Also, protein-biased diets should correspond to higher levels of nitrogen in the soma, and result in females with more and larger eggs. Carbohydrate-biased diets should correspond to higher lipid reserves, resulting in more flight fuel in the adult moth.

2.3 Methods and Materials

2.3.1 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. The egg bands were washed for 1 min 30 sec in 5% sodium hypochlorite, and then rinsed with water for 5 min. They were then rinsed again in 0.06% sodium hypochlorite and air-dried (as per Grisdale 1985). They were maintained on a 16:8 hour photoperiod at 22°C.

During their first instar the caterpillars were reared on a meridic artificial diet (Addy 1969) with approximately equal dry weight concentrations of protein and carbohydrate. Once they moulted to the second instar, the larvae were randomly split into four treatments, with 5 replicates (containers) of 12 larvae per treatment. The treatments consisted of artificial diets with different protein to carbohydrate dry weight concentrations: 21% protein: 21% carbohydrate; 14% protein: 28% carbohydrate, and 28% protein: 14% carbohydrate, as well as trembling aspen foliage collected from Parc Écologique des Sansonnets in Brossard, Québec. Protein was given in the form of casein, while the carbohydrate consisted of dextrose. Casein has a good balance of most amino acids and has been widely used in insect diets (Nation 2002). 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%). Cellulose was used as a filling agent for the remainder of the diet. The diets were a composite of 6: 1 agar solution: dry ingredients ratio. Leaves were collected every two days (or when

necessary), and were washed in 1% bleach (0.06% sodium hypochlorite) for 5 min and then rinsed in water (Hemming and Lindroth 2000). They were placed in flower picks containing water.

2.3.2 Larval development

The caterpillars were kept in 43 oz. containers lined with a layer of paper towel and wax paper to maintain moisture. The caterpillars were checked daily and their moulting dates were recorded for each instar. At pupation, they were placed in individual Petri dishes. Approximately 24 hours after the larvae pupated, their pupal weight and sex were determined. Once the adult moths emerged they were frozen for later biochemical analysis.

The larval development characteristics that were measured included pupal weight, larval and pupal development time. Larval development time is the amount of time (days) it took the larvae to reach pupation, while pupal development time is the amount of time it took the pupae to eclose into moths.

2.3.3 Adult reproductive characteristics

To determine the fecundity of the female moths, the adult females were dissected. The female moths were taken out of the freezer and allowed to thaw. Once thawed, the moths were weighed on an analytical balance. The ovaries were carefully removed from the female abdomen and the eggs were counted under a microscope. The ovaries and associated accessory glands were then rinsed with distilled water. Each ovary has four

ovarioles filled with the moth's whole complement of mature oocytes. The accessory glands are large reservoirs attached to the ovaries of the forest tent caterpillars and contain a frothy substance called spumaline, which is used to protect and cover the eggs during the winter (Fitzgerald 1995). The ovaries, accessory glands and remaining soma (the remaining non-reproductive tissue) were placed in a drying oven at 35°C for approximately 48 hours until the weights were constant. Once dry, the samples were placed in pre-marked microcentrifuge tubes and stored in a dessicator in the freezer (Parry *et al.* 2001).

The female reproductive characteristics examined included the weight allocation to the accessory glands, ovaries, and soma, as well as fecundity and individual egg mass. Individual egg weight was calculated as the total ovary weight divided by the number of eggs.

2.3.4 Lipid extraction

Lipid extraction was performed on male and female moths in order to determine the amount of lipid across the different body parts of the female, and in the male. The lipid analysis was performed using a chloroform extraction (Lee *et al.* 2002). The samples were thawed for 10 minutes and weighed. They were then placed in labelled glass test tubes (12 x 75 mm) and the lipid was extracted in three, 24-hour changes of chloroform (approximately 4 ml of chloroform each time). Marbles placed on top of the test tubes were used as stoppers. The lipid dissolved into the chloroform and was extracted when the chloroform was removed from the tubes. Following the last extraction, the samples were filtered through Whatman #1 paper to remove all excess

lipid and chloroform. The samples were dried overnight in the fume hood and then re-weighed to determine how much lipid was extracted. The samples were then placed back in the test tubes, covered with parafilm and frozen.

2.3.5 Nitrogen analysis

Elemental analysis was performed to determine the concentration of nitrogen in the samples. The lipid-free samples were crushed, weighed on an electrobalance (1-2 mg) and sealed in pressed 6 x 4 mm tin capsules. The tin capsules were dropped into a combustion chamber filled with catalytic material at approximately 1000°C (Perkin-Elmer Series II CHNS/O Analyzer 2400). As the sample entered the combustion chamber, a fixed amount of oxygen was injected into the constant stream of helium, which was used as the carrier gas. All of the reaction products including nitrogen, carbon dioxide, water and sulfur dioxide were then separated and detected. The chromatographic responses were calibrated against pre-analyzed standards (cystine: Perkin Elmer organic analytical standard), and the CHN elemental contents were reported in weight percent (Gnaiger and Bitterlich 1984).

2.3.6 Statistical analysis

Nested two-way analyses of variance (ANOVAs) were performed to determine the effects of the different diets and sex on larval development characteristics (nesting replicates within treatments), with Tukey's Post-Hoc tests used to determine differences

between the treatments. Survivorship of larvae and pupae were determined by Pearson chi-squared analysis.

Nested analyses of co-variance (ANCOVAs) were performed to test the effects of the diets on female adult reproductive characteristics, and on adult lipid content (for females and males). For the female adult reproductive characteristics: the dry weight of the total moth was used as a covariate, with the number of eggs, accessory gland dry weight, ovary dry weight, soma dry weight and individual egg weight as dependent variables, treatment as the independent variable, and replicate as the nested variable. To determine whether the regression slopes for the treatments were homogeneous, a statistical model was run. In this model only the F-test for the interaction term treatment X dry moth weight was of interest. If it is not significant, the slopes are homologous and the ANCOVA can be used to test for differences between treatments (represented by differences in intercepts) (Sokal and Rohlf 1995). To test for the homogeneity of intercepts, the model was run again without the interaction term (Parry *et al.* 2001). To determine whether the treatments had an effect on the amount of lipid content, the weights of the body parts in question before lipid extraction (e.g whole body for males; ovaries, accessory glands and soma for females) were used as a co-variate against each of the respective weights after lipid extraction.

A multiple nested analysis of variance (MANOVA) was performed to determine whether the treatments influenced the proportion of nitrogen contained in the different female body parts. A nested analysis of variance was conducted to determine treatment effects on the percent nitrogen in the male moth. ANCOVAs were not used for these analyses because when conducting this experiment the percentage nitrogen was

determined for a fraction of the sample, therefore, the total nitrogen content of the whole sample could not be found. Thus, an ANCOVA could not be used.

All statistical tests were conducted with SPSS for Windows (v. 10-12, SPSS Inc. Chicago, U.S.A).

2.4 Results

2.4.1 Larval development

The Nested two-way ANOVA with Type III sum of squares showed that the diet the larvae were reared on and the sex of the larvae significantly influenced their pupal weight (effects of treatment, $F_{3,17} = 87.574$, $p < 0.001$; effects of sex, $F_{1,147} = 274.391$, $p < 0.001$, Fig. 1). There was no effect of containers (replicates) ($F_{16,147} = 0.591$, $p = 0.887$). The interaction between treatment and sex was also significant ($F_{3,147} = 5.548$, $p = 0.001$). Females are heavier than males when they reach pupation. For both sexes, the caterpillars that were reared on the aspen foliage were the heaviest and those reared on the low protein: high sugar diet (p14:c28) were the lightest. The caterpillars fed the p21:c21 and p28:c14 diets were of similar weights and were not significantly different after performing a Tukey post-hoc test (Fig. 1). The interaction between sex and treatment suggests that there is a larger variation in pupal weight for females among the different treatments (Fig. 1).

Treatment and sex significantly affected the time it took the larvae to become pupae (effects of treatment, $F_{3,16} = 49.709$, $p < 0.001$; effects of sex, $F_{1,147} = 19.611$, $p < 0.001$, Fig. 2). The container (replicate) that the caterpillars were reared in affected the time that it took them to pupate ($F_{16,147} = 3.356$, $p < 0.001$), while no interaction between treatment and sex was seen ($F_{3,147} = 1.635$, $p = 1.87$). These results suggest that the females developed slightly slower, as can be seen in Fig. 2. For both sexes, however, the larvae reared on the foliage developed faster, and those on the p14:c28 diet developed the slowest. Those on p21:c21 and p28:c14 developed at similar rates (Fig. 2).

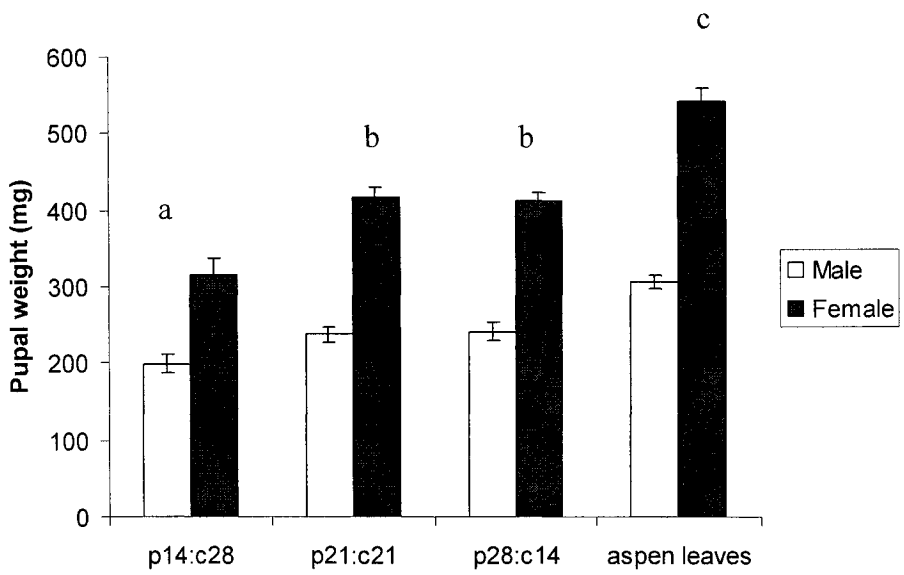


Fig. 1: The weight of male and female pupae (mg) reared on the 4 experimental diets. The letters represent differences based on Tukey's Post-Hoc tests ($p < 0.05$) between diets, with both sexes included. The bars are the standard error of the mean.

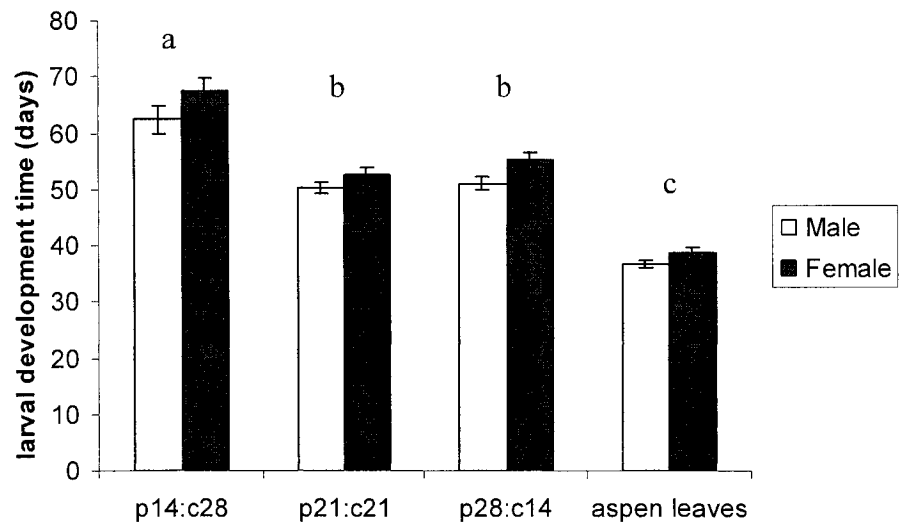


Fig. 2: The number of days from hatch to pupation for male and female caterpillars reared on the 4 diets. The letters represent differences between diets based on Tukey's Post-Hoc tests ($p < 0.05$), with both sexes included. The bars are the standard error of the mean.

Looking at the time that it took the pupae to eclose to moths, no significant differences between treatments and sexes were seen (effects of treatment, $F_{3,16} = 0.393$, $p = 0.759$; effects of sex, $F_{1,131} = 0.072$, $p = 0.789$; effects of interaction, $F_{3,131} = 1.943$, $p = 0.126$; effects of container, $F_{16,131} = 1.713$, $p = 0.052$).

The survivorship of the larvae was influenced by the rearing treatments ($\chi^2_{3\ d.f} = 30.445$, $p < 0.001$) (Fig. 3). More larvae survived when fed the aspen leaves, while only 40% of the caterpillars initially reared on the low protein: high sugar diet reached pupation. However, when looking at the number of pupae that became moths, no difference was noted between the treatments ($\chi^2_{3\ d.f} = 7.402$, $p = 0.060$).

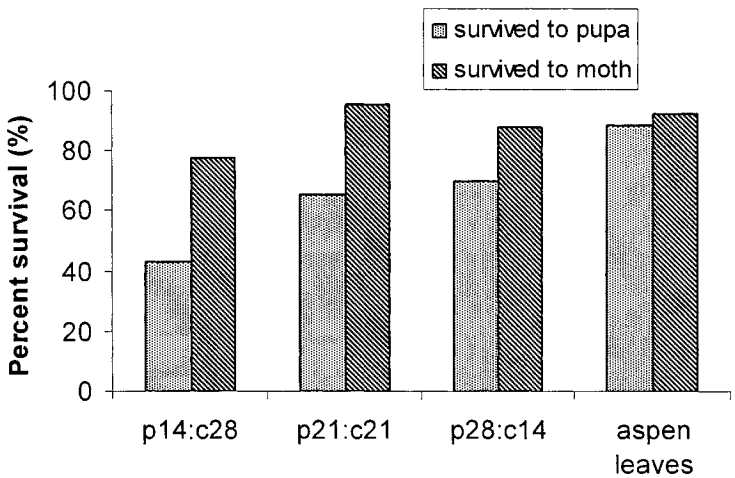


Fig. 3: The percent of larvae that survived to pupation reared on the 4 diets, and the percent of pupae that survived to adult reared on the 4 diets.

2.4.2 Adult reproductive characteristics

To determine the effects of the diets on the female adult body parts Nested analyses of co-variance were performed. For the weight of the accessory glands, there

was no difference between the slopes of the 4 treatments (as seen in interaction term in Table 1, and Fig. 4a). No difference in intercepts was seen, indicating that there was no difference between moths of equivalent sizes in the weight of the accessory glands between the treatments and replicates (Table 1: treatment and replicate terms). Only the total moth weight affected the weight of the accessory glands; those that were heavier had bigger accessory glands (Table 1: moth weight term, and Fig. 4).

a) b)

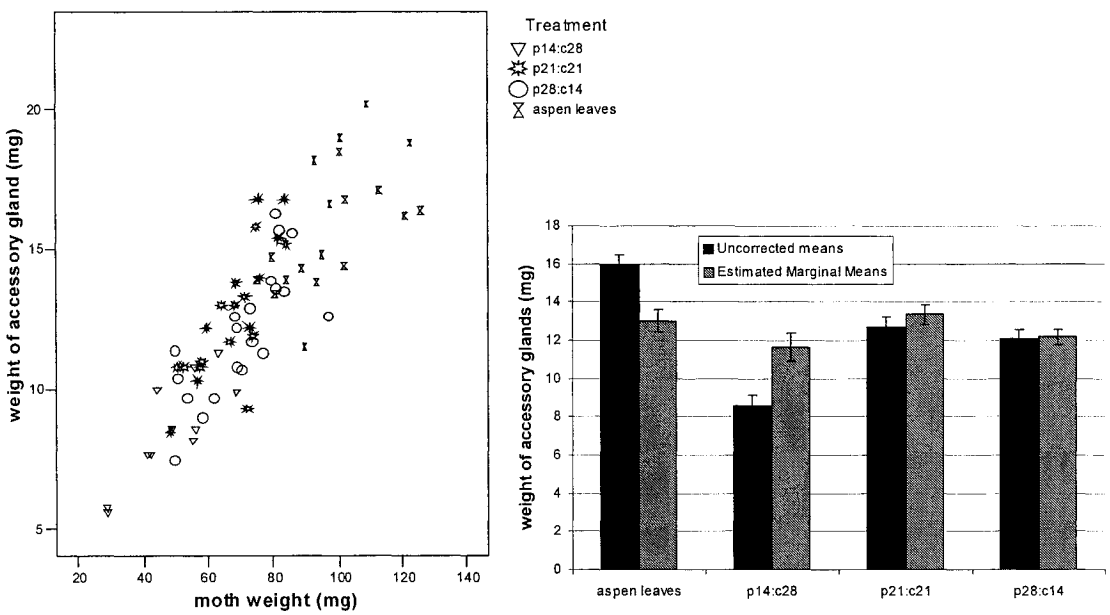


Fig. 4: a) Scatter plot of the weight of accessory glands on the female moth weight for forest tent caterpillars reared on different diets, b) The weight of accessory glands of female moths. The uncorrected means is the average accessory gland weight, while the estimated marginal means is the average weight corrected for the total moth weight (co-variate). Bars are the standard error of the mean.

Dependent Variable: accessory gland weight

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction moth weight * treatment	Hypothesis	8.855	3	2.952	1.146	.340
	Error	123.675	48	2.577		
Moth weight	Hypothesis	137.488	1	137.488	52.908	*<.001*
	Error	132.530	51	2.599		
Treatment	Hypothesis	12.613	3	4.204	1.695	.183
	Error	101.026	40.738	2.480		
Replicate	Hypothesis	35.895	15	2.393	.921	.547
	Error	132.530	51	2.599		

Table 1: Nested ANCOVA results for the effect of treatment on the weight of the accessory glands, standardized for total moth weight and nested between replicates. The fixed factor was the treatments, co-variate was the moth weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The moth weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the soma weight, there was no difference between the slopes of the 4 treatments (Table 2, and Fig. 5a). Also, there was no difference in soma weights for moths of equivalent sizes between the replicates (Table 2). The total moth weight and the different treatments affected the weight of the soma (Table 2). Moths that were heavier had bigger somas, such that moths reared on aspen had larger somas. However, when correcting for body weight, moths reared on the p14:c28 diet allocated a relatively larger amount to the soma, and moths reared on aspen allocated relatively little (Fig. 5b).

a) b)

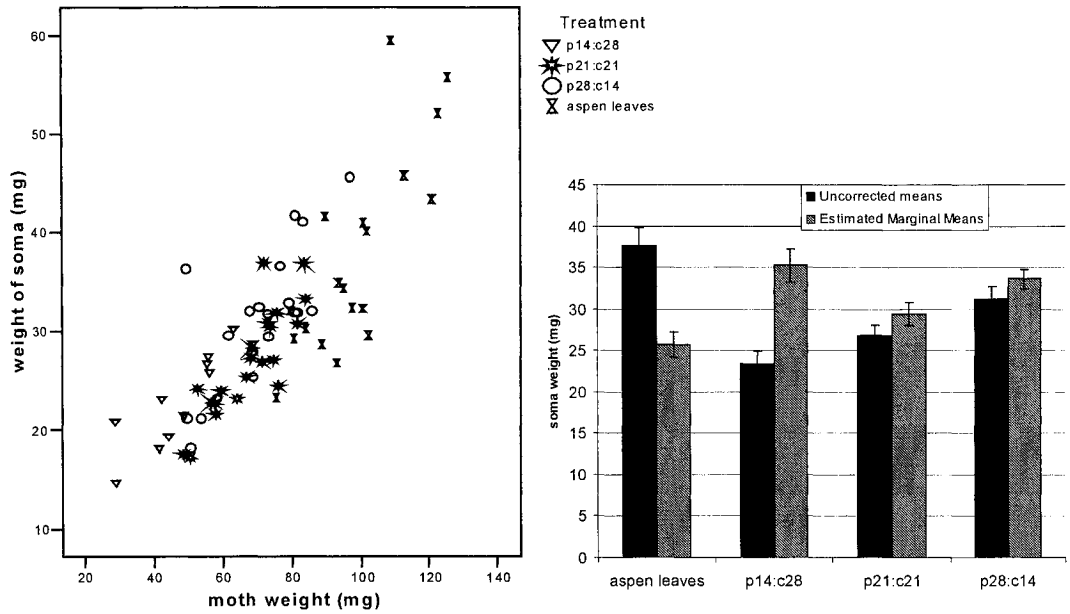


Fig. 5: a) Scatter plot of the weight of the soma on the female moth weight for forest tent caterpillars reared on different diets, b) The weight of the soma of female moths. The uncorrected means is the average soma weights, while the estimated marginal means is the average weight corrected for the total moth weight (co-variate). Bars are the standard error of the mean.

Dependent Variable: Soma weight

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction Moth weight * treatment	Hypothesis	34.724	3	11.575	.597	.620
	Error	930.690	48	19.389		
Moth weight	Hypothesis	1850.712	1	1850.712	97.768	*<.001*
	Error	965.413	51	18.930		
Treatment	Hypothesis	344.358	3	114.786	4.797	*.007*
	Error	753.875	31.508	23.927		
Replicate	Hypothesis	413.739	15	27.583	1.457	.158
	Error	965.413	51	18.930		

Table 2: Nested ANCOVA results for the effect of treatment on the weight of the soma, standardized for total moth weight and nested between replicates. The fixed factor was the treatments, co-variate was the moth weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The moth weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the weight of the ovaries there was no difference between the slopes of the 4 treatments (Table 3, and Fig. 6a). Also, there was no difference in the weight of the ovaries between the replicates. The moth weight and the different treatments affected the weight of the ovaries (Table 3). The heavier moths had heavier ovaries (Fig. 6). Overall, moths that were caterpillars reared on aspen allocated relatively more mass to the eggs, while those on p14:c28 allocated little to their eggs (Fig. 6b).

a) b)

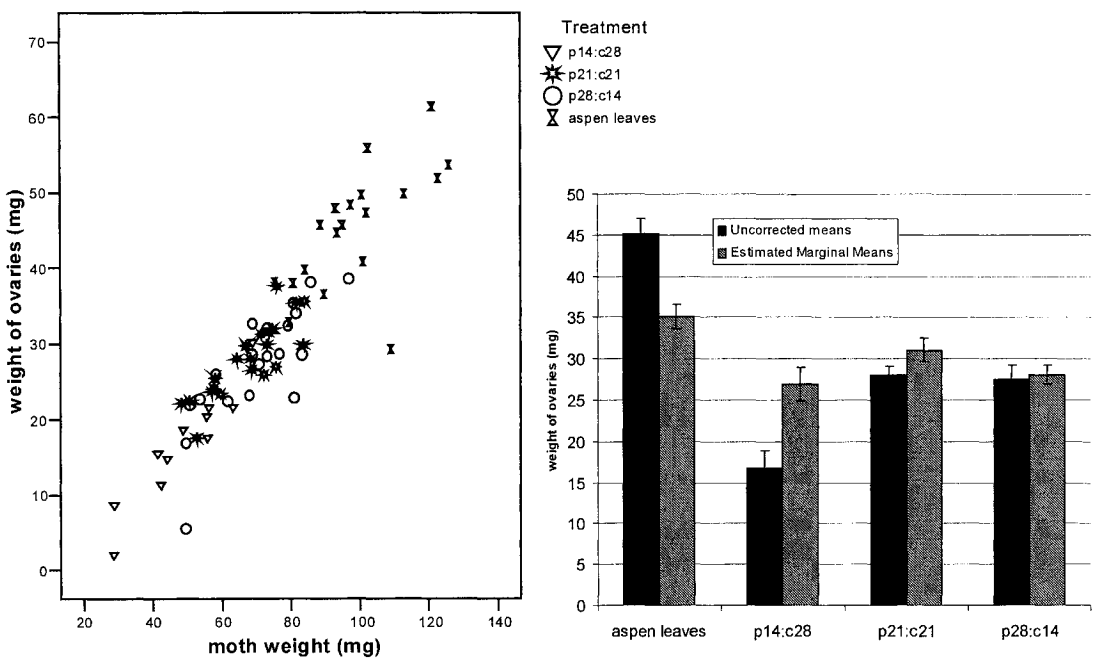


Fig. 6: a) Scatter plot of the weight of the ovaries on the female moth weight for forest tent caterpillars reared on different diets, b) The weight of the ovaries of female moths. The uncorrected means is the average weight, while the estimated marginal means is the average weight corrected for the total moth weight (co-variate). Bars are the standard error of the mean.

Dependent Variable: Ovary weight

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction Moth weight * Treatment	Hypothesis	18.318	3	6.106	.299	.826
	Error	981.753	48	20.453		
Moth weight	Hypothesis	1742.708	1	1742.708	88.872	*< .001*
	Error	1000.071	51	19.609		
Treatment	Hypothesis	254.190	3	84.730	3.888	*.017*
	Error	766.089	35.149	21.795		
Replicate	Hypothesis	350.918	15	23.395	1.193	.307
	Error	1000.071	51	19.609		

Table 3: Nested ANCOVA results for the effect of treatment on the ovary weight, standardized for total moth weight and nested between replicates. The fixed factor was the treatments, co-variate was the moth weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The moth weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the number of eggs, there was no difference between the slopes of the 4 treatments (Table 4, and Fig. 7a). Also, there was no difference in the number of eggs for moths of equivalent sizes between the treatments and between the replicates (Table 4). Only the moth weight affected the number of eggs found in each moth (Table 4). The heavier the moth, the more eggs it contained, leading to an overall larger number of eggs in the aspen treatment (Fig. 7).

a) b)

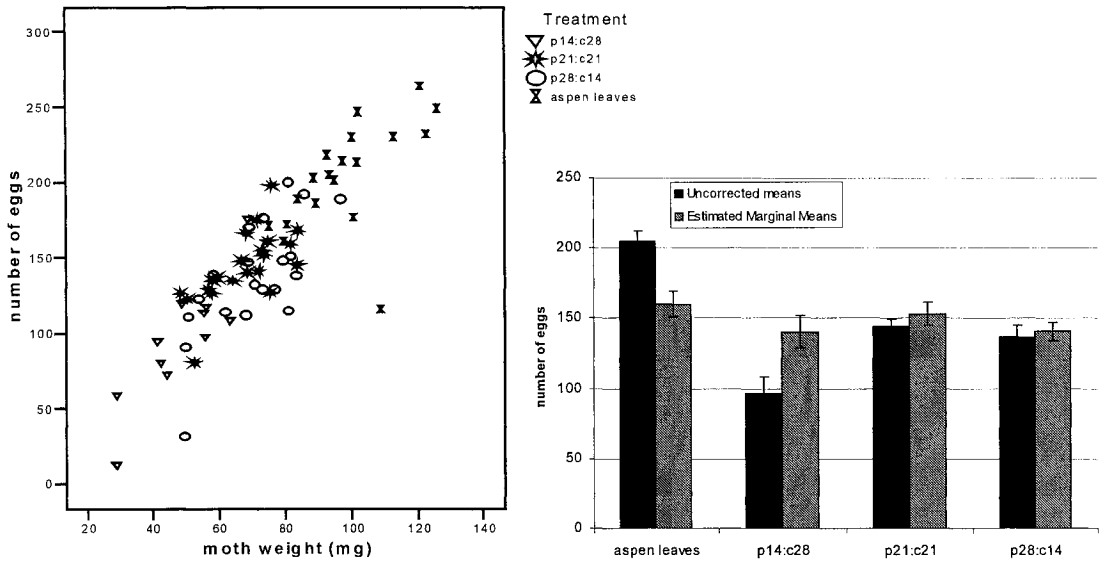


Fig. 7: a) Scatter plot of the number of eggs (fecundity) on the female moth weight for forest tent caterpillars reared on different diets, b) The number of eggs of female moths. The uncorrected means is the average number of eggs, while the estimated marginal means is the average number corrected for the total moth weight (co-variate). Bars are the standard error of the mean.

Dependent Variable: Number of eggs

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction moth weight * treatment	Hypothesis	1031.887	3	343.962	.517	.672
	Error	31924.123	48	665.086		
Moth weight	Hypothesis	33066.799	1	33066.799	51.171	*<.001*
	Error	32956.010	51	646.196		
Treatment	Hypothesis	2215.061	3	738.354	1.167	.334
	Error	25110.590	39.704	632.438		
Replicate	Hypothesis	9335.590	15	622.373	.963	.505
	Error	32956.010	51	646.196		

Table 4: Nested ANCOVA results for the effect of treatment on the number of eggs (fecundity), standardized for total moth weight and nested between replicates. The fixed factor was the treatments, co-variate was the moth weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The moth weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the weight of an individual egg, there was no difference between the slopes of the 4 treatments (Table 5, and Fig. 8a). Also, there was no difference in the weight of one egg when looking between the replicates. The moth weight and the different treatments affected the weight of an individual egg (Table 5). The heavier moths had heavier individual eggs (Fig. 8). Looking at the p14:c28 diet it can be seen that these moths had relatively lighter eggs for their body weight (Fig. 8b).

a) b)

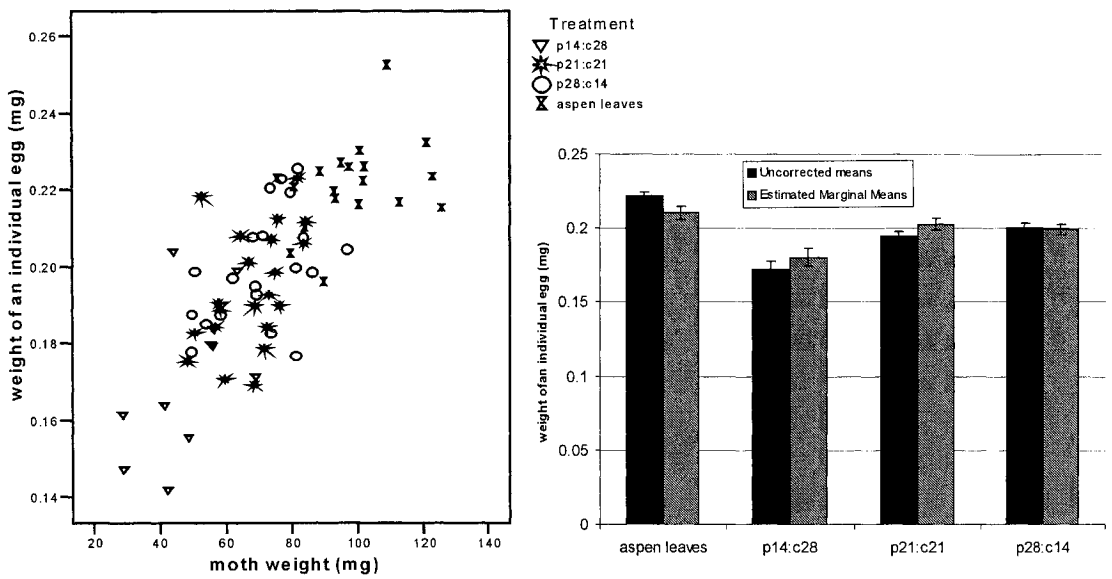


Fig. 8: a) Scatter plot of the weight of the an individual egg on the female moth weight for forest tent caterpillars reared on different diets, b) The weight of an individual egg of female moths. The uncorrected means is the average weight, while the estimated marginal means is the average number corrected for the total moth weight (co-variate). Bars are the standard error of the mean.

Dependent Variable: Weight of individual egg

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction Moth weight * Treatment	Hypothesis	.001	3	<.001	1.175	.174
	Error	.008	48	<.001		
Moth weight	Hypothesis	.002	1	.002	11.790	*.001*
	Error	.009	51	<.001		
Treatment	Hypothesis	.002	3	.001	3.203	*.037*
	Error	.007	30.827	<.001		
Replicate	Hypothesis	.004	15	<.001	1.519	.134
	Error	.009	51	<.001		

Table 5: Nested ANCOVA results for the effect of treatment on the weight of an individual egg, standardized for total moth weight and nested between replicates. The fixed factor was the treatments, co-variate was the moth weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The moth weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

Therefore, the caterpillars on foliage were heavier overall and the caterpillars fed the p14:c28 diet were the lightest. The number of eggs depended only on body mass and this relationship did not vary between diets. However, egg weight did change with diet (heaviest on aspen and lightest on p14:c28), such that the total mass allocated to the eggs was highest on aspen and lowest on p14:c28. As a result, the total mass allocated to the soma was lowest on aspen and highest on p14:c28 (Fig. 9).

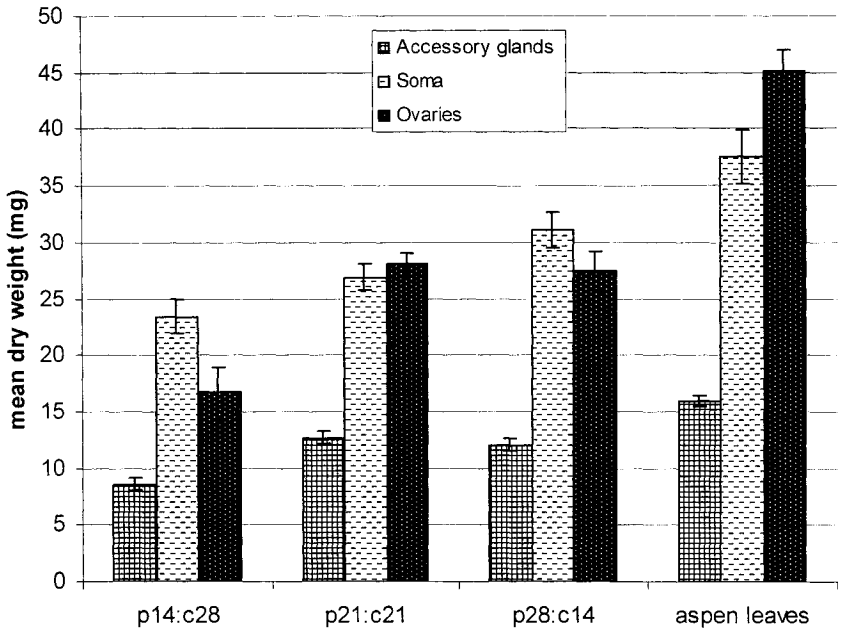


Fig. 9: The effects of diet on female adult reproductive characteristics. The graph demonstrates the total weight allocated to the accessory glands, egg mass, and soma. The bars are standard errors of the mean.

2.4.3 Lipid extraction

For the amount of lipid in the soma, the interaction term initial soma weight (before lipid extraction) X treatment in the test for homogeneity of slopes was significant (Table 6), indicating that there is a difference between the slopes of the 4 treatments. This can be seen in Fig. 10a. If the assumption that the slopes of the regression lines are the same is violated, as in this case, an increase in the likelihood of Type II errors occurs (thinking that there is no relationship when in fact there is, a false negative). The more the assumption is violated, the more conservative the test becomes (Rutherford 2001). However, intercepts for treatment were significantly different (Table 6) when looking at the model without the interaction, thus, we can be confident of the result since even an

excessively conservative test gives a significant difference. The weight of the soma before lipid extraction and the different treatments affected the amount of lipid in the soma (soma weight and treatment terms in Table 6, and Fig. 10). Those that had heavier somas contained more lipid, and when correcting for initial soma weight it was seen that moths on the aspen diet contained the highest amount of lipid, but there was no difference between the three artificial diets despite differences in dietary sugar (Fig. 10b).

a) b)

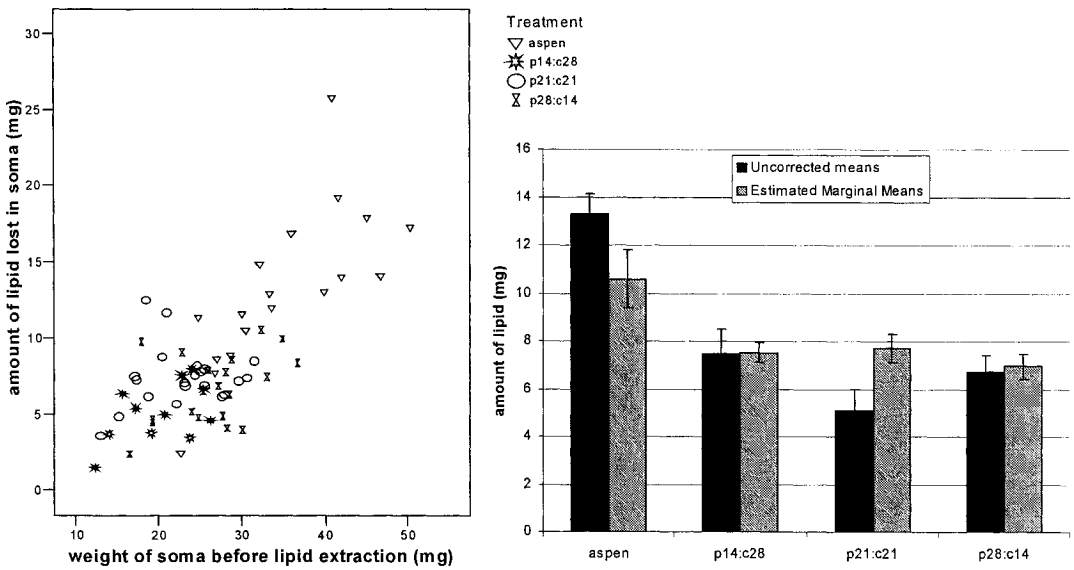


Fig. 10: a) Scatter plot of the amount of lipid lost in the soma on the female soma weight before the lipid extraction for forest tent caterpillars reared on different diets, b) The amount of lipid found in the soma of female moths. The uncorrected means is the average amount of lipid, while the estimated marginal means is the average amount of lipid corrected for the initial soma weight before the lipid extraction (co-variate). Bars are the standard error of the mean.

Dependent Variable: Amount of lipid lost in the soma

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction initial soma weight * interaction	Hypothesis	91.783	3	30.594	5.429	*.003*
	Error	264.838	47	5.635		
Initial weight of soma	Hypothesis	192.648	1	192.648	27.010	*<.001*
	Error	356.621	50	7.132		
Treatment	Hypothesis	91.244	3	30.415	4.224	*.012*
	Error	235.618	32.719	7.201		
Replicate	Hypothesis	108.583	15	7.239	1.015	.456
	Error	356.621	50	7.132		

Table 6: Nested ANCOVA results for the effect of treatment on the amount of lipid in the soma, standardized for soma weight before lipid extraction and nested between replicates. The fixed factor was the treatments, co-variate was the soma weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The soma weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the amount of lipid allocated to the accessory glands, the interaction term initial accessory gland weight X treatment in the test for homogeneity of slopes was significant (Table 7), indicating that there is a difference between the slopes of the 4 treatments (seen in Fig. 11a). Fig. 11a shows that the covariate does not affect the lipid content, and hence a simple ANOVA rather than the ANCOVA is the appropriate test. There was no difference in the amount of lipid in the accessory glands when looking at accessory glands of equivalent weights between the treatments (Fig. 11b).

a) b)

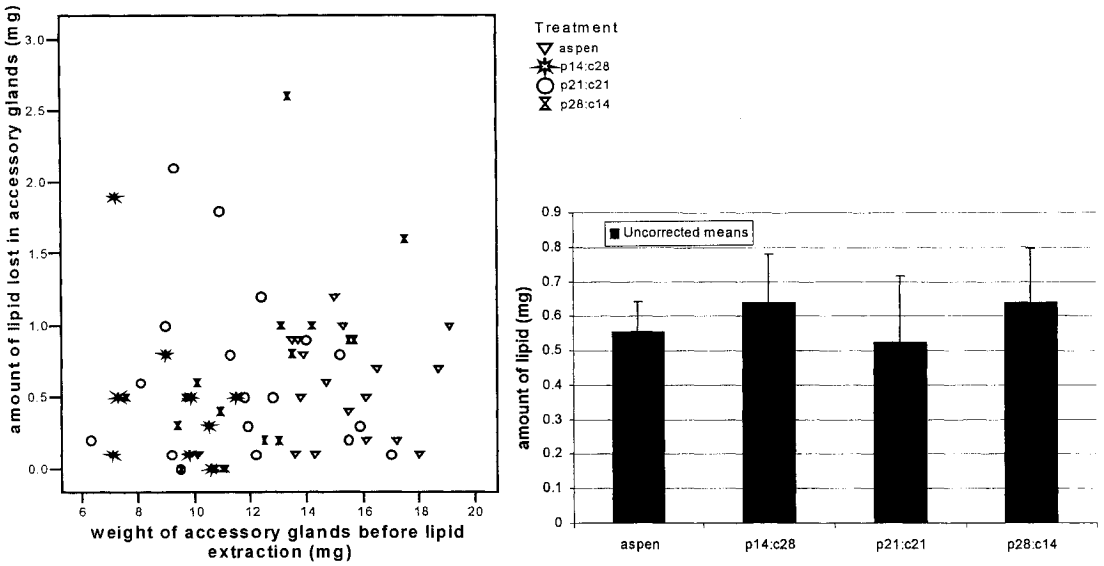


Fig. 11: a) Scatter plot of the amount of lipid lost in the accessory glands on the female accessory gland weight before the lipid extraction for forest tent caterpillars reared on different diets, b) The amount of lipid found in the accessory glands of female moths. The uncorrected means is the average amount of lipid. Bars are the standard error of the mean.

Dependent Variable: Amount of lipid in the accessory glands

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction initial accessory gland weight * Treatment	Hypothesis	3.451	3	1.150	3.940	*.015*
	Error	11.677	40	.292		
Initial accessory gland weight	Hypothesis	.608	1	.608	1.727	.196
	Error	15.128	43	.352		
Treatment	Hypothesis	.150	3	.050	.195	.899
	Error	12.170	47.606	.256		
Replicate	Hypothesis	2.912	15	.194	.552	.894
	Error	15.128	43	.352		

Table 7: Nested ANCOVA results for the effect of treatment on the amount of lipid in the accessory glands, standardized for accessory gland weight before lipid extraction and nested between replicates. The fixed factor was the treatments, co-variate was the accessory gland weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The accessory gland weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the amount of lipid allocated to the ovaries, there was no difference between the slopes of the 4 treatments (Table 8, and Fig. 12a). Also, there was no difference in the amount of lipid in the ovaries when looking at equivalent weights of ovaries between the treatments (Table 8, and Fig. 12), but differences due to replicates were noticed. The weight of the ovaries before lipid extraction affected the amount of lipid in the ovaries (Table 8), such that those that have heavier ovaries contained more lipid.

a) b)

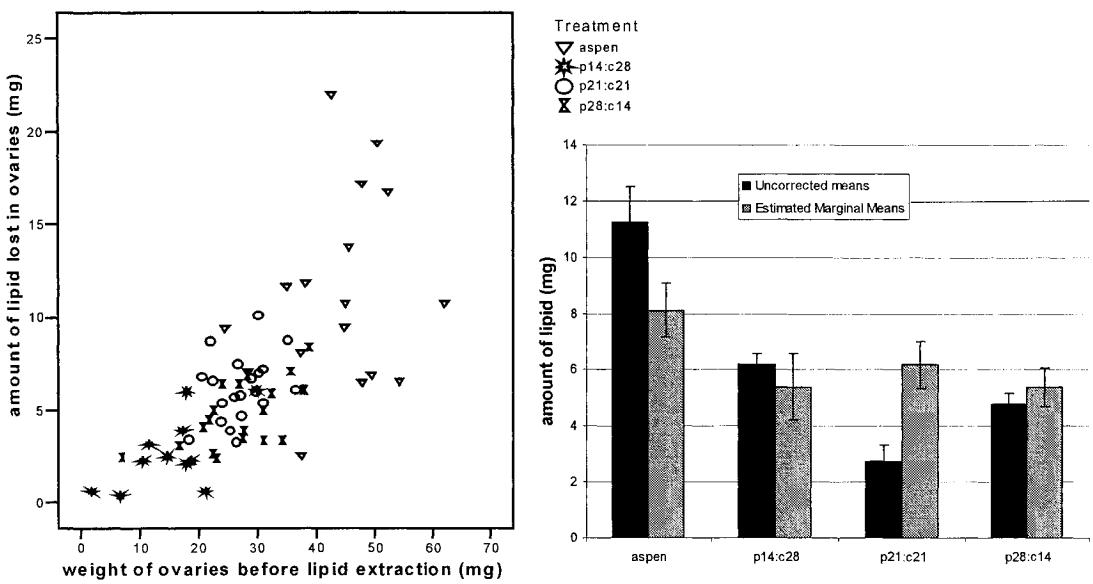


Fig. 12: a) Scatter plot of the amount of lipid lost in the ovaries on the female ovaries weight before the lipid extraction for forest tent caterpillars reared on different diets, b) The amount of lipid found in the ovaries of female moths. The uncorrected means is the average amount of lipid, while the estimated marginal means is the average amount of lipid corrected for the initial ovaries weight before the lipid extraction (co-variate). Bars are the standard error of the mean.

Dependent Variable: Amount of lipid in the ovaries

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction Initial ovaries weight * treatment	Hypothesis	3.014	3	1.005	.142	.934
	Error	311.431	44	7.078		
Initial ovaries weight	Hypothesis	79.282	1	79.282	11.850	*.001*
	Error	314.445	47	6.690		
Treatment	Hypothesis	32.262	3	10.754	1.008	.405
	Error	281.165	26.357	10.668		
Replicate	Hypothesis	202.274	15	13.485	2.016	*.034*
	Error	314.445	47	6.690		

Table 8: Nested ANCOVA results for the effect of treatment on the amount of lipid in the ovaries, standardized for ovary weight before lipid extraction and nested between replicates. The fixed factor was the treatments, co-variate was the ovary weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The ovary weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

For the amount of lipid allocated to the body of the male moth, there was no difference between the slopes of the 4 treatments (Table 9, and Fig. 13a). Furthermore, there was no difference in the amount of lipid in the body of the male moths between the replicates and treatments (Table 9). Only the weight of the moth before lipid extraction affected the amount of lipid in the male moths (Table 9, Fig. 13).

a) b)

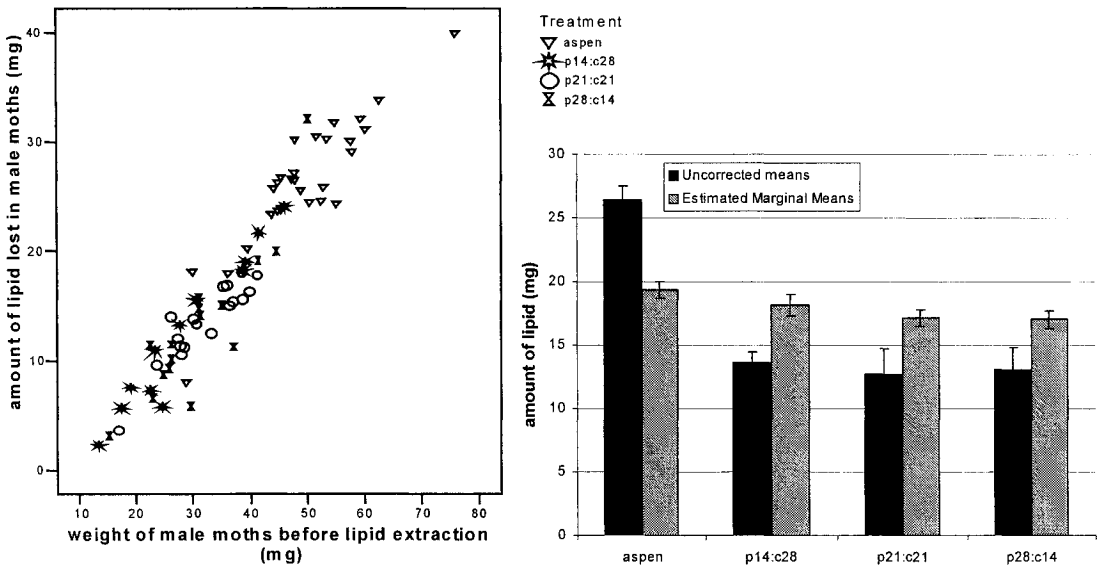


Fig. 13: a) Scatter plot of the amount of lipid lost in the male moth on the moth weight before the lipid extraction for forest tent caterpillars reared on different diets, b) The amount of lipid found in the male moths. The uncorrected means is the average amount of lipid, while the estimated marginal means is the average amount of lipid corrected for the initial moth weight before the lipid extraction (co-variate). Bars are the standard error of the mean.

Dependent Variable: Amount of lipid in the male moth

		Type III Sum of Squares	df	Mean Square	F	Sig.
Interaction moth weight * treatment	Hypothesis	46.569	3	15.523	2.624	.060
	Error	301.668	51	5.915		
Moth weight	Hypothesis	1684.268	1	1684.268	261.180	*<.001*
	Error	348.230	54	6.449		
Treatment	Hypothesis	41.561	3	13.854	2.705	.060
	Error	181.055	35.352	5.121		
Replicate	Hypothesis	68.211	15	4.547	.705	.768
	Error	348.230	54	6.449		

Table 9: Nested ANCOVA results for the effect of treatment on the amount of lipid in male moth, standardized for moth weight before lipid extraction and nested between replicates. The fixed factor was the treatments, co-variate was the moth weight, and replicates were the nested factor. The interaction term indicates the homogeneity of the slopes of the 4 treatments. The moth weight, treatment and replicate factors indicate the significance of the intercepts. df= degrees of freedom, F is the test statistic, and Sig. is the p value ($\alpha > 0.05$).

2.4.4 Nitrogen analysis

There was no significant effect of treatment on nitrogen content in the female moth soma, ovaries and accessory glands (soma: $F_{3, 20}= 2.991$, $P = 0.06$; accessory glands: $F_{3, 20}= 1.389$, $P = 0.275$; ovaries: $F_{3, 20}= 2.566$, $P = 0.083$) (Fig. 14).

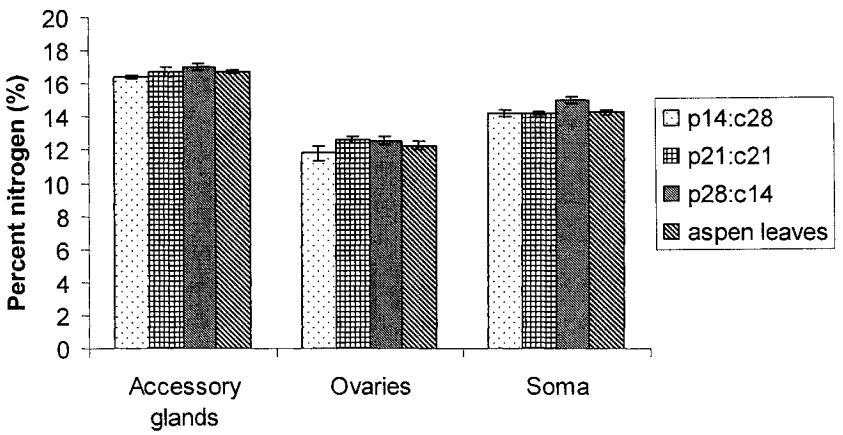


Fig. 14: The percent of nitrogen found in the accessory glands, ovaries and soma of the female moths reared on different diets. Bars are the standard error of the mean.

Additionally, there was no significant effect of treatment on the amount of nitrogen in the male moths ($F_{3, 52}= 1.226$, $p = 0.309$, see Fig. 15).

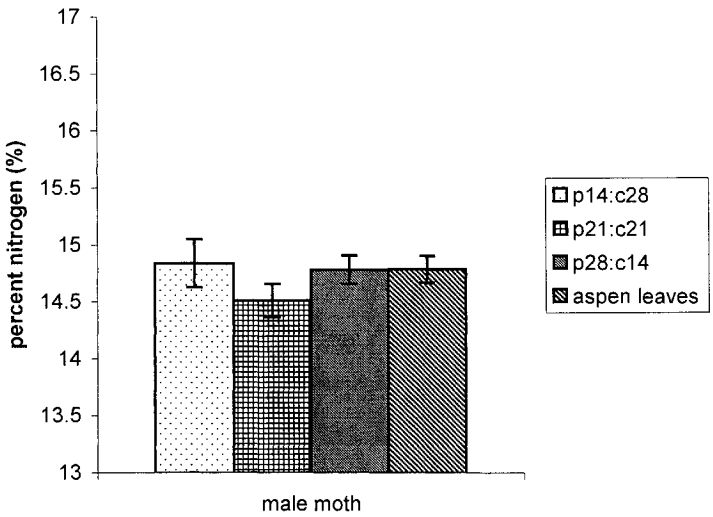


Fig. 15: The effects of the four diets on the percent of nitrogen found in male adults. The bars are standard errors of the mean.

2.5 Discussion

This study demonstrated that forest tent caterpillars perform better on their natural foliage when compared to artificial diets, in terms of survival, growth and offspring quality, as determined by egg weight. Female adults, who were reared on foliage as larvae, contained more lipid in their soma, with no difference in lipid content in their accessory glands and ovaries. This trend was also observed for male body lipid content, although not significant. Aspen, despite its variability in protein: carbohydrate ratio (Hwang and Lindroth 1997) and secondary metabolites, allows forest tent caterpillars to perform better. However, it has been shown that caterpillars can differentiate between specific tree parts. The leaves used in this experiment were sun leaves of the preferred host plant. Tent caterpillars prefer sun leaves compared to leaves found in the shade (Fitzgerald 1995, Panzuto *et al.* 2001), while leaves that are a week old have adverse effects on early development (Jones and Despland 2006). Also, they perform better on aspen than they do on maple (Lorenzetti 1993), but have further been shown to develop better on maple sun leaves than shade leaves (Lévesque *et al.* 2002, Panzuto *et al.* 2001). It has been established that it is difficult to create adequate diets that contain the right balance of nutrients to imitate what can be found in nature (Cohen 2004), however the diet used in this experiment is based on Addy's (1969) study and contains the appropriate proportions of necessary nutrients for forest tent caterpillars. It has been the artificial diet of choice in many experiments where caterpillars have been successfully reared (Grisdale 1985, Despland and Hamzeh 2004, Colasurdo and Despland 2005).

When looking between the artificial diets in this study, we observed that forest tent caterpillars do not perform as well on the carbohydrate-biased diet when compared to the balanced and protein-biased diets. They had lower survivorship, lower pupal weight and slower development times. Female adults whose caterpillars were reared on the low protein diet were smaller, but for each mg of body weight they allocated more weight to their soma, and less to their eggs (Fig. 9). The caterpillars on diets that contained high protein and balanced nutrients (with medium protein) attained similar performance in terms of growth, survival and egg weight. Therefore, this further supports a previous study with this species of caterpillars showing that they attained the best performance on balanced diets and slightly protein-biased diets (Despland and Noseworthy 2006).

Other lepidopterans have also been shown to have a protein-biased intake target. For example, the optimal P:C ratio for *Manduca sexta* is 80% protein: 20% carbohydrate, and for *Helicoverpa zea* this is 79% protein: 21% carbohydrate (Waldbauer *et al.* 1984). *Heliothis virescens* have also been shown to self-select high protein diets (Lee *et al.* 2006). Waldbauer and Friedman (1991) suggest that since lepidopterans have short life cycles, the high proportion of protein during the larval stage permits rapid growth to the pupal stage. Forest tent caterpillars acquire all nutrients for adults as larvae, since the adult does not feed; therefore, protein at this stage is important.

Furthermore, this study demonstrated that body composition was tightly regulated. We were able to further investigate fitness parameters by looking at the allocation of weight, lipid and nitrogen to the adult body. When studying the artificial diets differing in nutrient quantity, no difference in amount of body lipid and nitrogen found in the body parts was observed. Although the caterpillars were fed diets that

different proportions of protein and digestible sugars, in the end they attained the same proportion of lipid and nitrogen within the adult bodies. Thus, this suggests that the forest tent caterpillars seem to be maintaining their body composition despite the different diets by regulating protein and carbohydrate closely. However, it is not known whether this regulation occurs during the pupal phase, or during metamorphosis. Such post-ingestive compensatory responses have also been reported in a number of other caterpillars. For example, Lee *et al.* (2002) found that for *Spodoptera littoralis* pupae, despite having eaten different amounts of protein, arrived at similar points of body nitrogen content. Also, there was slight evidence that the caterpillars regulated body lipid content, as shown by the efficiency with which ingested carbohydrate was converted to lipid decreased as carbohydrate content increased. The authors suggest that this is due to a post-ingestive regulatory mechanism allowing the caterpillars to release excess carbohydrate from their body (Lee *et al.* 2002). In addition, *Heliothis virescens* rely on post-ingestive processing of nutrients to regulate growth (Telang *et al.* 2001).

The novelty of this study is that the differences seen between the body components on the different artificial treatments varying in nutrient ratio are weight related, and not based on body composition. It was found that the weight and composition of the accessory glands were the same between treatments; only the total moth weight affected the weight of the accessory glands. Surprisingly, the fecundity of the females depended only on the mass of the adult moth and not diet, while the composition of nitrogen and lipid in the eggs remained the same across treatments. This is in contrast to studies that have demonstrated the variation of egg weight depends on geographic location within species (Harvey 1983, Parry *et al.* 2001).

Despite egg numbers being the same across treatments, the weight of the eggs did vary. When caterpillars were reared on low protein diets, eggs were lighter. Soma weights between treatments were also different. Accordingly, the caterpillars who fed on low protein diets had the lightest somas as adults, however, they allocated more to their somas in proportion to their weight thus, less to their eggs. Consequently, their eggs were of a smaller mass, and the total weight of the ovaries was smaller. This suggests that allocation of weight to the soma and accessory glands are slightly more regulated than the distribution in the eggs. Fig. 9 demonstrates that the ovary weight is more variable among the different body parts. The soma and accessory gland weights varied by 1.6 and 1.8 times, respectively, between the low protein and aspen diets, while ovary weight varied by 2.8 times between the treatments.

Thus, protein deficiency did have negative consequences, in terms of larval survivorship and the weight of individual eggs. Several studies have demonstrated that protein is a growth-limiting factor (McNeill and Southwood 1978, Mattson 1980). In many cases, low total soluble sugar content in diets tends to slow larval development (Bidon 1993, Carisey and Bauce 2002, Lee *et al.* 2004). As well, moulting to the next instar appears linked to protein content instead of carbohydrate (Despland and Noseworthy 2006). Other lepidopterans have been shown to be negatively affected by the concentration of dietary nutrients in their food in terms of pupal mass (*S. littoralis*: Lee *et al.* 2002; *Heliothis virescens*: Telang *et al.* 2002; *Spodoptera exigua* and *Heliothis zea*: Broadway and Duffey 1986). Here, we confirm effects on pupal mass and show that additional effects are due to differences in weight allocation. Protein-limited caterpillars give rise to moths that are lighter, and have smaller eggs.

Overall, this protein deficiency may lead to fitness implications due to nutrient deficiencies. Carisey and Bauce (2002) show even farther reaching effects of diet on the next generation. The impact of poor quality diets on the parental generation can affect subsequent early progeny development. This was seen in the case of spruce budworm populations, where parental nutrition influenced the quality and size of the progeny. However, the authors suggest that the benefit for the progeny whose mothers were fed poor quality diets is that they are able to withstand longer periods of starvation just after emergence from diapause, and may have higher chances of surviving to adulthood (Carisey and Bauce 2002). Additionally, the nutritional history of the maternal *Lymantria dispar* (gypsy moth) parent affected the tendency of the offspring to disperse. When the mothers are nutritionally stressed they tend to have lower pupal weights and small numbers of eggs, and the offspring have a lower tendency to disperse (Diss *et al.* 1996). Therefore, inadequate diets have effects beyond fecundity and can influence not only the quantity but also the quality of offspring.

Egg size is correlated with the fitness of the second generation (Sinervo 1993, Stearns 1992), and can therefore be used as an index of the success of progeny. Female reproductive success is generally nutrient-limited (Wheeler 1996), with the amount of nutrients available to the maternal parent influencing the weight of eggs (Rossiter *et al.* 1988). It has been suggested that smaller eggs lead to lower probability of hatching (McCowan 1952). In the European grapevine moth *Lobesia botrana,* the larger the size of the eggs that were laid, the larger the size of the larvae that hatched. These larger larvae had a better ability to endure starvation than smaller ones (Torres-Vila and Rodriguez-Molina 2002). Thus, such life-history traits influenced by inadequate diets and

transmitted to offspring *via* maternal effects could lead to herbivore outbreaks (Rossiter 1994, 1996), which would be of great importance in understanding population dynamics for defoliator insects. Thus, this establishes the possibility that eggs in this study may be affected by nutritional stress.

Furthermore, lipid reserves in the soma seem to be less regulated than lipid allocation to the eggs and accessory glands. The adult females whose caterpillars were reared on aspen contained more lipid than those on the artificial diets, while a similar trend was observed in males but not as significant.

No clear evidence was found for the oogenesis-flight trade-off. When the caterpillars were reared on high protein diet, the eggs were heavier relative to the soma. But the soma is overall larger so it is unclear if this would diminish flight. It seems that when they have enough protein they invest the surplus in bigger eggs, as evidenced by the moths being larger overall but allocating more to their eggs than soma. The caterpillars only slightly invest surplus carbohydrates into more body lipid, seen by only the slightly greater amount of lipid in the soma. Thus, not a great deal of evidence for a diet mediated trade-off is seen because soma lipid does not greatly vary.

Although this study suggests that forest tent caterpillars are able to regulate their protein and carbohydrate post-ingestive use, it is not known at which stage in the development this occurs. The nutritional requirements of insects change over their lifetime because of changing demands for growth, reproduction and adult dispersal (Stockhoff 1993, Slansky and Scriber 1985). A shift in the relative importance of protein and lipid is compatible with the common observation that later-instar larvae contain a greater proportion of lipid in their bodies (Slansky and Scriber 1985). In tent caterpillars

it has been established that total lipids in the larvae gradually increase as the larvae develop, until the formation of the pupae. Lipids decrease rather rapidly in the eggs after deposition to extremely small quantities for the maintenance of the larvae while overwintering in the egg cases, and continue to decrease slowly until the larvae hatch (Rudolfs 1926a). Nitrogen content also varies throughout the insect's life cycle; Rudolfs (1926b) observed that total nitrogen increases rapidly during the period when the larvae are forming inside the eggs and decreases as larvae hatch and begin feeding. Therefore, this variation in lipid and nitrogen throughout the life cycle of the tent caterpillar demonstrates ontogenetic changes. This indicates that body composition changes in time and is regulated at certain levels during development. Therefore, diet effects on body composition of caterpillars might not be a good predictor of composition in the adults.

These findings confirm that forest tent caterpillars perform better on their natural foliage, and protein-biased artificial foods. Protein deficiency did have negative fitness consequences, in terms of larval survivorship, fecundity and quality of offspring (predicted from individual egg mass). Post-ingestive mechanisms can compensate for the variation in food nutrient content, such that the insects on high and medium protein diets attained the same performance. We were able to establish that forest tent caterpillars regulate their body composition despite variation in food nutrient ratios, and that this is more variable for eggs. Fitness implications of low protein in the diets are small body weight and lighter eggs (and likely lower offspring survival), but no significant differences in lipid reserves for flight energy were observed.

Chapter 3
The feeding pattern and time budget of the forest tent caterpillar *Malacosoma disstria* on aspen and artificial diet

3.1 Abstract

The feeding behaviour of mid-fourth instar forest tent caterpillars was evaluated on three artificial diets, as well as their preferred plant host, aspen. Our primary focus was on their taste preference during the first 2 hours, and then their feeding pattern for 48 hours. Their exploratory behaviour was also monitored to determine the amount of time it takes for the caterpillars to leave a nutritionally poor food source for a nutritionally rich source.

Our results demonstrate that the caterpillars do not initially differentiate between diets varying in carbohydrate and protein concentration in terms of their overall time spent eating, which was also verified in the long-term. When exposed to diets poor in protein content, the caterpillars are more active and have short feeding bouts. As protein content increases they eat less frequently, due to longer interfeed durations, but they eat for longer within a feeding period. These differences in feeding patterns between the nutritionally varying diets can be related to post-ingestive effects *via* haemolymph trehalose levels. Furthermore, there was no difference in exploratory behaviour between the artificial diets.

Although aspen diets support better growth, it was seen that when on aspen, caterpillars are active, have short feeding durations and short interfeed pauses. In addition, the caterpillars on the aspen diet are more likely to leave the trail and consume

more of the artificial diet when given a choice. This might possibly be explained by the lack of familiarity with foliage.

3.2 Introduction

Although caterpillars are known as eating machines, they do not spend their entire time feeding. Most will eat intermittently, alternating between periods of rest, activity and eating. Feeding depends on phagostimulatory input from the food source being eaten; thus an inadequate concentration of phagostimulants may lead to early cessation of feeding (Bernays and Simpson 1982). Primary cues used by insects to detect food are chemical compounds, usually sugars in caterpillars (Panzuto *et al.* 2001). Compensatory feeding experiments have demonstrated that caterpillars show an initial preference for foods containing carbohydrates even despite prior nutritional deficiencies (Despland and Noseworthy 2006). Lee *et al.* (2002) established that total food consumption for *Spodoptera littoralis* is greatest on carbohydrate-biased food. Furthermore, *S. littoralis* (Simmonds *et al.* 1992; Simpson *et al.* 1988) and *Grammia geneura* (Bernays *et al.* 2004) have been shown to have longer first feeding bouts on carbohydrate-containing foods. However, the attractiveness of the food does not necessarily mirror its appropriateness for larval or adult performance. In some situations the ingestion of a toxic food that may taste good leads to temporary sickness and consequent food-aversion learning or even possible death (Slansky and Rodriguez 1987).

Feeding patterns vary greatly among individual insects (Simpson 1995). Individual feeding rhythms depend mostly on factors that determine when feeding is initiated, including the developmental and nutritional state of the insect, environment, and the type and availability of the food (Simpson 1995). For example, the time since the previous feeding event, size and nutritional quality of the previous meal, defecation and surrounding neighbours can all affect when meals begin (see references in Simpson

1995). The likelihood that an insect provided with food will begin to feed increases as a function of time since the end of the last feeding event; large meals reduce feeding frequency for longer periods than small ones. Feeding cessation depends on volumetric factors, the size of the preceding meal and the nutritionally quality of the meal, while the length of the interval between feeding bouts depends on interactions occurring physiologically within the insect (ex. gut emptying, haemolymph concentrations) and behaviourally in relation to the environment (ex. temperature) (Bernays 1985, Simpson 1995).

The main question asked when studying feeding patterns is what constitutes a feeding bout? Feeding bout determination can be achieved through the use of log-survivorship analyses. Log-survivorship analyses of the distribution of gaps between periods of feeding indicate the presence of a clear bout criterion for distinguishing intrafeed pauses from interfeed intervals (Simpson 1995). Thus, using this, feeding bouts can be established, and analysing the distribution of feeding bouts will show a distinction between periods of feeding versus sampling events (Simpson 1995).

After having established what constitutes a feeding bout, feeding rhythms can be investigated. These can provide information about the insect's physiological responses to a food. The probability of initiating feeding after contact with a food reflects its phagostimulatory power. The duration of the first feeding event reflects both the food's taste and its effects on digestive physiology (Simpson and Raubenheimer 2000). The duration of the interval between feeding bouts reflects post-ingestive responses to the previous meal.

Most of the temporal analysis of feeding behaviour in insects has concentrated on *Locusta migratoria* (Simpson 1982, Simpson 1995). Locusts' feeding behaviour is known to occur in bouts that are separated by periods of activity without feeding (Simpson 1982). The difference between caterpillars and locusts is that there is a clear meal termination - locusts leave their feeding site between feeding bouts, while caterpillars remain relatively close (Reynolds *et al.* 1986). The feeding rhythm of the caterpillar *Manduca sexta* has been quantified. On inadequate diets Timmins *et al.* (1988) demonstrated that *M. sexta* compensated for low food quality by spending more time eating. On diets diluted with water they spent more time eating by having longer individual feeding bouts, but the frequency of these bouts was not affected. This would be expected, since when the food contains more water it will take the caterpillars longer to eat the amount required to supply the appropriate quantity of nutrients. On diet diluted with cellulose, the caterpillars showed increased bout lengths, as well as increased frequency of bouts. Contrary to *M. sexta,* locusts respond to differences in levels of dietary protein by reducing the time between meals on low protein diets but keeping the same meal size (Simpson and Abisgold 1985). When fed food with 14% protein the locusts ate the same size meals, just more frequently than those fed the 28% protein food. The authors suggest that this was due to haemolymph osmolality and the concentration of various free amino acids regulating the time between meals (Abisgold and Simpson 1987).

Foraging patterns in forest tent caterpillars are achieved through the use of trails, to which they closely adhere (Colasurdo and Despland 2005). When individuals travel they deposit silk marked with pheromone. The silk is produced in the labial glands, while

pheromone is secreted from the posterior end of abdomen (Fitzgerald 1995). Forest tent caterpillars use this chemical communication to facilitate group foraging, to maintain colony cohesion and to orient their conspecifics (Costa and Pierce 1997, Fitzgerald 1995), leading recruited caterpillars to reinforce the pheromone trails. The long-standing nature of their trails and their gregarious behaviour leads to the eventual regrouping of the caterpillars to new locations (Fitzgerald and Costa 1999).

The objective of this study was to investigate individual feeding patterns of fourth instar forest tent caterpillars eating aspen and artificial diets, and to determine whether these diets affect their exploration. It is known that caterpillars initially prefer the taste of sugar, but it has been established in the first part of this thesis that they have greater fitness on the p28:c14 diet when compared to the p14:c28 diet (heavier pupal weight, greater larval survivorship, and heavier individual eggs). However, in the long-term, when given a choice, these caterpillars consume 50:50 of the two diets (Despland and Noseworthy 2006). Therefore, differences in performance have been demonstrated based on these diets, thus we want to determine what effects these diets have on foraging patterns. A shorter latency to start a meal is expected on the high carbohydrate diet due to its phagostimulatory influence, while the patterns of feeding (the duration of the feeding bouts and the interfeed intervals between feeding periods) are expected to be different between adequate and inadequate diets.

Furthermore, forest tent caterpillars have been shown to be more active on food that is suboptimal (Dussutour *et al.* unpublished data), thus have a greater tendency to leave the trail. It has been observed that when faced with inadequate nutrient sources, increasing exploration is a way in which insects can locate a nutritionally superior source

(Barton Browne 1993). We wanted to determine whether forest tent caterpillars on poor foods are more likely to leave a trail that has already been established and go explore a new area. It is predicted that caterpillars on inadequate food sources will be more likely to leave the trail and find the better food source.

3.3 Method and Materials

3.3.1 Experimental insects and diets

Laboratory colonies of the forest tent caterpillar were reared from egg masses collected October 2005, 40 km North East of Wabasca, Alberta. The egg bands were washed for 1 min 30 sec in 5% sodium hypochlorite, and then rinsed in water for 5 min. They were then rinsed again in 0.06% sodium hypochlorite and air-dried (Grisdale 1985). They were maintained on a 16:8 hour photoperiod in growth chambers.

During the first instars (instar 1 to instar 4) 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 four treatments. The treatments consisted of artificial diets with different protein to carbohydrate dry weight concentrations: 14% protein: 28% carbohydrate, 28% protein: 14% carbohydrate, and 35% protein: 7% carbohydrate, as well as trembling aspen foliage collected from Parc Écologique des Sansonnets in Brossard, Québec. The high protein diet (p35:c7) was chosen for this experiment because it was thought not to be a phagostimulatory diet due to the low concentration of sugar. Furthermore, in choice tests forest tent caterpillars consume less of this diet compared to the others (Despland and Noseworthy 2006). As described earlier, protein was given in the form of casein, while the carbohydrate consisted of dextrose, and the diets were a composite of 6: 1 agar solution: dry ingredients ratio. Leaves were washed in 1% bleach (0.06 % sodium hypochlorite) for 5 min and then rinsed in water (Hemming and Lindroth 2000). They were placed in microcentrifuge tubes containing water.

3.3.2 Short-term behaviour

3.3.2 a. Observations

Observations were done on mid-fourth instar caterpillars. Caterpillars were starved individually in Petri dishes (4 inches) for 2, 4 or 18 hours. A small square piece of food or the leaf was placed into the Petri dish. Using the pocket Observer 5 program (Noldus Information Technology 2005, Wageningen, Netherlands) interval scans of 40 seconds documented the behaviour of the caterpillar for 2 hours. The types of behaviours recorded were: contacting the food, eating the food, and off the food.

3.3.2 b. Statistical analysis

To determine whether there were any differences in the duration of the first feeding bouts between the different treatments (a measure of phagostimulatory power) and the amount of time that the caterpillars were starved, a two-way analysis of variance (ANOVA) was performed. A Tukey post-hoc test was conducted to determine the differences between the starvation times. Kruskal-Wallis analyses were performed to determine whether there are differences between the treatments in the number of caterpillars that contacted and ate the food, that contacted the food but did not eat, and that did not contact the food. ($N_{aspen} = 82$, $N_{p14:c14} = 108$, $N_{p28:c14} = 108$, $N_{p35:c7} = 102$). All statistical tests were conducted with SPSS for Windows (v. 10-12, SPSS Inc. Chicago, U.S.A).

3.3.3 Long-term Behaviour

3.3.3 a. Feeding pattern

Experimental insects were reared as above. Individual mid-fourth instar caterpillars were placed in 1 compartment of a Petri dish (14 cm) that was divided into 4 sections using cardboard separators. A piece of food or the leaf 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 hours (using 1 image per second). (N_{aspen} = 17, $N_{p14:c28}$ = 17, $N_{p28:c14}$ = 19, $N_{p35:c7}$ = 17).

3.3.3 b. Effect of diets on exploratory behaviour

After the 48 hours, the experimental insects from above were then tested to determine their exploratory behaviour. The cardboard barrier separating the compartments (zone 1 from zone 2) in the Petri dish (14 cm) was removed, and a piece of balanced artificial diet (21% dry weight protein: 21% dry weight carbohydrate) was placed at the other end of the dish (in zone 2). The caterpillars were filmed using 1 image per second for 24 hours, and humidity was kept constant. This part of the experiment was done in order to determine how long it takes the caterpillars to make the decision to leave the pre-established trail and move to the new (food 2) source. (Number of caterpillars that contacted the second source: N_{aspen} = 13, $N_{p14:c28}$ = 13, $N_{p28:c14}$ = 9, $N_{p35:c7}$ = 6).

3.3.4 Statistical analysis

3.3.4 a. Feeding pattern

Eight-hour observations were conducted on all caterpillars to test the latency between contact and feeding and the duration of the first feeding event as measures of phagostimulatory power. Forty-eight hour observations were conducted for six to eight caterpillars per treatment ($N_{aspen} = 8$, $N_{p14:c14} = 7$, $N_{p28:c14} = 7$, $N_{p35:c7} = 6$). The parameters tested were feeding event durations, interfeed pauses (periods of no eating between feeding periods), number of feeding events, total time spent eating, total time spent resting, total time spent in motion, and latency to first rest event. This was done by recording both the start and end times of the events. These parameters were tested for normality and then analyzed to determine if there were significant differences between the treatments using Kruskal-Wallis tests for the non-parametric data, and one-way ANOVAs for the parametric data.

To calculate the above parameters that describe the pattern of feeding of the caterpillars, log-survivorship analyses were conducted. By looking at the frequency distribution of pauses (gaps) between feeding events it is possible to distinguish intrafeed pauses (pauses within a feeding period) from interfeed pauses (pauses between feeding periods) by means of determining a "bout criterion". Pauses that are shorter than the "bout criterion" are considered intrafeed pauses, while pauses greater than the bout criterion are interfeed pauses. Using these intrafeed and interfeed pauses the feeding pattern can be determined by breaking an individual's feeding rhythm into a series of feeding periods. Then looking at the frequency distribution of the feeding bouts will

demonstrate a "meal criterion", distinguishing between feeding and sampling events (Simpson 1995). In order to create a log-survivorship distribution, the data for each treatment was pooled across all individuals and a plot of the fraction of individuals still performing an event (log transformed) as a function of time was performed. If feeding occurred randomly with time during an observation period, then gaps between feeding episodes would follow an exponential distribution. Therefore, the probability of starting to eat after a gap would be independent of the length of that gap. However, behaviour tends to be structured in bouts and gaps, where short gaps represent pauses within bouts, and long gaps represent pauses between bouts (Sibly *et al.* 1990). If gaps are divided between intrafeed pauses and interfeed intervals, the log survivorship curve should resemble a broken-stick model (the sum of two exponentials) and the bout criterion is given by the break in the curve (Simpson 1995, Sibly *et al.* 1990). The linearity of the log-survivorship curves was confirmed using linear regressions. To obtain these slopes many regressions of the pauses between feeding events per treatment are conducted and the one that best fits (with the best R^2) is the one chosen (Jeanson *et al.* 2003). All slopes indicated in the text are given with $\pm$ $C.I._{0.95}$ (95% confidence intervals).

The analyses were done to compare the artificial diets to each other, and then were re-done to determine the effects of the aspen treatment relative to the artificial diets. All statistical tests were conducted with SPSS for Windows (v. 10-12, SPSS Inc. Chicago, U.S.A).

3.3.4 b. Effects of diets on exploratory behaviour

Eight-hour observations were conducted on all caterpillars for the second part of the experiment, after the cardboard barrier was removed. The parameters tested were the number of feeding events on the original food source, the number of feeding events on the second food source, total time spent eating both sources, and the latency to contact the second food source. These parameters were analyzed using one-way ANOVAs or non- parametric Kruskal–Wallis tests. The analyses were done to compare the artificial diets to each other, and then were re-done to determine the effects of the aspen treatment relative to the artificial diets. All statistical tests were conducted with SPSS for Windows (v. 10-12, SPSS Inc. Chicago, U.S.A).

3.4 Results

Kolmogorov-Smirnov tests were performed to determine whether the data were normally distributed ($p > 0.05$). The corresponding parametric or non-parametric analyses were then conducted.

3.4.1 Short-term behaviour

A two-way analysis of variance demonstrated that there was no significant difference ($p > 0.05$) in the duration of the first contact with the food between the different diets, but there was a difference due to starvation time ($F_{2,388} = 11.985$, $p < 0.001$) (Fig. 16). Caterpillars that were previously starved for 18 hours took longer first feeding events than those deprived of food for 2 or 4 hours.

The amount of time that the caterpillars spent eating during the 2 hours was not significantly different between treatments, however, there was a difference due to the amount of time that the caterpillars were deprived of food ($F_{2,388} = 23.923$, $p < 0.001$). The caterpillars that were previously starved for 18 hours spent the longest time eating regardless of food source (Fig. 17).

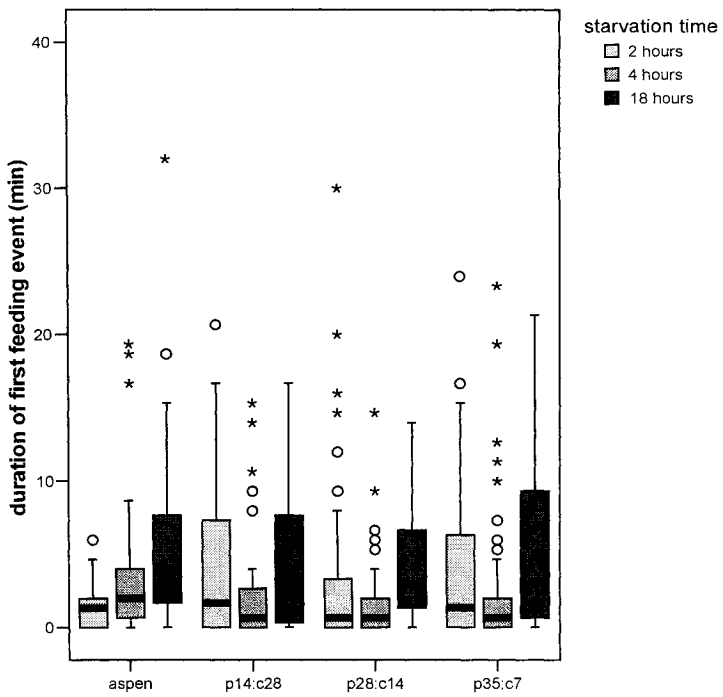


Fig. 16: The duration of the first feeding event (min) per treatment when the caterpillars have been starved for 2, 4 or 18 hours. The dark bars within the boxes represent the median, while the box is the first quartile, the whiskers are the second quartile, the circles are outliers (cases with values 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).

No significant differences were observed between treatments for the number of caterpillars that contacted and ate the food ($\chi^2 = 2.178$, df= 3, $p = 0.536$), the number of caterpillars that contacted the food but did not eat ($\chi^2 = 2.031$, df= 3, $p = 0.566$), and the number of caterpillars that did not contact the food ($\chi^2 = 4.981$, df= 3, $p = 0.173$) (Table 10).

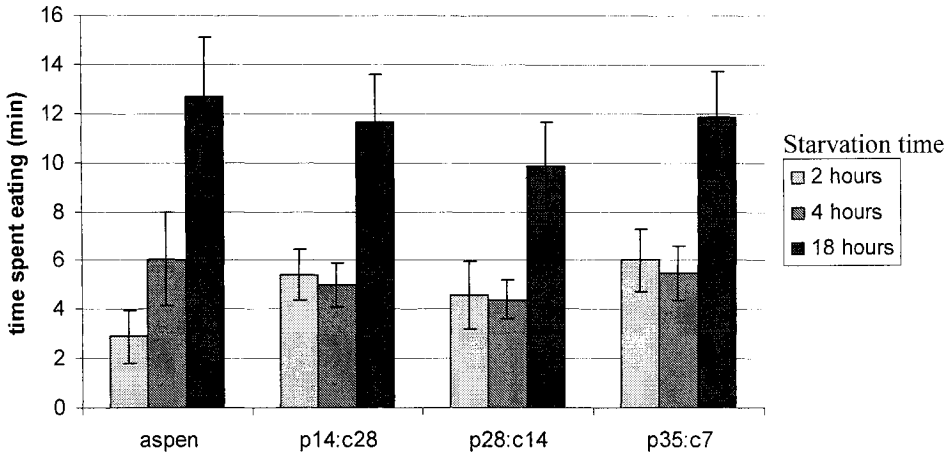


Fig. 17: The amount of the that the caterpillars spent eating the food source during a 2 hour period once they were previously food deprived for 2, 4 or 18 hours. The bars are the standard error of the mean.

Treatment	# caterpillars	# caterpillars that contacted and ate the food	# caterpillars that contacted but did not eat food	# caterpillars that did not contact food
Aspen	82	59	10	13
p14:c28	108	68	11	29
p28:c14	108	68	15	25
p35:c7	102	68	17	17

Table 10: The number of replicates (caterpillars) per treatment during a two-hour observation period, pooling starvation times.

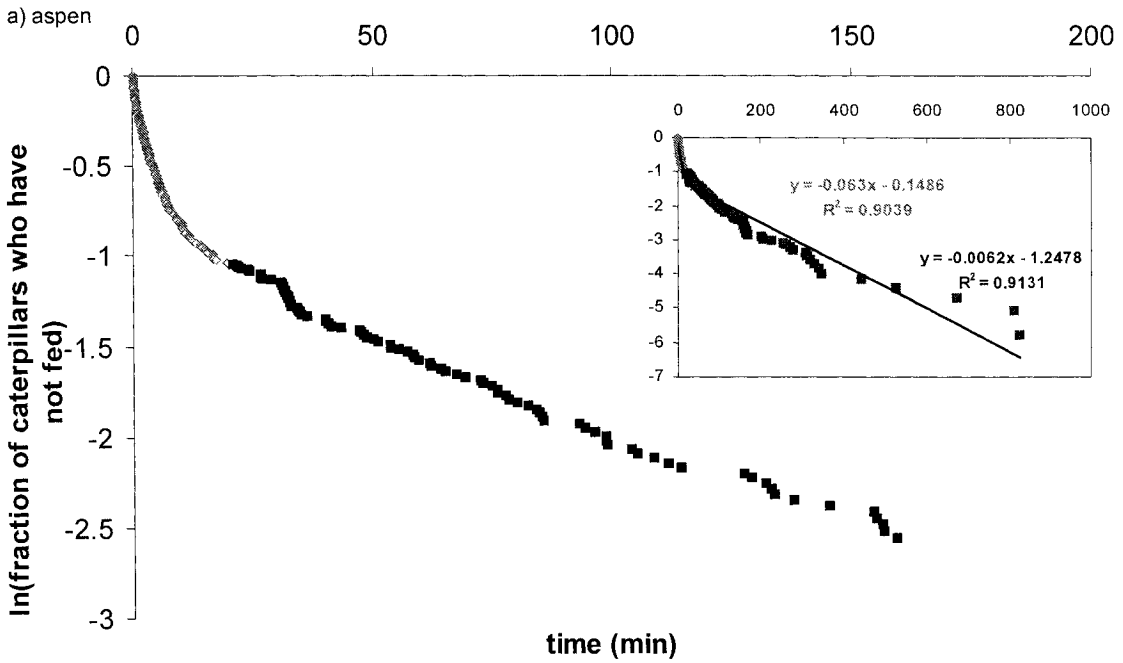
Thus, in the short-term, forest tent caterpillars do not show a difference in the duration of their first feeding events and in the total time spent feeding on the different food sources.

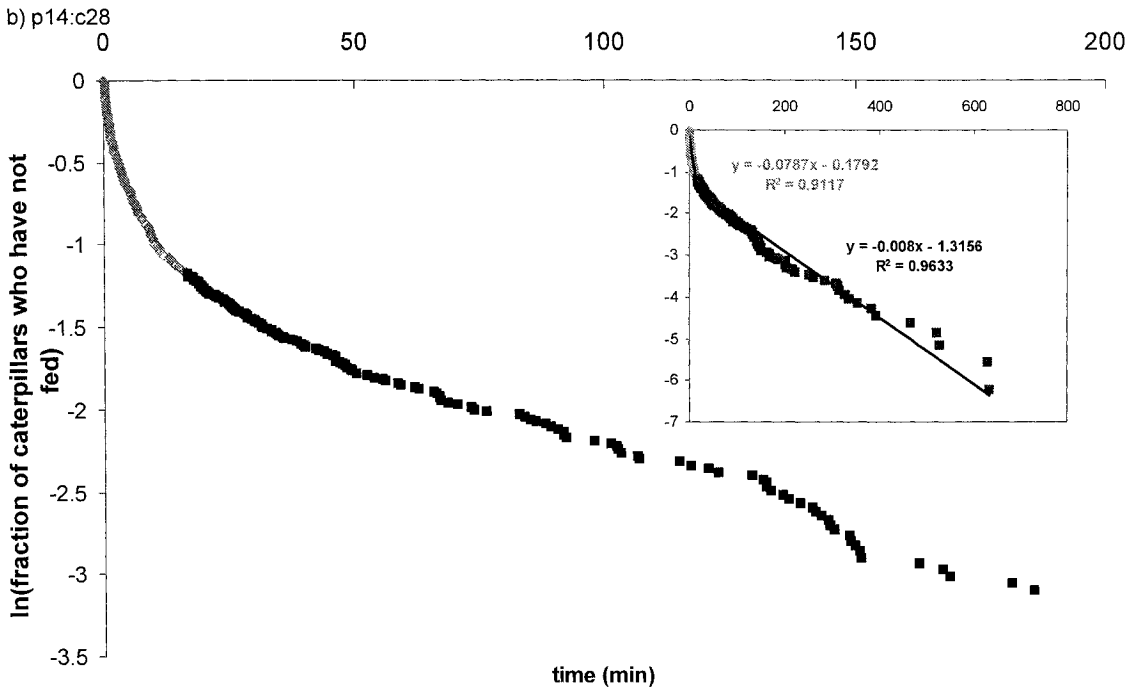
3.4.2 Long-term behaviour

3.4.2 a. Description of feeding pattern

To determine the bout criterion, log survivorship analysis was performed on the pauses between the feeding events. From this, the bout criterion was found to be approximately 16.67 min (1000 sec) (see breaks for each treatment in Fig. 18). Therefore, two types of pauses were characterized: intrafeed pauses (pauses within a feeding period) of less than 16.67 min, and interfeed pauses (pauses between feeding periods) greater than 16.67 min. These survivorship curves also allow us to determine the probability at which the caterpillars will start eating again after their pause. The probability to start eating for the first 16.67 min is high, and is similar for all the treatments. After 16.67 min, the probability to start eating decreased, with those on the p28:c14 diet having the lowest (8.6×10^{-5}/s, see Fig. 18). The duration of the interfeed pauses will be compared between treatments later.

Using the bout criterion of 16.67 min, feeding events were grouped into a series of feeding periods separated by interfeed intervals of more than 16.67 min. Using the log-survivorship analysis to look at feeding bouts (the feeding events occurring during a feeding period) over the experimentation period, no meal criterion could be established (Fig. 19). In other words, feeding events are not divided between feeding and sampling events, but instead their durations are randomly distributed. After about 15 minutes, the fraction of caterpillars that are still eating decreases dramatically (Fig. 19). Here the caterpillars reach a point of satiation and the probability to stop eating becomes high. Comparisons of the duration of feeding bouts between the diets will be mentioned later.


a) aspen
0
50
100
150
200
0
-0.5
-1
-1.5
-2
-2.5
-3
ln(fraction of caterpillars who have not fed)
time (min)
0
200
400
600
800
1000
y = -0.063x - 0.1486
R² = 0.9039
y = -0.0062x - 1.2478
R² = 0.9131


b) p14:c28
0
50
100
150
200
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
ln(fraction of caterpillars who have not fed)
time (min)
0
200
400
600
800
y = -0.0787x - 0.1792
R² = 0.9117
y = -0.008x - 1.3156
R² = 0.9633

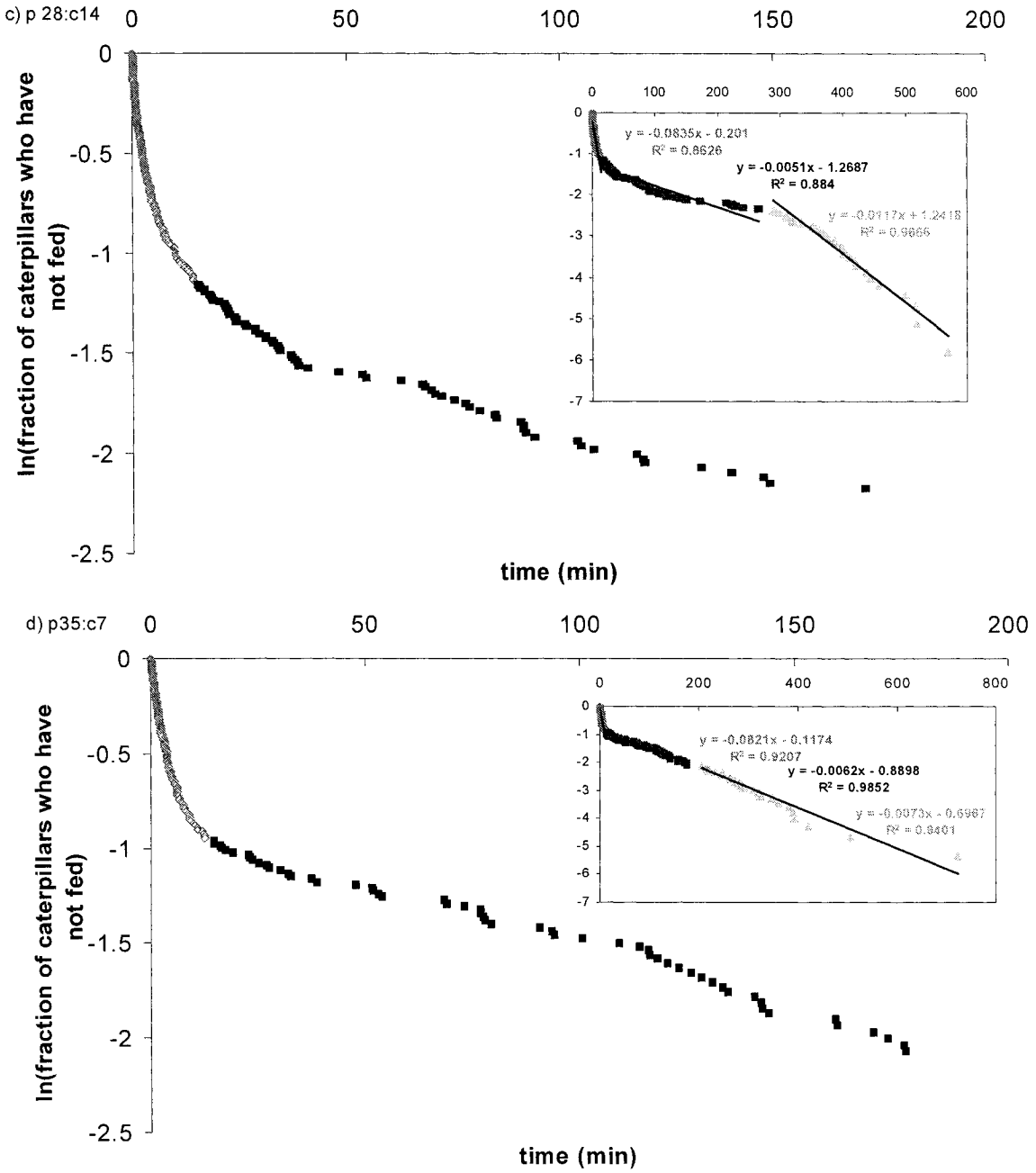


Fig. 18: Log-survivorship curves for the pauses between feeding events when feeding on a) aspen, b) p14:c28, c) p28:c14 and d) p35:c7. Two types of intervals were noticed demonstrated by the break in the log curves (intrafeed: pauses < 16.67 min and interfeed: pauses > 16.67 min). A linear regression demonstrated the linearity of the distributions for each treatment (equations shown on the inserted figures). The probability of the caterpillars to start feeding during the first 16.67 min were a) 1.1 x 10^{-3}/s +/- 4.0 x 10^{-5}C.I.$_{95}$, b) 1.3 x 10^{-3}/s +/- 4.0 x 10^{-5} C.I.$_{95}$, c) 1.4 x 10^{-3}/s +/- 7.0 x 10^{-5} C.I.$_{95}$ and d) 1.4 x 10^{-3}/s +/- 7.0 x 10^{-5} C.I.$_{95}$. These probabilities decrease after 16.67 min: a) 1.2 x 10^{-4}/s +/- 6.0 x 10^{-6} C.I.$_{95}$, b) 1.3 x 10^{-4}/s +/- 4.0 x 10^{-6} C.I.$_{95}$, c) 8.6 x 10^{-5}/s +/- 7.4 x 10^{-6} C.I.$_{95}$, and d) 1.0 x 10^{-4}/s +/- 4.0 x 10^{-6} C.I.$_{95}$. After 200 min the probabilities to start feeding increase again for c) 2.0 x 10^{-4}/s +/- 5.0 x 10^{-6} C.I.$_{95}$ and d) 1.3 x 10^{-4}/s +/- 1.3 x 10^{-5} C.I.$_{95}$.

Using the information found about the pauses between feeding events, the following feeding behaviour for the forest tent caterpillar has been established (Fig. 20). Feeding bouts are considered the sum of feeding events separated by intrafeed pauses less than 16.67 min. A feeding period is the sum of the feeding activity, as well as the intrafeed pause, and is separated from other feeding periods by interfeed intervals greater than 16.67 min (Fig. 20).

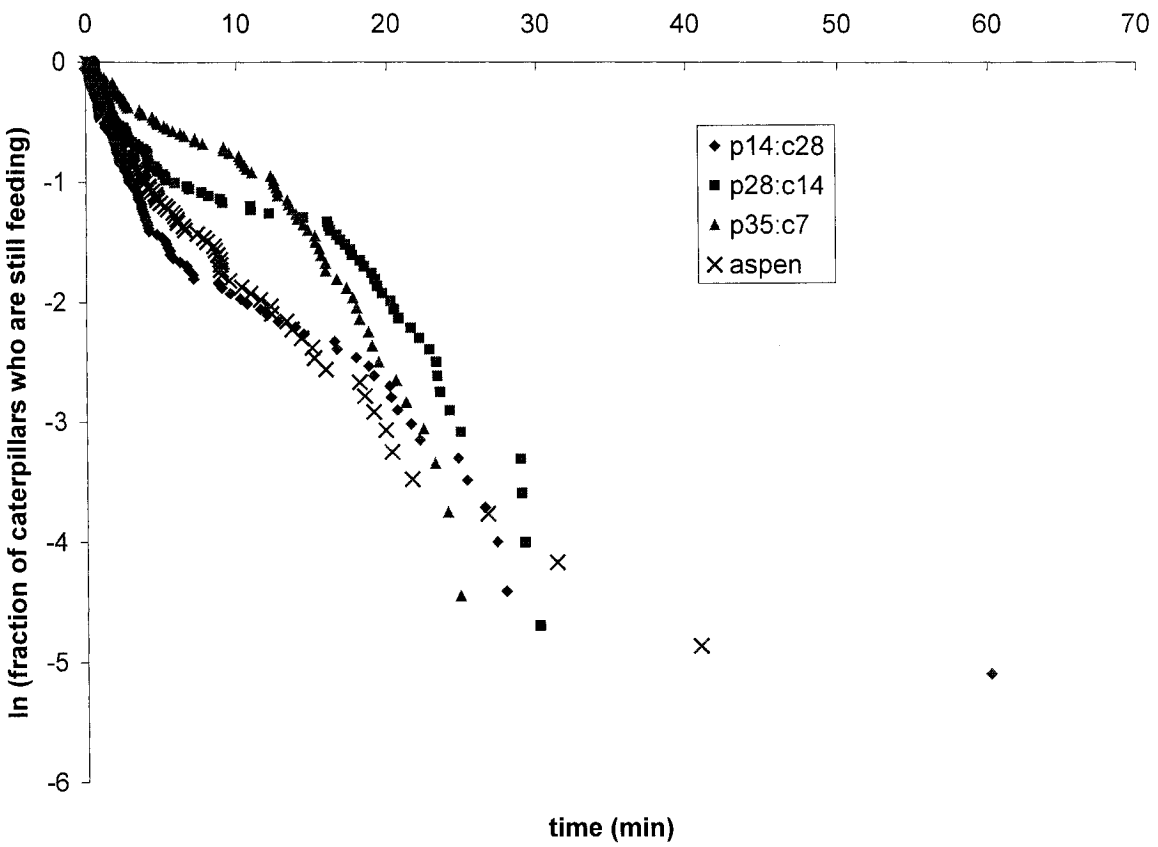


Fig. 19: Log-survivorship curves for feeding bout durations when feeding on aspen, p14:c28, p28:c14, and p35:c7. The linear regressions were as follows- aspen: y= -0.1368x – 0.2724 (R^2 = 0.9601); p14:c28: y= -0.1157x – 0.4229 (R^2 = 0.8677); p28:c14: y= -0.1026x – 0.1671 (R^2 = 0.9225) and p35:c7: y= -0.1239x + 0.1491 (R^2 = 0.8977).

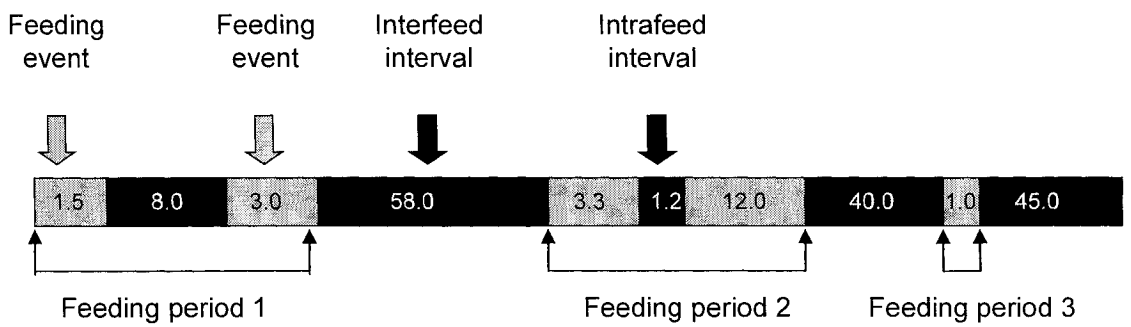


Fig. 20: The pattern of feeding for forest tent caterpillars. The numbers indicate the duration of the event in minutes. A feeding bout is the sum of feeding events that are separated by intervals less than 16.67 min. For example, the duration of feeding bout 1 would be 1.5 min + 3.0 min. A feeding period is the sum of feeding events and the intrafeed interval. The first feeding period duration would be 1.5 + 8.0 + 3.0 min. Intrafeed pauses are periods of "no eating" less than 16.67 min; interfeed pauses are periods of "no eating" longer than 16.67 min between feeding periods.

3.4.2 b. Phagostimulatory power

A one-way ANOVA demonstrated that there was no difference in the latency to eat the food source following a direct contact between the treatments ($F_{3,56} = 1.133$, $p = 0.344$). However, a slight trend was noticed; the proportion of caterpillars that eat immediately after contacting the food is highest for those on the p14:c28 food source. On p14:c28, 55% of the caterpillars ate the food right after they contacted it (see Table 11). Furthermore, a Kruskal – Wallis analysis demonstrated that there was no difference in the duration of the first feeding event between the different treatments ($\chi^2 = 0.944$, $df = 3$, $p = 0.815$).

Treatment	# caterpillars that contacted food	# caterpillars that ate food	# caterpillars that ate at first contact	Percent caterpillars that ate at first contact (%)
Aspen	17	14	0	0
p14:c28	17	11	6	55
p28:c14	19	16	3	19
p35:c7	17	16	5	31

Table 11: The number of caterpillars that contacted and eventually ate the food sources provided to them, as well as the percent of caterpillars that ate the food as they contacted it (at first contact).

3.4.2 c. Caterpillar behaviour within feeding periods

The duration of feeding periods (intrafeed pauses + feeding bouts) was significantly different between treatments ($\chi^2 = 11.922$, df= 3, $p = 0.008$). The caterpillars on the aspen diet had the shortest feeding periods (Fig. 21). There is no difference in the duration of feeding periods between the artificial diets when aspen was removed from the analysis, however a trend was seen; the duration of feeding periods increases as the diets become protein-biased.

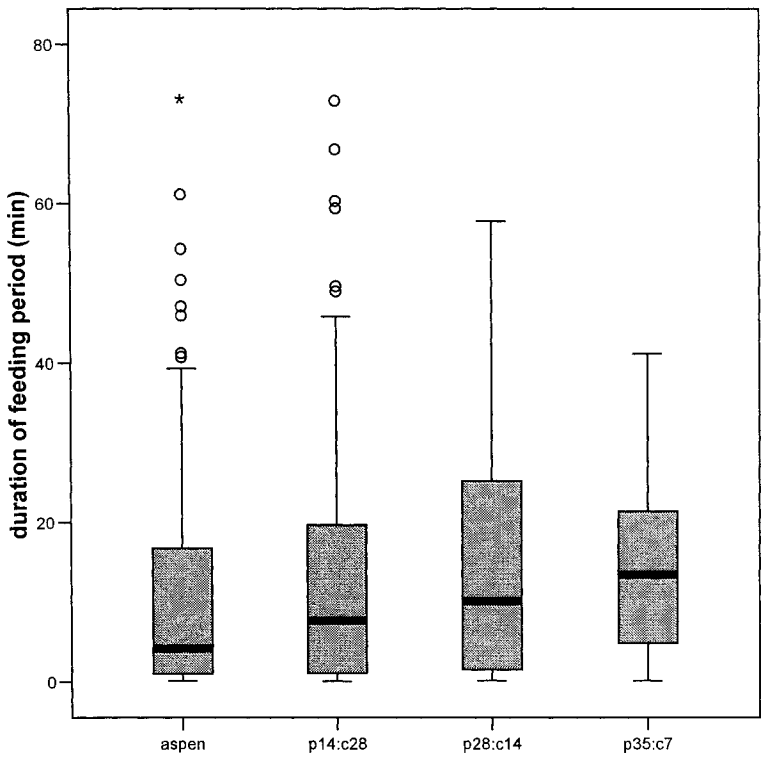


Fig. 21: The duration of feeding periods between treatments. See Fig. 16 for the explanation of the box plot graph.

The amount of time spent eating during a feeding period (i.e. the duration of the feeding bout) was significantly different between treatments (χ^2 = 31.373, df= 3, p < 0.001). The caterpillars on p14:c28 and aspen spent less time eating during their feeding periods (Fig. 22). These differences are still significant when removing aspen. Here again a trend is noticed; the duration of feeding increases as the diets become more protein-biased. There was no significant difference in the number of intrafeed pauses and number of feeding events per feeding period between the treatments (intrafeed pauses: χ^2 = 2.975, df= 3, p = 0.396; feeding events: χ^2 = 3.025, df= 3, p = 0.361).

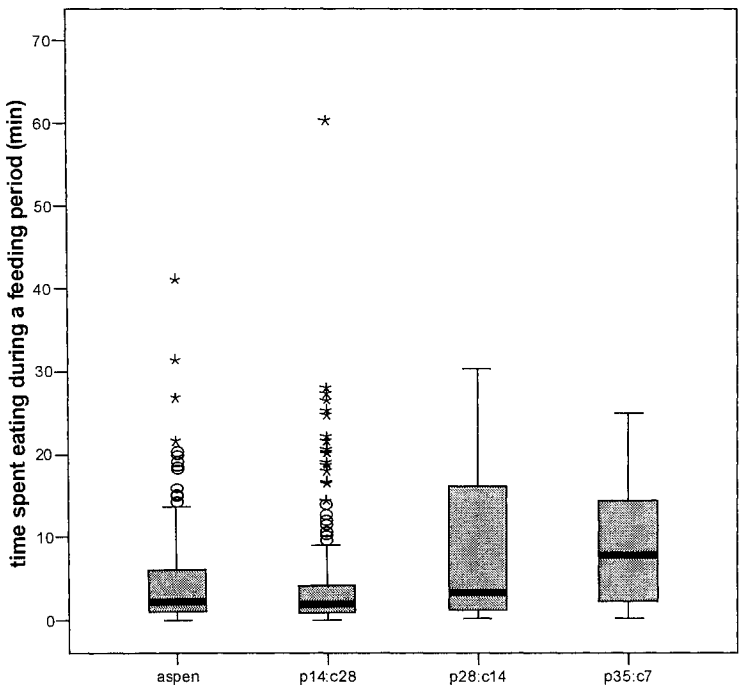


Fig. 22: The duration of feeding bouts for each treatment. See Fig. 16 for the explanation of the box plot graph.

Fig. 23 demonstrates that the caterpillars on p14:c28 spend less time eating overall because they have more short feeding bouts, with few bouts over 10 minutes. The caterpillars on p35:c7 have more long feeding bouts that last for over 10 minutes and few bouts less than that. Again the trend was noticed; as the amount of protein in the diet increases so does the number of longer feeding bouts.

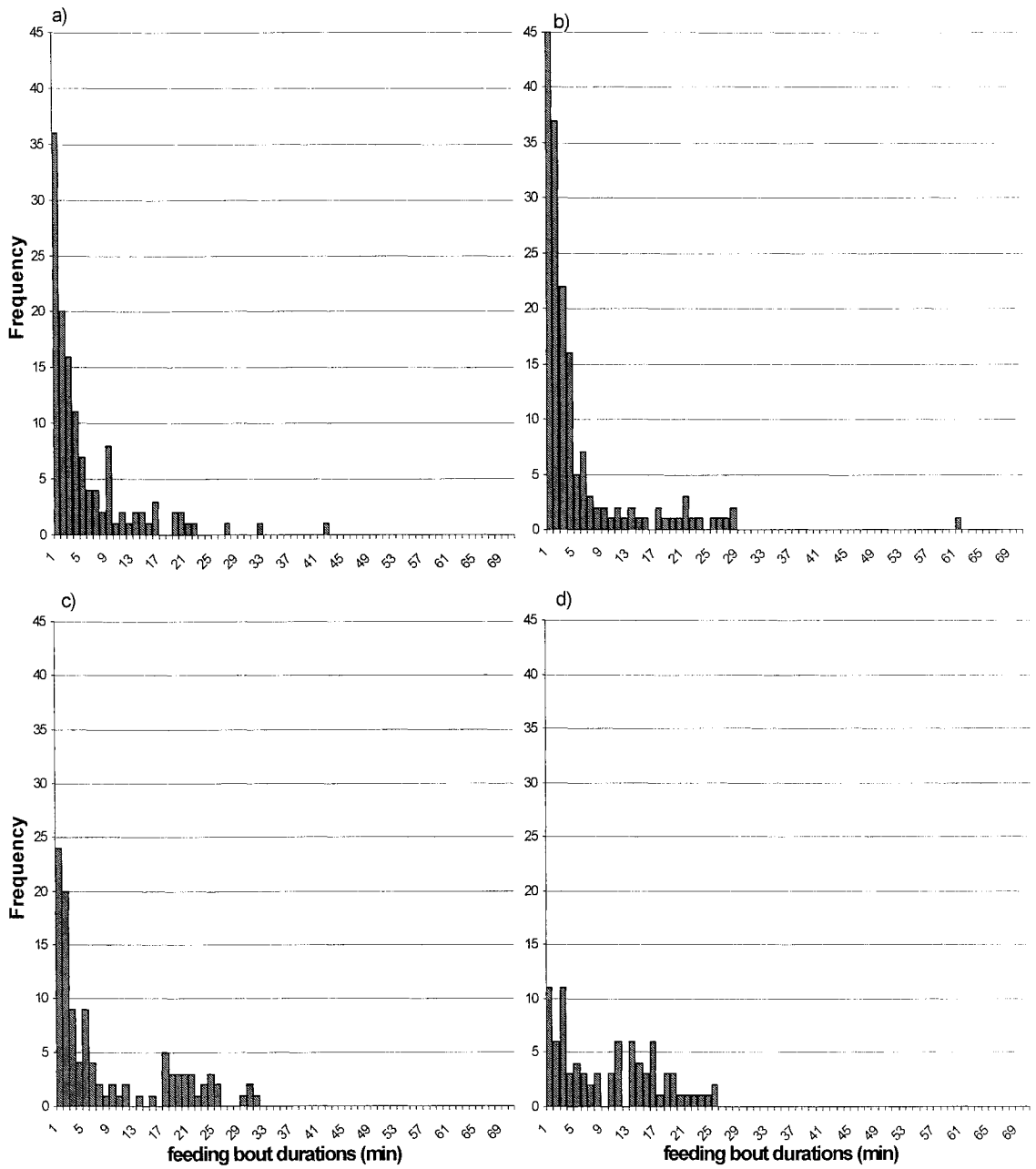


Fig. 23: Frequency distributions for the duration of feeding bouts for a) aspen, b) p14:c28, c) p28:c14 and d) p35:c7.

Thus, on artificial diet, feeding periods are of similar durations (slightly longer with higher protein content), but on low protein: high carbohydrate they are made up of

short feeding bouts whereas on high protein: low carbohydrate there tend to be longer feeding bouts, such that the total proportion of the feeding period spent eating tends to be higher (Fig. 22).

On aspen, the feeding behaviour is most similar to low protein: high carbohydrate diet with short feeding periods, and short feeding bouts.

3.4.2 d. Occurrence of feeding periods

A one-way ANOVA demonstrated no significant differences between treatments in the number of feeding periods (feeding bouts + intrafeed pauses) per hour ($F_{3,24}$ = 1.521, p = 0.235).

A Kruskal-Wallis analysis demonstrated that there was a significant difference in the duration of interfeed pauses between the treatments (χ^2 = 20.184, df= 3, p < 0.001, Fig. 24). The caterpillars on the p35:c7 diet had the longest interfeed durations when compared to the other treatments. This was still significant once the aspen treatment was removed from the analysis; the caterpillars on p35:c7 have the longest interfeed duration among the artificial diets. Here again the trend was noticed; as the diets become more protein-biased, the duration of pauses increase between feeding periods.

The alternation between feeding events and "non- eating" pauses can be visualized for each caterpillar per treatment in the plots seen in Appendix 1.

Thus, on artificial diets, the interfeed pauses are longer on high protein diets. On aspen the caterpillars had short interfeed intervals.

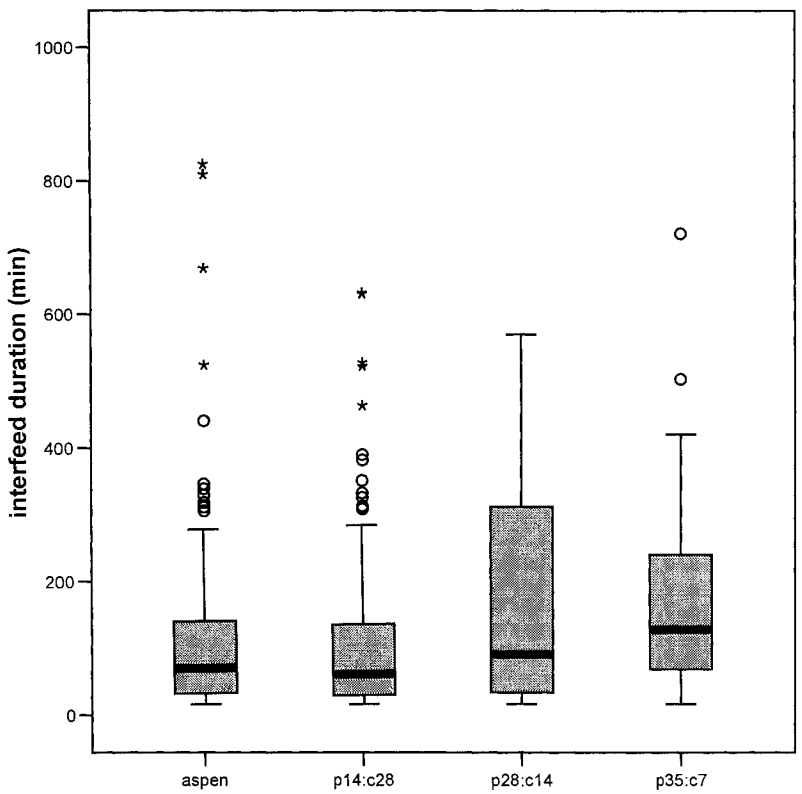


Fig. 24: The duration of interfeed pauses (> 16.67 min) for each treatment. See Fig. 16 for the explanation of the box plot graph.

3.4.2 e. Time budgets

No significant difference in the latency to rest was noticed between the treatments after performing a one-way ANOVA ($p > 0.05$). Rest is when the caterpillars are inactive and are clearly not in motion – most of the non-feeding time is spent active. A Kruskal-Wallis test demonstrated a significant difference in the duration of resting periods between treatments ($\chi^2 = 22.517$, df= 3, $p<0.001$). Resting periods are shorter for caterpillars on aspen (see Fig. 25), however there was no difference among the artificial diets (seen after removing aspen from the analysis).

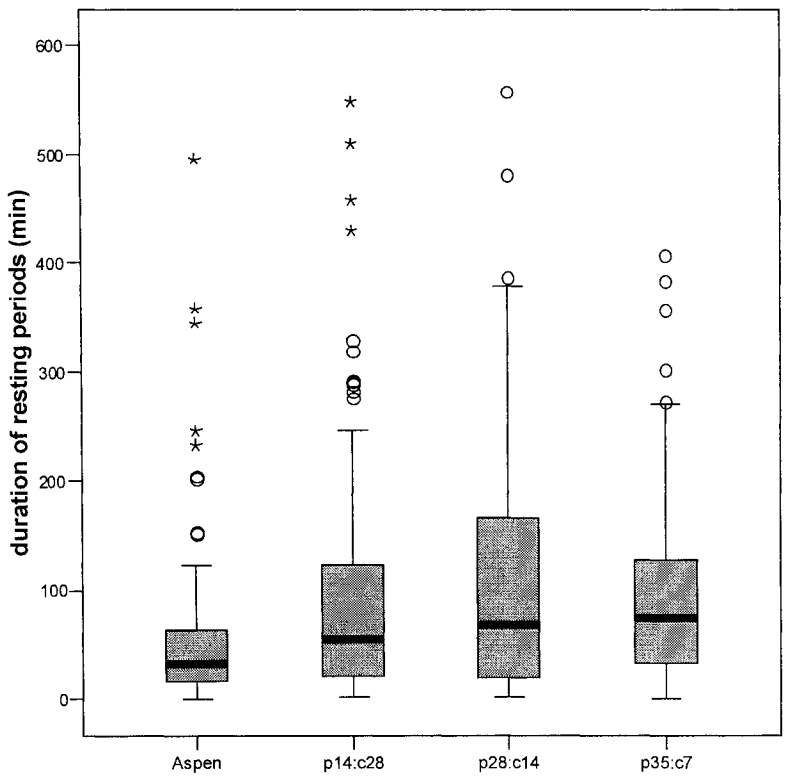


Fig. 25: The duration of resting periods for each treatment. See Fig. 16 for the explanation of the box plot graph.

There is a significant difference in the duration of activity periods (which includes feeding and non-feeding activity) between treatments (χ^2 = 23.995, df = 3, $p<0.001$). Caterpillars on p28:c14 and p35:c7 have shorter activity periods. Activity periods are longer for caterpillars on aspen and on p14:c28 (see Fig. 26). Significant differences were still seen between the artificial diets when the aspen treatment was removed from the analysis (χ^2 = 8.684, df = 2, p=0.013); longer activity periods were observed for caterpillars on the p14:c28 diet.

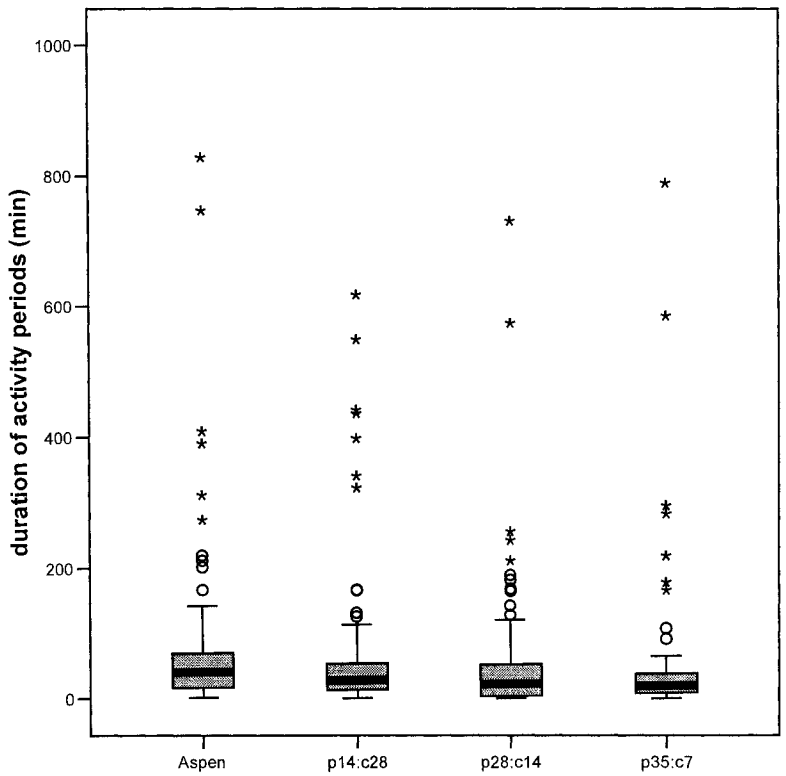


Fig. 26: The duration of activity periods (feeding and non-feeding events) for each treatment. See Fig. 16 for the explanation of the box plot graph.

One-way ANOVAs were performed to determine whether the total time spent eating, in motion and resting per hour were different between the treatments. No significant difference was noticed for the total time spent eating ($p > 0.05$), however, the total time spent resting and in motion were different between the treatments (resting: $F_{3,24} = 10.595$, $p < 0.001$; active: $F_{3,24} = 8.822$, $p < 0.001$). The caterpillars that fed on aspen spent the least time resting and the most time in motion (see Fig. 27). There are no significant differences in the total time spent eating, resting and in motion between the artificial diets when removing aspen from the analysis.

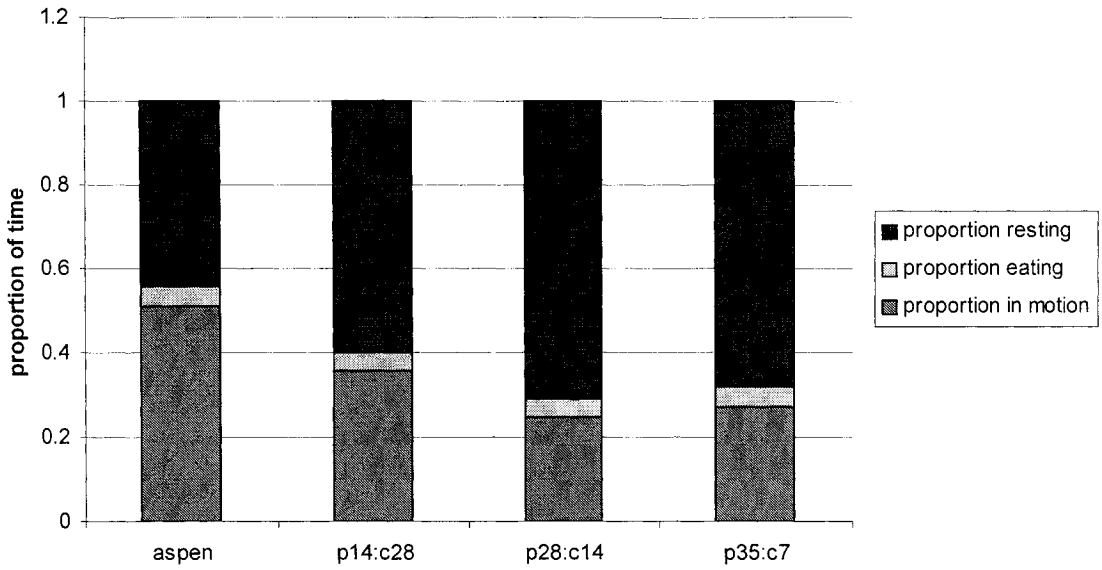


Fig. 27: Proportion of time spent eating, resting and in motion between the different treatments.

3.4.2 f. Diet effects on exploration

The proportion of caterpillars that contacted the second food source (the balanced artificial diet) differed between treatments (χ^2 = 9.268, df = 3, p=0.026), however the proportion that ate the second food source after having contacted it was not significantly different between treatments (χ^2 = 2.278, df = 3, p=0.517). A greater number of caterpillars on aspen and the p14:c28 diet contacted the second food source when compared to the p28:c14 and p35:c7 diets (Table 12). However, the latency to enter the second zone after the removal of the barrier, and the latency to contact the second food source after the removal of the barrier and after having entered the second zone were found not to be significantly different between the treatments ($p > 0.05$). The total time

spent resting and the latency to rest were also not significantly different between the 4 treatments ($p > 0.05$).

Treatment	Total # caterpillars that contacted food 1 during first 48 hours	Total # caterpillars that ate food 1 during first 48 hours	# caterpillars that fed on food 1 after removal of barrier	# caterpillars that contacted food 2 after removal of barrier	# caterpillars that fed on food 2 after removal of barrier
Aspen	17	14	9	13	12
p14:c28	17	11	12	13	9
p28:c14	19	16	14	9	7
p35:c7	17	16	10	6	5

Table 12: The original number of caterpillars that contacted the first food source during the first part of the experiment (during 48 hours), and who ate it. Also, the number of caterpillars that fed on the original food source, and that contacted and ate the second balanced artificial food source given to them once the barrier was removed in the second part of the experiment.

A Wilcoxon test determined that the number of feeding events on food 1 vs. food 2 (after contacting food 2) was significantly different between the treatments ($Z = -3.068$, $p = 0.002$). The caterpillars on aspen took more meals on food 2 than on food 1 compared to the other caterpillars on the artificial diets (see Fig. 28). No significant difference was seen between the artificial diets. Furthermore, there was no difference between the treatments in the number of feeding events on the first food source after contacting the second ($p > 0.05$).

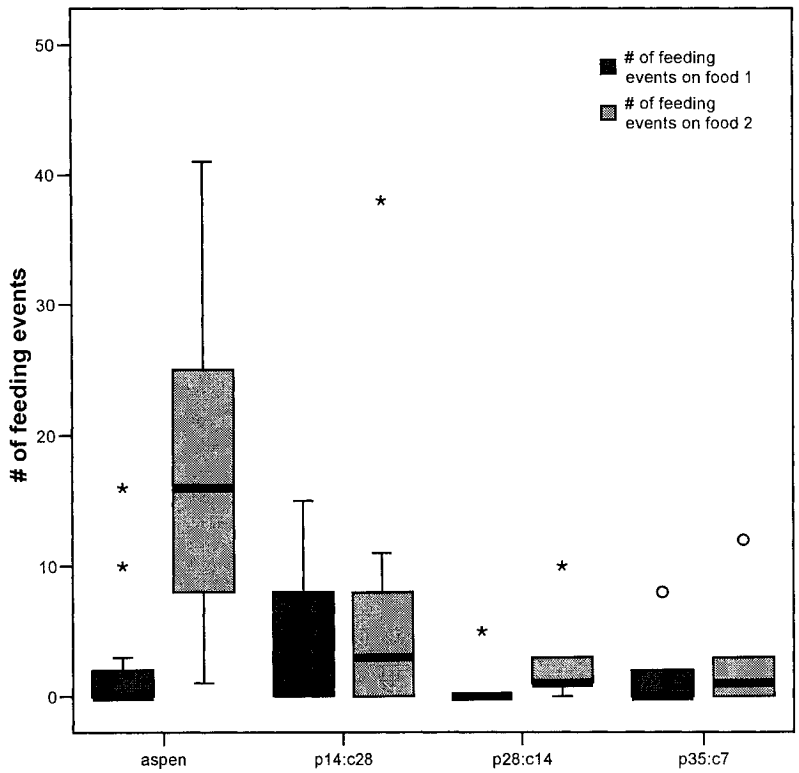


Fig. 28: The number of feeding events on the initial food (food 1) and the second food source (food 2: p21:c21) during an 8-hour period once the caterpillars had contacted the second food source. See Fig. 16 for the explanation of the box plot graph.

A Wilcoxon test determined that the time spent eating food 1 vs. food 2 (after contacting food 2) was significantly different between treatments ($Z = -3.472$, $p = 0.001$). The caterpillars initially feeding on aspen spent more time feeding on p21:c21 than the caterpillars pre-treated on the artificial diets (see Fig. 29). No differences were seen between the artificial diets. Also, there was no significant difference between the treatments for the duration of eating on the original food source (after contact with the second).

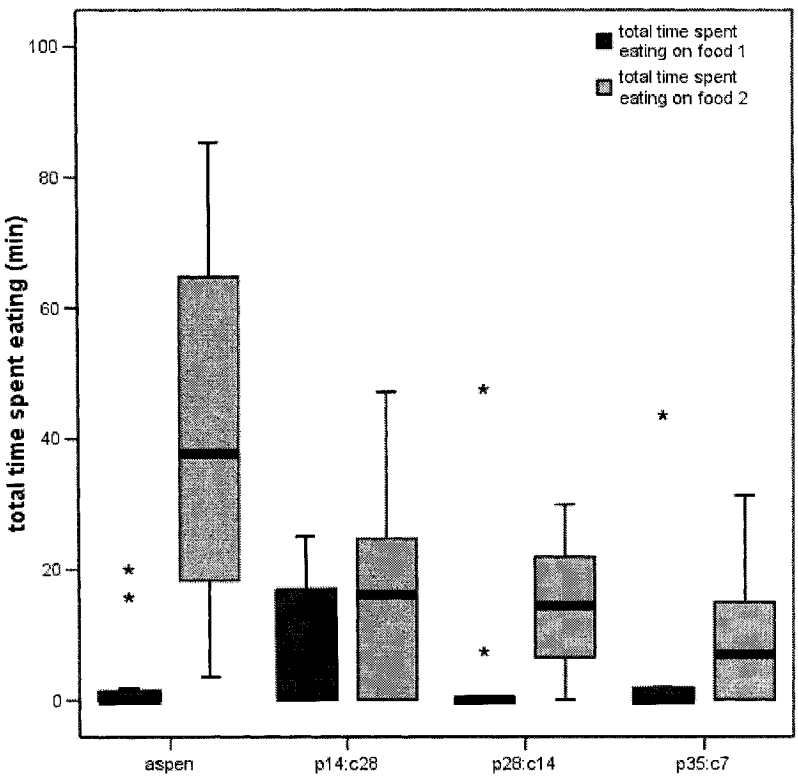


Fig. 29: The duration of time spent eating the initial food source and the second food source after contacting food 2. See Fig. 16 for the explanation of the box plot graph.

Thus, the caterpillars on aspen had more feeding events on the balanced artificial food source, resulting in longer total time eating, although there were no differences between treatments in the amount of time they spent eating the first food source. The caterpillars on aspen and p14:c28 were more likely to leave the trail and contact the second food. The second food source was a balanced diet; therefore, once the caterpillars established a trail to it (contacting it) they ate it, no matter which diet they were initially eating.

3.5 Discussion

Feeding pattern

After analyzing the feeding pattern of the caterpillars, a differentiation between pauses was found. Intrafeed pauses are gaps of less than 16.67 min within a feeding period where the caterpillars do not eat, while interfeed pauses are gaps greater than 16.67 min between feeding periods. However, no difference between sampling events and feeding was found (no meal criterion). This is contrary to the feeding patterns seen in locusts where distinct sampling events occur. For these insects, feeding occurs in bouts separated by periods where they distinctively stop eating and get into a perching position (Simpson 1995). However, the feeding patterns of caterpillars are not as straightforward as that; it has been demonstrated that *Manduca sexta* do not have clear daily feeding behaviour patterns. Feeding is not continuous, and thus also occurs in bouts; however, the bouts and pauses between feeding appear to be randomly distributed. This caterpillar does not leave the food between pauses making the distribution of gaps unclear (Reynolds *et al.* 1986).

Phagostimulatory power

The latency from contact with the food to the first feeding event, as well as the duration of the first feeding event are usual measures of phagostimulatory power of the food. Once insects come into contact with a food item that they like, they will be more likely to remain and eat it than they would with food that does not appeal to them. In this study it was seen that the latency to eat the food after contacting it was shortest, though not significantly different, for caterpillars on the high carbohydrate food among the

artificial diets, which may reflect a high phagostimulatory power. The percentage of caterpillars that ate immediately once they had contacted the food was 55% for those on p14:c28. In spite of this, there was no difference among the diets in the duration of the first feeding event. This suggests there is no clear trend for the preference of one food varying in nutrients over the other, which is contrary to other studies that have indicated that carbohydrates are strong phagostimulants (Despland and Noseworthy 2006, Panzuto *et al.* 2001). Perhaps carbohydrate differences between the foods in the present experiment were not different enough to elicit clear differences in taste response.

Surprisingly, none of the caterpillars that contacted aspen went on to eat it right away despite it being the preferred host tree, and leading to greater performance (seen in Chapter 2). This may be attributed to the caterpillars not being acclimatized to eating foliage. They were hatched and reared on artificial diet, therefore, when faced with a leaf during their fourth instar they may not be used to the different texture and structure. Thus, 48 hours might not be enough time for insects to adjust to eating foliage after having been reared on artificial diet.

Feeding behaviour on artificial diet

A trend was found when looking at the feeding behaviour of forest tent caterpillars on artificial diet. As the amount of carbohydrate in the diet increases, feeding bout durations and interfeed intervals become shorter.

In locusts, interfeed intervals are affected by protein but not carbohydrate. Simpson and Abisgold (1985) saw longer intervals on high protein diet, similar to this experiment. In locusts the mechanism behind such feeding is explained by an increase in levels of ten free amino acids in the haemolymph, as well as to the rise of haemolymph

osmolality (Abisgold and Simpson 1987). These levels of free amino acids fall with time since the last protein-containing meal. As the concentration of amino acids in the haemolymph fall, activity increases, along with the sensitivity of taste receptors. Thus, the locust is more prone to accept and consume the food containing amino acids (Abisgold and Simpson 1987). In caterpillars the main mechanism regulating feeding is haemolymph trehalose. The longer feeding bouts on more high protein, low carbohydrate food seen in this experiment may reflect a post-ingestive protein effect *via* influences of haemolymph trehalose – the 'blood sugar' of insects (Thompson 2003). Haemolymph plays a central role in the regulation of nutrient intake and provides internal information on the nutritional status of the insect because its composition fluctuates dramatically with changing nutritional conditions (Thompson 2003). Haemolymph levels vary with time since a meal, with quantity and quality of previous meals, and with the metabolic and growth demands of the tissues (Simpson and Raubenheimer 1993). Freidman *et al.* (1991) observed dietary selection behaviour due to trehalose in *Heliothis zea*. Larvae with low haemolymph trehalose levels chose food high in carbohydrate instead of food high in protein, and they showed the reversion of this choice with the injection of trehalose to raise haemolymph sugar. Thompson and Redak (2000) showed that larvae reared on diet with different amounts of sucrose and protein displayed variable haemolymph trehalose levels, depending on the relative amounts of these nutrients, where larvae on high sugar diets had high haemolymph trehalose and larvae on low protein had low haemolymph trehalose. Most larvae with low haemolymph trehalose selected the high carbohydrate diet, and then later switched over to the high protein diet,

seemingly having reached a threshold trehalose level, while the larvae with high haemolymph trehalose selected the high protein diet.

Thus, in this study, on diets with high carbohydrates, the insects' trehalose levels would be predicted to be elevated; therefore, they have a higher probability to stop eating because the haemolymph becomes saturated with trehalose. For this reason, feeding bouts are shorter on these diets and longer on diets that are protein-biased. This was also seen when *M. sexta* caterpillars were given balanced protein: carbohydrate diets; they had lower haemolymph trehalose levels than those fed carbohydrate-rich diets, and therefore they consumed more food (Thompson and Redak 2000). Similarly, *Spodoptera littoralis* had small feeding bouts on deficient (no protein or sugar) diets, but longer bouts on balanced food resulting in a higher consumption on the balanced diet (Simpson *et al.* 1988). Also on the high protein diet, the caterpillars have longer interfeed gaps, and hence process food longer before resuming feeding. This could be due to the effect of protein content in the meal. Protein might be slowing the passage of nutrients through the midgut, thus, it takes these caterpillars longer to digest (Bernays 1985).

Time budget on artificial diet

The overall total time spent eating in this experiment was the same regardless of diet, which was already evidenced after 2 hours of observation (during the short-term observations). This is contrary to previous studies conducted on this species of caterpillar (Despland and Noseworthy 2006, Dussutour *et al.* unpublished data). Despland and Noseworthy (2006) demonstrated that the overall consumption rate on a protein- biased diet (p35:c7) is lower per day when compared to p28:c14 and p14:c28. However, Despland and Noseworthy (2006) conducted the experiment over the span of an instar.

Thus, perhaps 48 hours is not enough time to determine a significant difference in the consumption of foods varying in different nutrient ratios, if it is due to lower growth on the extreme protein-biased food. However, our study suggests that the lack of eating the p35:c7 diet shown by Despland and Noseworthy (2006) is not due to lack of phagostimulatory power of the food.

Furthermore, Dussutour *et al.* (unpublished data) demonstrated that individual forest tent caterpillars fed more readily and for longer when the food source was nutritionally balanced. However, the experiment was conducted with diets containing no sugar. Therefore, because we did not notice a difference in feeding consumption for caterpillars on diet with 7 % carbohydrate, perhaps there is a threshold amount of sugar that is attractive to forest tent caterpillars between 0 % and 7 %.

Feeding behaviour on aspen

Our findings for caterpillars that were exposed to aspen were opposite from what were expected. Chapter 2 demonstrated that the natural foliage supports better growth of the forest tent caterpillars, thus it was expected that they would consume more of this diet compared to unbalanced artificial diet. Furthermore, the caterpillars on aspen show different feeding patterns than they do on the more adequate artificial diets; the duration of feeding periods, feeding bouts, intrafeed pauses and resting periods were shorter. Perhaps they have short feeding events on this food because they are not accustomed to it, and are more active, so eat frequently by returning multiple times. This is similar behaviour to what was found for the caterpillars on the p14:c28 diet, however the nutrient content of aspen is variable. Nutrient concentrations vary by approximately a factor of 2

among aspen trees (Hwang and Lindroth 1997), between 12 and 24% dry weight for protein and 8 to 20 % dry weight for digestible carbohydrate, fructose being the main sugar (Miller 1987; Lorenzetti 1993; Hemming and Lindroth 1999). Perhaps the caterpillars do not eat aspen because it is novel for them at this stage in development. Food intake can be regulated by the physical structure of the food. The physical structure between aspen and artificial diet is different, as are nutrient and water content. Phytophagous insects have to take into account the hardness and coarseness of foods that they ingest. Tough food might reduce feeding or increase the time taken to ingest the food (Bernays 1985). However, regardless of their unexpected feeding behaviour on aspen, in the end the caterpillars spent the same amount of time eating it.

Studies have demonstrated the variability in the foraging patterns of other caterpillars. Reynolds *et al.* (1986) demonstrated differences in feeding patterns on two types of diet for *Manduca sexta*: the host plant (tobacco) and artificial diet. Feeding behaviour in *M. sexta* caterpillars is organized into bouts of active feeding separated by periods of inactivity, whether the food is tobacco leaves or artificial diet. The results differed from our experiment in that *M. sexta* spent a greater proportion of time feeding on tobacco than on artificial diet. However, similar to our study, on tobacco the caterpillars' feeding periods were separated by shorter interfeed pauses than on artificial diet. Bernays and Singer (1998) also demonstrated that even though two plants are the primary hosts for *G. geneura* and support good growth and survival, the caterpillars' feeding activities were different, suggesting that pattern of feeding is not a simple measure of the quality of a food plant.

Diet effect on exploratory behaviour

During the first 48 hours of this experiment the caterpillars on aspen were more active (longer activity periods and shorter resting periods), therefore they would be more likely to reach the new artificial food source made available to them and consume it. After the barrier was removed, a difference was seen in the proportion of caterpillars that left the pre-established trail between the different diets. Those on aspen and the p14:c28 diet were more likely to leave the trail and contact the second food source. Although there was no difference in the latency to reach the balanced food source, those that were initially exposed to aspen spent more time eating the artificial diet once they contacted it, thus establishing an exploratory trail to that site. This suggests that forest tent caterpillars do not prefer aspen to the artificial diet, which is contrary to what was expected. Again, this could be because they are not accustomed to feeding on foliage, which is qualitatively different, and perhaps mechanically more difficult to eat.

When looking between the artificial diets differing in nutrient ratios, no difference in the amount of time spent eating the original food vs. the new balanced food was found. It has already been established that the p35:c7 diet does not support good growth for forest tent caterpillars (Despland and Noseworthy 2006) while p21:c21 and p28:c14 diets support better performance than p14:c28 (in terms of growth and survivorship from Chapter 2). However, when the caterpillars are given a choice between these diets, they do not eat more of the new food (p21:c21), which is nutritionally better for them. This suggests that forest tent caterpillars are not good at pre-ingestively regulating food choices. This is in accordance with the study done by Despland and Noseworthy (2006).

They did show reduced consumption of p35:c7 when paired with a carbohydrate-biased diet, but again this is over a much longer time period.

Chapter 4
General Discussion

In conclusion, this thesis gives an indication of the effects of nutritionally different diets on caterpillar performance and feeding behaviour.

The first part of this thesis confirmed that forest tent caterpillars perform better on their natural foliage, and also do better on protein-biased artificial foods. Fitness consequences were not only expressed as differences in larval survival, development time and pupal mass, but also included allocation to reproduction and offspring quality (as measured by individual egg mass). The novelty of this study was that the differences seen in the distribution of weight to the different body components between the treatments varying in nutrient ratio are due to the weight allocation to them, and not based on body composition. Body lipid and nitrogen were seen to be tightly post-ingestively regulated, similar to studies conducted on other caterpillars (Lee *et al.* 2002). Overall, the effects of protein deficient foods on the fitness of the female adults included reduced fecundity due to smaller weight and lower offspring quality (in terms of egg weight). Male fitness might be affected by a smaller body weight on carbohydrate-biased diets, but no effect on flight reserves was observed. In turn, these fitness repercussions may be related to the defoliation outbreaks and cycles of this pest species. Changes in population densities of these insects may be linked to changes in leaf nutritional quality over time (Haukioja 1980).

The second part of this thesis demonstrated that with higher protein in the diet, caterpillars take longer meals. This can be explained by the role of haemolymph trehalose

levels in regulating insect feeding (Thompson 2003). When protein content is high (and sugar is low) in diets, trehalose levels remain low, thus caterpillars need to consume more food in order to obtain the sufficient amount of sugar that they require (Thomspon 2003). Surprisingly, expected feeding patterns on natural foliage were not seen. On aspen, the feeding behaviour is most similar to that on low protein: high carbohydrate diet with short feeding periods, and short feeding durations. This suggests that these caterpillars do not prefer aspen when exposed to it for the first time for only 48 hours.

In many cases caterpillars perform badly on artificial diet compared to natural food, although artificial diets are formulated to the best of our ability to contain all the nutrients in the right quantities (Cohen 2004). Although the caterpillars may initially prefer artificial diets, in the end there is a vital component in the natural food that makes them grow better (higher survivorship, pupal weight and shorter development time).

Furthermore, artificial diets of varying nutritional ratio and aspen did not affect the exploratory behaviour of forest tent caterpillars. These caterpillars follow trails (Colasurdo and Despland 2005) in order to manoeuvre in nature and to maintain group cohesion (Fitzgerald 1995). Exploratory behaviour involves leaving the trail and covering new territory, which would be expected to increase in the presence of poor quality food (Barton Browne 1993, Dussutour *et al.* unpublished data). Caterpillars on aspen were more likely to leave the trail and discover a new food source. However, it was seen that there was no difference between treatments in the amount of time the caterpillars spent eating their original food source, after having contacted and made a trail to the new artificial diet given. However, those originally on aspen had more feeding events and overall ate more of the artificial diet. This implies that forest tent caterpillars do not make

the best decisions when given a choice of food, which confirms previous studies conducted with these caterpillars (Noseworthy and Despland 2006). However, forest tent caterpillars are efficient at post-ingestively regulating their carbohydrate and protein intake, such that they attain the same amount of body lipid and nitrogen.

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Appendix 1

Feeding patterns of individual caterpillars on the different diets

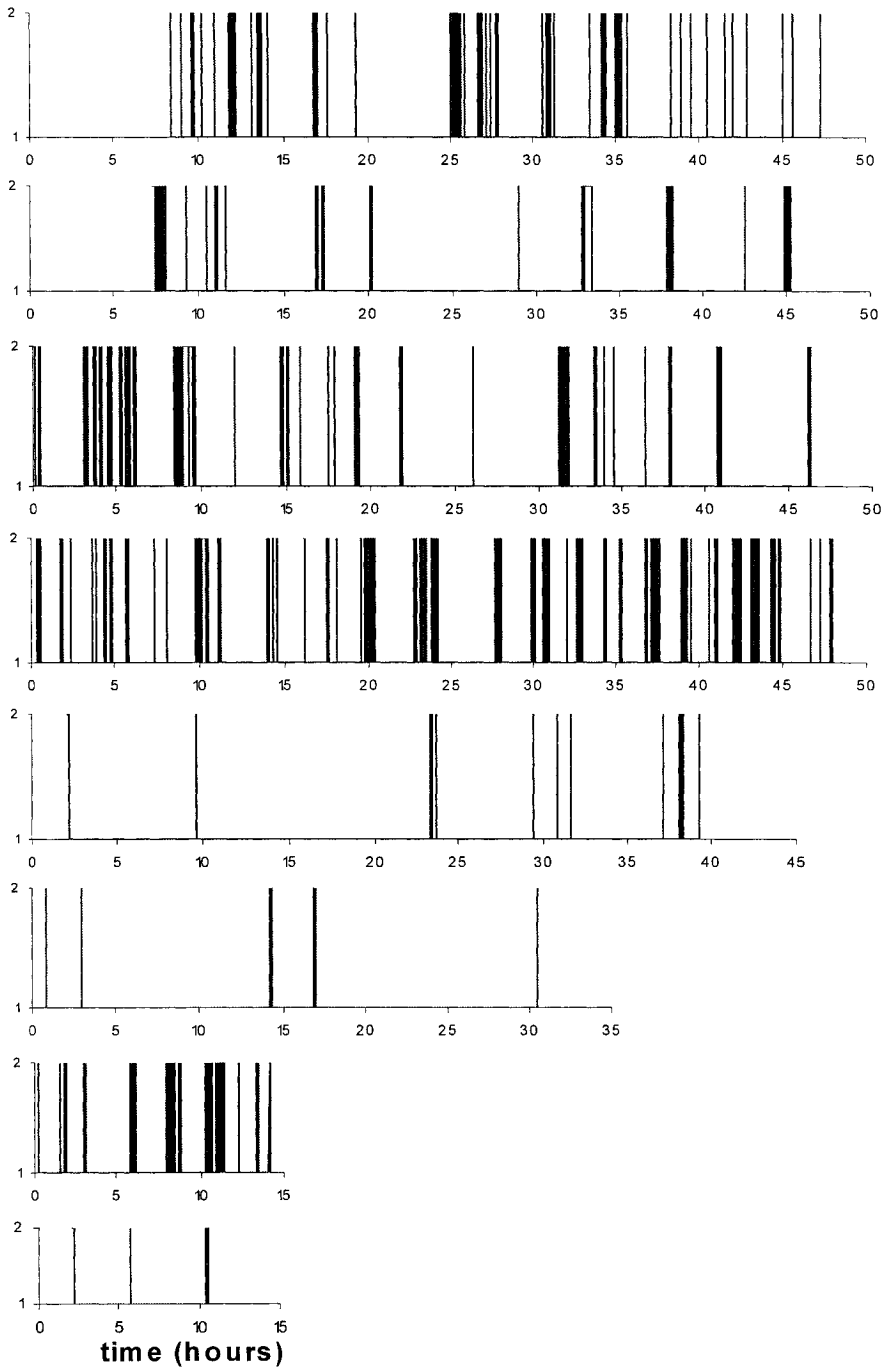

Fig. 30: Pattern of feeding for the eight forest tent caterpillars on the aspen diet over the experimental period (in hours). The vertical bars represent feeding events, with the gaps within and between feeding events shown. Feeding periods (separated by 16.67 min) are not distinguished.

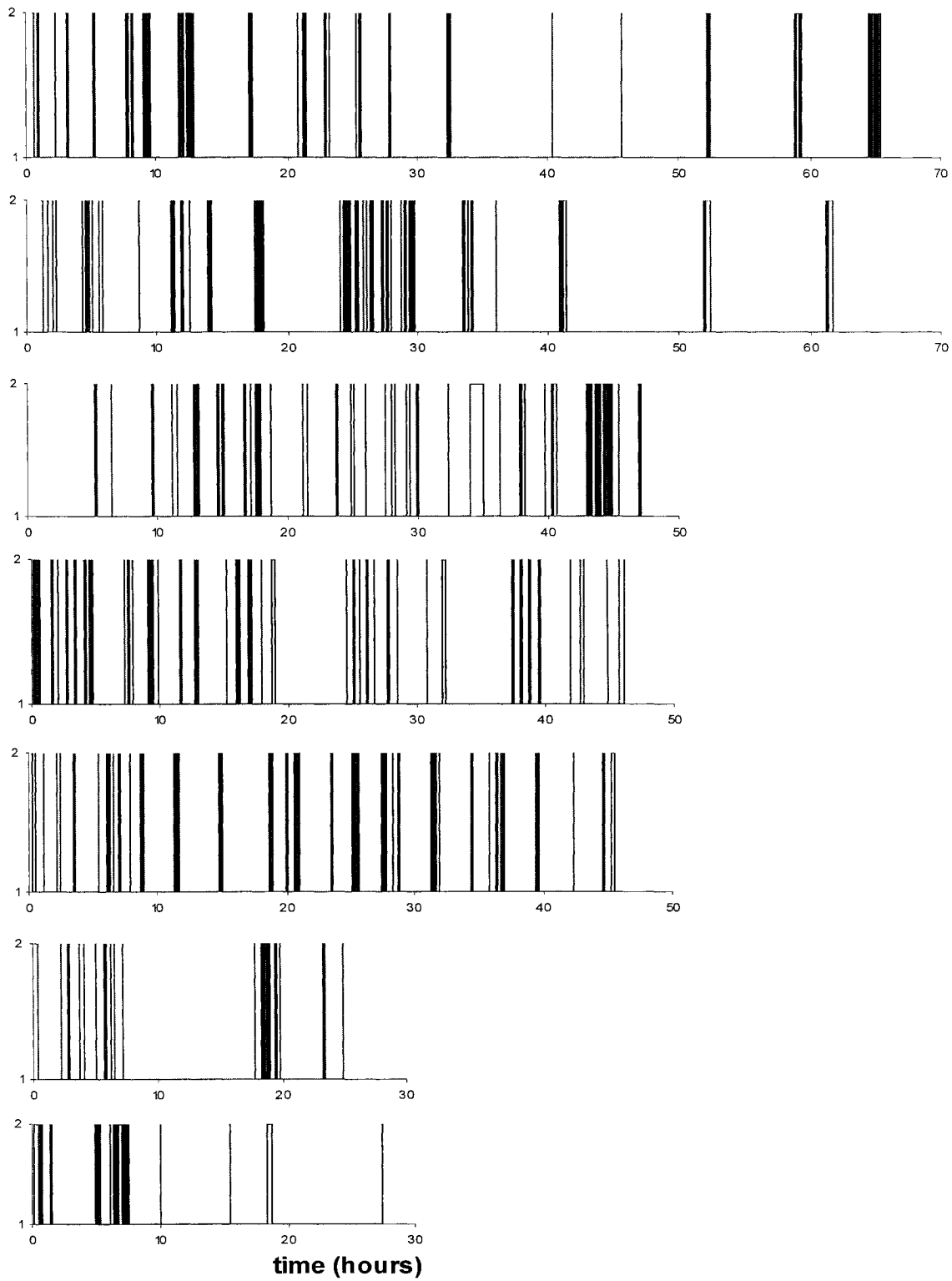

Fig. 31: Pattern of feeding for the seven forest tent caterpillars on the p14:c28 artificial diet over the experimental period (in hours). The vertical bars represent feeding events, with the gaps within and between feeding events shown. Feeding periods (separated by 16.67 min) are not distinguished.

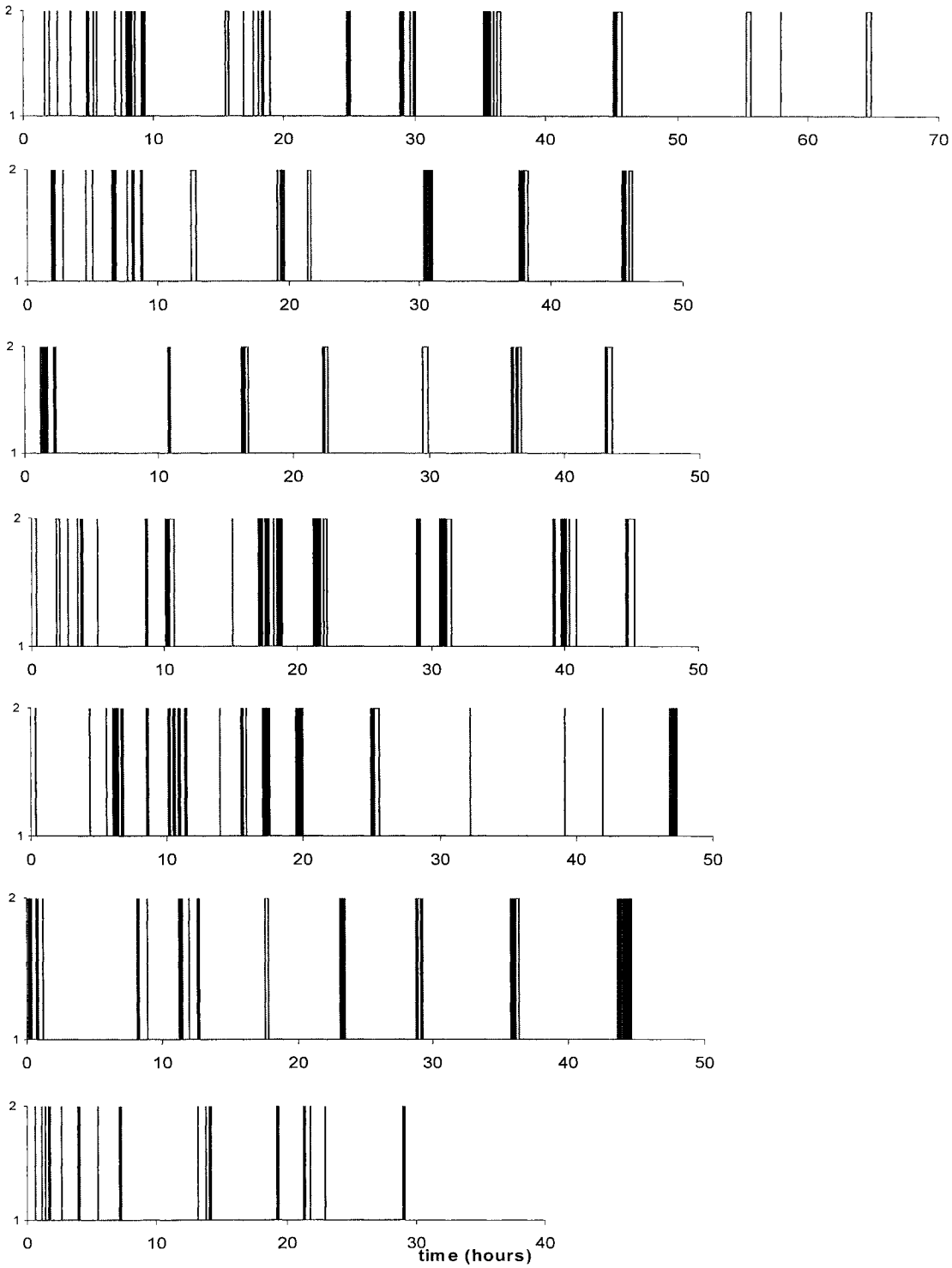

Fig. 32: Pattern of feeding for the seven forest tent caterpillars on the p28:c14 artificial diet over the experimental period (in hours).The vertical bars represent feeding events, with the gaps within and between feeding events shown. Feeding periods (separated by 16.67 min) are not distinguished.

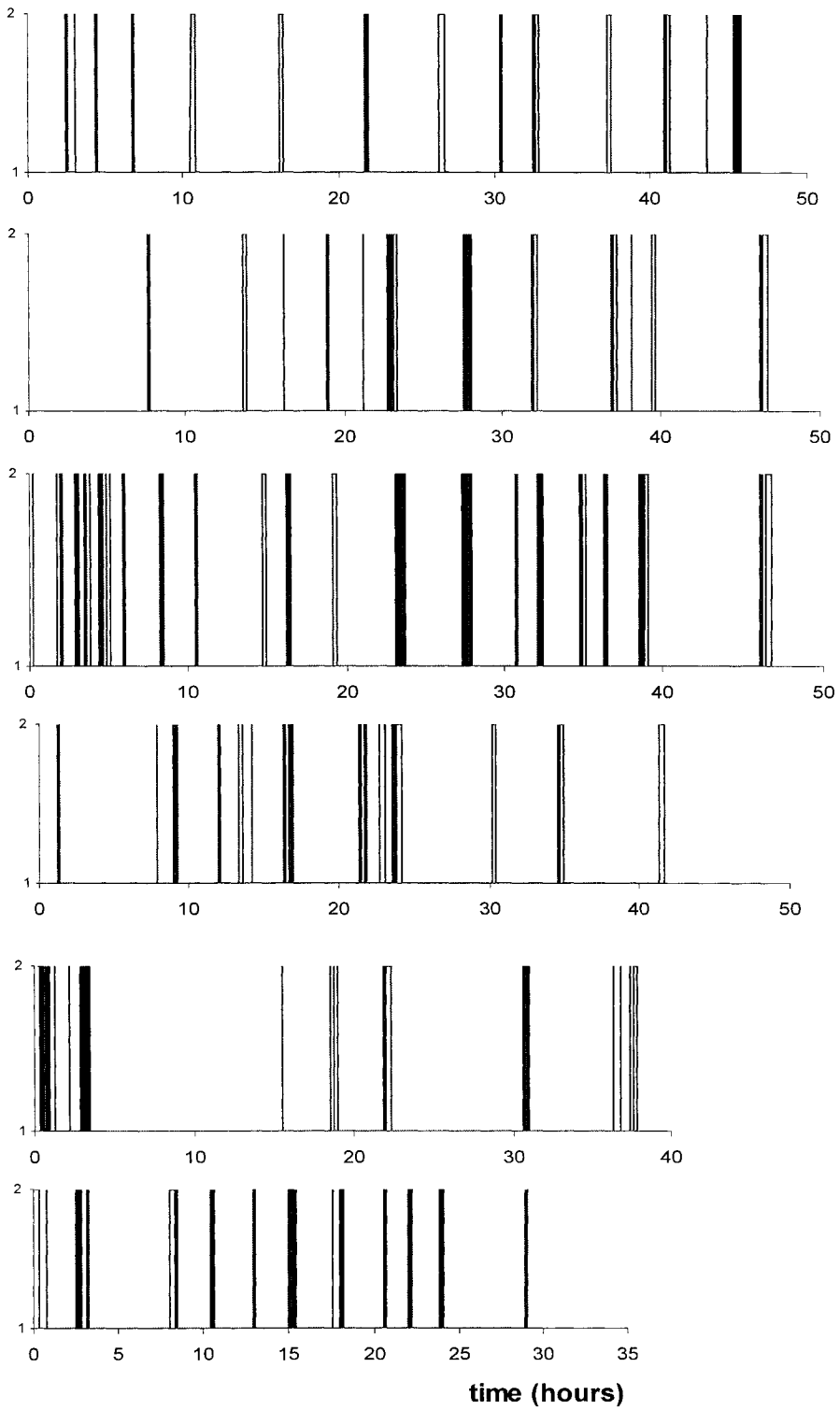

Fig. 33: Pattern of feeding for the six forest tent caterpillars on the p35:c7 artificial diet over the experimental period (in hours). The vertical bars represent feeding events, with the gaps within and between feeding events shown. Feeding periods (separated by 16.67 min) are not distinguished.