The Journal of Experimental Biology 208, 625-635
Published by The Company of Biologists 2005
doi:10.1242/jeb.01403

Host nutrition determines blood nutrient composition and mediates parasite developmental success: *Manduca sexta* L. parasitized by *Cotesia congregata* (Say)

S. N. Thompson*, R. A. Redak and L.-W. Wang

Department of Entomology, University of California, Riverside, California 92521, USA

*Author for correspondence (e-mail: nelsont@ucr.edu)

Accepted 24 November 2004

Summary

This investigation examined the influence of dietary protein and carbohydrate balance in a chemically defined artificial diet for *Manduca sexta* larvae on development of the gregarious parasite *Cotesia congregata*. Normal unparasitized larvae and larvae superparasitized in the fourth stadium were reared to the end of the fifth stadium on six diets, each having the same total amount of casein and sucrose but with different ratios ranging from high protein/no carbohydrate through to low protein/high carbohydrate. Levels of blood protein nitrogen and trehalose, nutrients supporting growth and development of *C. congregata*, varied with diet and were influenced by parasitism. Different levels of blood metabolites reflected differences in diet consumption, and the relationships between protein nitrogen and trehalose were very similar to those for protein and carbohydrate intake by parasitized and normal larvae on various diets. Dietary nutrient ratio had a significant effect on parasite burden, the numbers of parasites developing in individual host larvae and on parasite biomass. Parasites included individuals that developed and eventually emerged as second instar larvae, moulted to third instars and pupated. Many apparently mature second instar parasites, however, failed to emerge. The proportion of non-emerging individuals varied with diet, and in some cases, parasites failing to emerge were greater in number and total biomass than those that did emerge to complete development. On most diets, the mass of individual parasites was similar regardless of dietary nutrient ratio. Three dimensional models developed to demonstrate the relationships between blood protein nitrogen and trehalose levels and parasite burden and biomass established that the levels of both metabolites are important for supporting growth and development of emerged and non-emerged parasites. In the case of emerged parasites, however, the relationships are linear, and a quadratic function best describes the relationships with non-emerged parasites. Blood metabolite levels supporting the greatest parasite burden and biomass of emerged and non-emerged parasites occupy a region of two dimensional space corresponding to approximately 60–200 mg per insect of protein nitrogen and 60-100 mg per insect of trehalose. Despite the differences in the response of emerged and non-emerged parasites to host nutrition, the present results indicate that host nutrition is not the critical factor determining parasite emergence. The significance of these findings to the biology of *C. congregata* is discussed.

Key words: insect, development, nutrition, diet, parasitism, *Manduca sexta*, *Cotesia congregata*.

Introduction

Parasitism is the association of two organisms where one, the parasite, lives at the expense of the other, the host (Roberts and Janovy, 2000). Parasites are harmful and in many cases the host ultimately dies (Ewald, 1995). Nevertheless, host survival if only temporary is essential for the success of most parasites (Smyth, 1976; Thompson, 1985). Nourishment and development of hymenopteran insect endoparasites is highly integrated with the biology of their insect hosts (Mackauer and Sequeira, 1993; Quickie, 1997). Parasitized hosts undergo complex physiological alterations that ensure suitable conditions for parasite development (Vinson and Iwantsch, 1980; Thompson, 1993; Beckage and Gelman, 2001).

During the associations of many insect endoparasites, including gregarious braconid wasps of the genus *Cotesia*, with their lepidopteran larval hosts, the effects of parasitism are initially mediated by a variety of parasite-derived factors. These include polydnaviruses and/or venom, injected into the host by the adult female parasitoid during oviposition (Beckage et al., 1994; Nakamatsu et al., 2001; Nakamatsu and Tanaka, 2003). Also involved are teratocytes, specialized cells derived from the serosal membrane of the parasite egg (Dahlman and Vinson, 1993; Zhang et al., 1997). A critical role of such components is early suppression of host defense responses that would otherwise encapsulate and destroy the parasite egg or early larval stages (Schmidt et al., 2000). An adult female *Cotesia* spp. deposits between 50 and a few hundred eggs into

the body cavity or haemocoel of a single host larva. Host larvae, however, are often superparasitized, where more than one adult parasitoid oviposit eggs. The eggs hatch and parasite larvae develop, feeding principally on the host's haemolymph or blood, but also on the fat body following disintegration through the action of teratocyctes (Nakamatsu et al., 2002). In the case of *Manduca sexta* parasitized by *Cotesia congregata*, mature, second instar parasite larvae emerge from the host, moulting to the third stadium as they penetrate the cuticle (Fulton, 1940). Upon moulting, parasite larvae spin cocoons and pupate.

Parasitism also brings about long-term physiological effects, many of which may influence the ultimate success of parasite development. Depressed host growth and increased development time are common responses (Vinson and Iwantsch, 1980; Beckage and Riddiford, 1983). Delayed host development may be important for ensuring sufficient time for parasite growth and development (Smith and Smilowitz, 1976; Slansky, 1978; Lawrence and Lanzrein, 1993). Numerous studies, including investigations of a variety of lepidopteran insects parasitized by *Cotesia* spp. demonstrate that decreased food consumption accompanies the above effects (Tanaka et al., 1992; Alleyne and Beckage, 1997). Otherwise, little is understood of the potential effects of nutrition on parasitized host insects or of the importance of host nutrition to parasite success.

Recent studies establish that dietary nutrient balance influences growth and development of *Manduca sexta* over the last two larval stadia (Thompson et al., 2005). Feeding and nutrient intake differ markedly between normal and parasitized larvae and depend on the ratio of digestible protein and carbohydrate. Growth of parasitized larvae is equivalent to that of normal unparasitized larvae when insects are maintained on diets having a ratio of casein (C) to sucrose (S) between 1.0C:1.0S and 1.5C:0.5S, although development time of parasitized larvae is longer. On diets having nutrient ratios with greater or lesser protein, growth of parasitized larvae is severely depressed when compared with normal larvae even though development times of parasitized insects are greater on the less suitable diets. These results suggest that parasitized larvae may exhibit different long-term feeding preferences than normal larvae. That conclusion is consistent with results of a previous investigation demonstrating that parasitized larvae, offered a choice of diets having variable nutrient content, select a different ratio of nutrients from that preferred by normal larvae (Thompson et al., 2001).

Understanding how host responses to nutritional variation mediate parasite growth and development is important to assess whether nutritional factors play a critical role in successful parasitism. The present study addresses how dietary protein and carbohydrate balance influences the development of *C. congregata* in *M. sexta*. We establish how host dietary nutrient ratio affects the total levels of blood protein nitrogen and trehalose, and in turn, how these metabolites affect parasite burden, the numbers of parasite larvae developing in individual hosts and total parasite biomass. Furthermore, we examine the relationship between host dietary nutrient ratio, blood metabolites and the numbers of mature parasite larvae that emerge from the host to pupate and complete development and the numbers of larvae that fail to emerge. Based on the findings of studies cited above, we predicted that the nutritional status of host larvae as affected by dietary nutrient ratio would influence parasite burden and success through effects on host blood composition.

Materials and methods

Insect culture

Stock colonies of *Manduca sexta* L. were reared on a semi-defined wheat germ-based artificial diet as previously outlined (Thompson et al., 2005). Larvae used in the experiments were reared on the rearing diet until completion of the third stadium. Pharate fourth instar host larvae were synchronized as described by others (Baker et al., 1987) and superparasitized. For parasitization, larvae were placed individually in 3 l glass jars containing 100–150 *Cotesia congregata* (Say) parasitoids of both sexes. Larvae were parasitized two to four times (Alleyne, 1995), removed from the jars and placed on the experimental diets in 160 ml plastic cups.

Experimental diet and feeding protocol

Newly moulted fourth instar *M. sexta* larvae were fed a chemically defined artificial diet containing variable levels of casein and sucrose as digestible protein and carbohydrate, respectively (Ahmad et al., 1989). The diet also contained B vitamins, linseed oil, ascorbic acid and Wesson's salt mixture, obtained principally from Bioserve (Frenchtown, NJ, USA) and Nutritional Biochemicals (Cleveland, OH, USA). Six diets were employed, each having the same total amount of casein and sucrose, but with different casein to sucrose ratios as follows: 0.125C:1.875S, 0.25C:1.75S, 0.5C:1.50S, 1.0C:1.0S, 1.5C:0.5S and 2.0C:0S. The nutrient levels are indicated relative to the amount of casein and sucrose in the stock formulation, that is, 1C:1S relative to 90 g l^{-1} casein and 90 g l^{-1} sucrose. Groups of 10 randomly selected normal and parasitized larvae were maintained on each experimental diet for the feeding studies described below. Insects were housed in a Precision Scientific incubator at 28°C with a 16 h:8 h light:dark non-diapausing, long-day photocycle.

Larvae were fed on the experimental diets from the start of the fourth stadium until the end of the fifth stadium. In the case of normal larvae, the experiments were discontinued after approximately 25% of the larvae had stopped feeding and entered the wandering phase in preparation for pupation. At this point all larvae had reached approximately 8–10 g with a total development time between 7 and 15 days, depending upon diet. For parasitized larvae, the experiment was stopped at the time larvae ceased feeding prior to parasitoid emergence. The time also varied with diet, between 12 and 15 days.

Estimation of parasite burden and biomass

The number of parasites that emerged from individual host

larvae maintained on the various diets was determined at the end of the experiments by counting parasite larvae and cocoons. After dissection of the gut of parasitized larvae, the parasites failing to emerge were counted using a Wild dissecting microscope. To determine parasite biomass, the emerged and non-emerged parasites were collected and dried in an oven at 100°C for 24 h. Biomass was measured on a Sartorius microbalance.

Estimation of host blood metabolite levels

Previously, we reported the effects of dietary nutrient ratio and parasitism on equilibrium blood concentrations (mg ml^{-1}) of protein, total free amino acids and trehalose in normal and parasitized *M. sexta* larvae (Thompson et al., 2005). Here, we use those data to estimate the total quantities or levels of these metabolites in the blood of normal and parasitized larvae based on their final mass and water content, assuming 50% extracellular water (Chapman, 1998). Data are presented as total protein nitrogen level (protein plus free amino acids) and total trehalose (mg per insect).

Determination of host final mass and nutrient consumption

Diet consumption was determined as the difference between the total amount of diet offered to larvae and the amount remaining in the diet cups at the end of the experiment together with undigested diet remaining in the gut. Dry mass of the diet remaining in the cups was determined by drying the diet in an oven as described above. Initial dry mass of the diet offered to the insects was estimated from the known ratio of wet/dry mass. Protein and carbohydrate consumption were estimated based on the composition of each diet. The guts of normal and parasitized larvae were dissected at the end of the experiment and the diet remaining in the gut was removed. This diet was added to that remaining in the cups, for estimation of diet consumption. The individual carcasses of host larvae were dried as above and final host mass measured by weighing.

General statistical analyses

Data showing the effects of dietary nutrient ratio on parasite burden and biomass were examined by two-way analysis of variance (ANOVA). Analysis of covariance (ANCOVA) using various parameters as covariates, were applied in specific cases described below. The Shapiro-Wilk 'W' test and normal probability plots evaluated normality and homogeneity of variance. Except in the case of parasite burden, data met the assumptions for analysis of variance. Non-normally distributed data for parasite burden were square root transformed.

Results

Effect of dietary nutrient ratio and parasitism on host blood metabolite levels

Dietary nutrient ratio and parasitism each affected blood protein nitrogen level of *M. sexta* larvae and there was no interaction between dietary nutrient ratio and parasitism (Table 1). Trehalose level was also affected by dietary nutrients, but any effect of parasitism was marginal. Further, there was a significant interaction between parasitism and dietary nutrient ratio demonstrating that dietary nutrient ratio affects trehalose differently in normal and parasitized larvae (Table 1).

Table 1. *ANOVA summary demonstrating the effects of dietary nutrient ratio and parasitism by* Cotesia congregata *on the total levels (mg per insect) of blood protein nitrogen and trehalose in fifth instar* Manduca sexta *larvae reared on a chemically defined artificial diet*

Dependent variable	d.f.	Mean square	*F* value	Probability
Protein nitrogen (mg per insect)				
Dietary nutrient ratio	5	8839.8845	28.65	<0.0001
Parasitism	1	3927.8449	12.73	=0.0009
Interaction	5	439.9257	1.43	=0.2349
Error	42	308.5473		
Trehalose (mg per insect)				
Dietary nutrient ratio	5	3919.8908	40.62	<0.0001
Parasitism	1	376.7472	3.90	=0.0548
Interaction	5	547.9664	5.68	=0.0004
Error	42	96.4957		

The relationship between blood protein nitrogen and trehalose levels (mg/insect) relative to nutrient consumption by hosts on the various diets is shown in Fig. 1. For normal

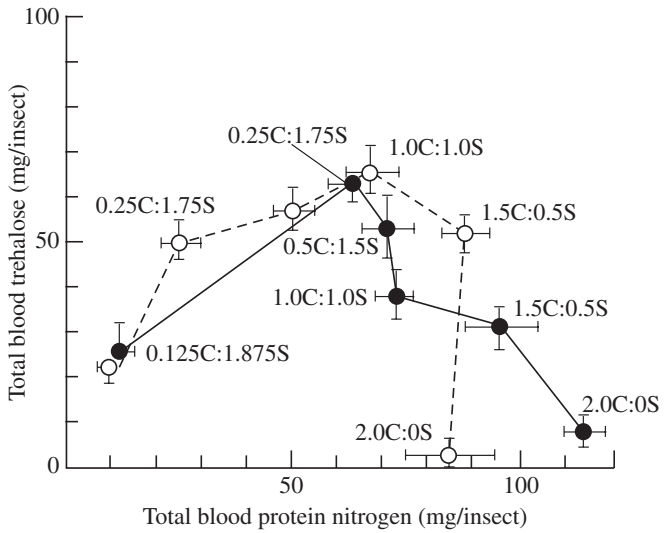

Fig. 1. Two-dimensional representation of blood protein nitrogen (protein and free amino acids) and trehalose levels (mg per insect) in normal (filled circles) fifth instar *M. sexta* larvae and larvae parasitized by *C. congregata* (open circles), maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. Values are means ± S.E.M. Dietary nutrient ratios shown relative to the level of each nutrient (C, casein; S, sucrose) in the basal chemically defined formulation where 1.0=90 g l^{-1}.

unparasitized larvae, total blood trehalose decreased as the dietary level of sucrose decreased, with the exception of the 0.125C:1.875S diet. This diet was previously judged 'pathological' (Raubenheimer and Simpson, 1999) based on earlier findings that normal host larvae fail to adjust consumption in response to the poor nutrient balance of this diet (Thompson et al., 2005). In contrast, with parasitized larvae total blood trehalose increased as dietary level of sucrose decreased reaching a maximum on the 1.0C:1.0S diet, thereafter decreasing with further decreases in dietary carbohydrate. Total blood nitrogen for both parasitized and normal larvae increased as dietary protein level increased and was maximal on the 1.5C:0.5S and 2.0C:0.5S diets.

Effect of host dietary nutrient ratio on parasite burden and biomass

Dietary nutrient ratio had a significant effect on parasite burden, the numbers of parasites developing in individual host larvae and total biomass, regardless of whether parasites emerged to complete development (Table 2; Fig. 2). The parasite burden and biomass of non-emerged parasites were greatest on the 1.0C:1.0S and the 1.5C:0.5S diets (Fig. 2AB). Non-emerged parasite burden and biomass were lower in host larvae on the other diets, but there was no significant difference between these diets. The burden and total biomass of emerged parasites was more uniform between diets and highest from host larvae on the 0.5C:1.5S, 1.0C:1.0S and 1.5C:0.5S diets (Fig. 2AB). No parasites emerged from larvae on the 0.125C:1.875S diet. On this diet parasite burden was very low and the individual parasites were very small (parasite parameters were not determined).

The mass of individual parasite larvae that emerged or that failed to emerge was similar for host larvae at all dietary nutrient ratios, except for

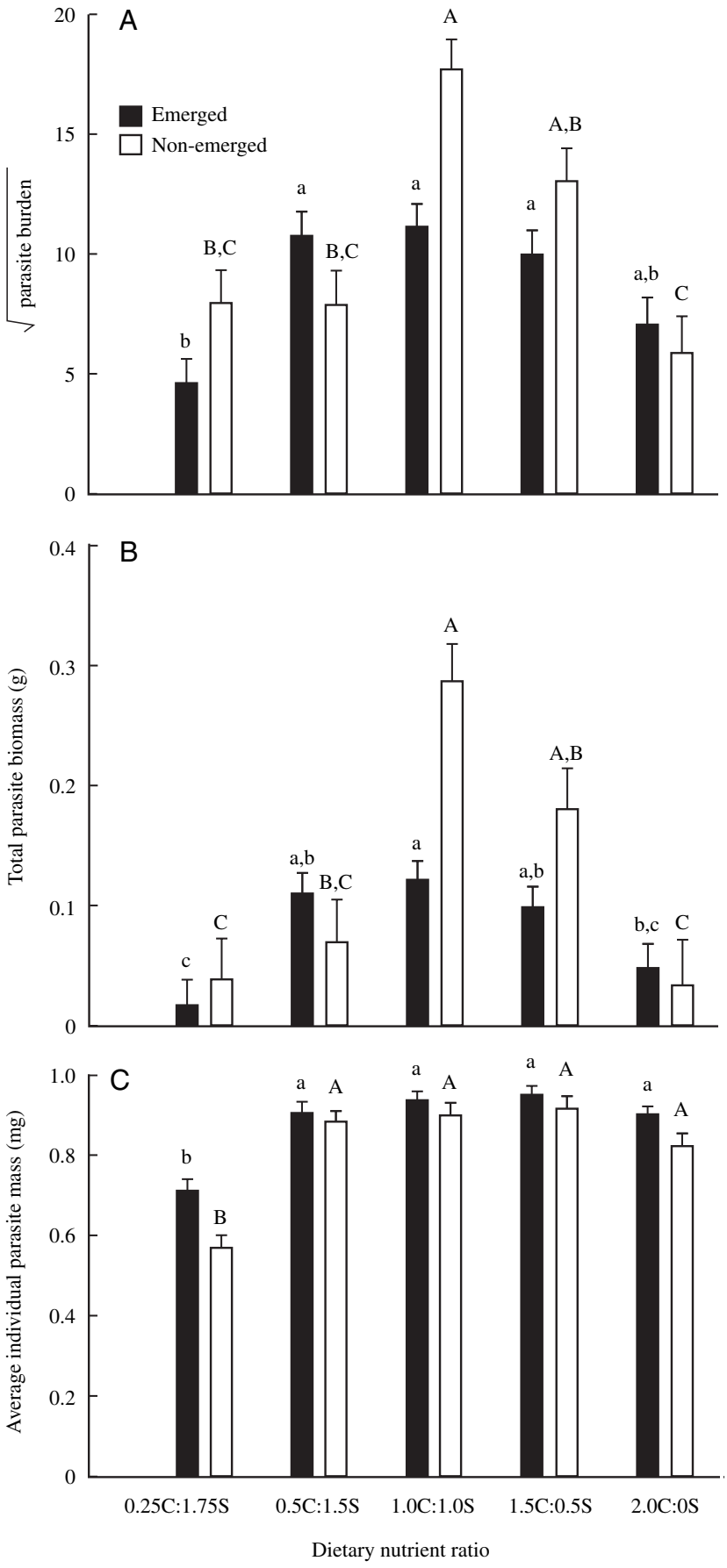

Fig. 2. Effects of host dietary nutrient ratio on parasite burden (A), total parasite biomass (B) and individual parasite mass (C) of *C. congregata*, emerged and non-emerged from *M. sexta* larvae maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose. Dietary nutrient ratios shown relative to the level of each nutrient (C, casein; S, sucrose) in the basal chemically defined formulation where 1.0=90 g l. Significant differences (P<0.05) between emerged parasites on different diets are indicated by different lower case letters and differences between non-emerged parasites by different upper case letters.

Table 2. *ANOVA summary demonstrating the effects of host dietary nutrient ratio on* Cotesia congregata *burden and biomass emerged and non-emerged from fifth instar* Manduca sexta *larvae maintained on a chemically defined artificial diet*

Dependent variable	d.f.	Mean square	*F* value	Probability
$\sqrt{}$Parasite burden – emerged				
Dietary nutrient ratio	4	47.3918	7.20	=0.0005
Error	25	6.5839		
$\sqrt{}$Parasite burden – non-emerged				
Dietary nutrient ratio	4	143.0120	12.89	<0.0001
Error	25	11.0945		
Total parasite biomass (g) – emerged				
Dietary nutrient ratio	4	0.0114	6.80	=0.0008
Error	25	0.0017		
Total parasite biomass (g) – non-emerged				
Dietary nutrient ratio	4	0.0783	10.81	<0.0001
Error	25	0.0072		
Individual parasite mass (mg) – emerged				
Dietary nutrient ratio	4	0.0409	16.46	<0.0001
Error	23	0.0025		
Individual parasite mass (mg) – non-emerged				
Dietary nutrient ratio	4	0.1268	21.45	<0.0001
Error	25	0.0059		

those from hosts on the 0.25C:1.75S diet (Fig. 2C), where both emerged and non-emerged parasites weighed less. A large portion of non-emerged parasites appear to be mature second instar larvae, an observation previously reported by Alleyne and Beckage (1997).

We compared the burden and the biomass of emerged and non-emerged parasites from hosts on the individual diets by conducting an ANOVA analysis on the arithmetic differences between the two parasite populations. The results (not shown) demonstrate a significant effect of dietary nutrient ratio on the mean difference scores for both burden and biomass. Only in the case of the 1.0C:1.0S diet, however, were the difference scores between non-emerged and emerged parasite burden and biomass significantly different from 0, with emerged parasites being fewer and having less total biomass.

Effect of host blood nutrient levels on parasite burden and biomass

We estimated the total levels of protein nitrogen and trehalose in the insects for which parasite data were available based on the total metabolite levels reported above. First, we established the relationships between metabolite levels and host final mass by applying ANCOVA using blood protein nitrogen and trehalose levels as dependent variables, dietary nutrient ratio as a main-effect treatment and final mass as the covariate. Dietary nutrient ratio had a significant effect on both metabolites and in each case, there was a significant covariate effect of final mass (Table 3). Using the metabolite levels predicted from linear regression equations, we modeled the effect of protein nitrogen and trehalose on emerged and non-emerged parasite burden and biomass (PROC REG followed by PROC G3 GRID and PROC G3D. SAS version 8.02. 2001.

Table 3. *ANCOVA summary demonstrating the effects of dietary nutrient ratio on protein nitrogen and trehalose levels (mg per insect) in fifth instar* Manduca sexta *larvae maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein and sucrose*

Dependent variable	d.f.	Mean square	*F* value	Probability
Protein nitrogen (mg per insect)				
Dietary ratio	5	2150.7572	43.21	<0.0001
Final mass (covariate)	1	2266.9880	45.54	<0.0001
Error	20	49.7771		
Trehalose (mg per insect)				
Dietary ratio	5	2536.6192	48.35	<0.0001
Final mass (covariate)	1	450.2863	8.58	=0.0080
Error	21	52.4661		

Final mass (g) was the covariate.

SAS Institute Inc., Cary, NC, USA). Data for protein nitrogen and trehalose levels were first standardized (mean of 0.0 and standard deviation of 1.0) and then used as dependent model variables. Standard variable selection techniques (Freund and Little, 2000) were used to generate a best-fit model that considered both linear and quadratic terms as independent variables and parasite burden or biomass as dependent variables. Four predictive models were constructed. Additionally, contour maps were generated from these data (PROC GCONTOUR) from a 22×10^3 point matrix by interpolating a simple linear function for the relationship between blood metabolite levels and final mass (PROC G3 GRID).

A linear model provided the best fit for emerged parasite burden and biomass, and quadratic and interaction terms involving protein nitrogen and trehalose were not included in these models (Table 4). Blood protein nitrogen and trehalose levels both predicted emerged parasite biomass (Fig. 3A). The relationship for parasite burden is not shown. Both protein nitrogen and trehalose levels were positively associated with increased emerged parasite burden and biomass (Table 5). Based on the standard regression coefficient, trehalose was the more important predictor of parasite biomass, while protein nitrogen was the more important in the case of parasite burden. With non-emerged parasite burden and biomass (Fig. 3C), both trehalose and protein nitrogen levels were important, but the relationships were more complex than with emerged parasite parameters (Fig. 3A). Here, the quadratic terms for both protein nitrogen and trehalose significantly contributed to the precision of the models in predicting non-emerged parasite burden and biomass (Table 5). The model component describing the interaction between trehalose and nitrogen did not contribute to the ability of the models to predict non-emerged parasite burden and biomass. Trehalose level (both linear and quadratic estimates) was the most important predictor of non-emerged parasite parameters. However, the overall effect of trehalose was similar to that of nitrogen. At intermediate levels of both metabolites, fewer numbers of non-emerged parasites are produced. Low and high levels of metabolites lead to greater numbers and biomass (Fig. 3C) of parasites failing to emerge.

The contour maps illustrate the optimal levels of protein nitrogen and trehalose supporting parasite growth and development. The relationships between blood metabolite levels and emerged and non-emerged parasite biomass are shown in Fig. 3C,D. Relationships for parasite burden were similar but are not shown. Nutrient levels for the 0.5C:1.5S, 1.0C:1.0S and 1.5C:0.5S diets supporting the greatest numbers and biomass of parasites appear to occupy a common region in two dimensional space. Within this space, the nutrient levels varied between approximately 60 and 110 mg per insect

Table 4. *ANOVA summary for models estimating the relationship between blood protein nitrogen and trehalose levels (mg per insect) and emerged parasite burden and biomass*

Parasite burden

Source	d.f.	Mean square	*F* value	Probability	r^2
Model	2	7.1124	13.42	0.0001	50.80
Error	26	0.5298			

Model parameter estimates

Variable	Standardized regression coefficient	Standard error of coefficient
Trehalose	0.0089	0.0051
Nitrogen	0.0107	0.0035
Intercept	–1.6085	0.3394

Parasite biomass

Source	d.f.	Mean square	*F* value	Probability	r^2
Model	2	6.7631	12.15	0.0002	48.31
Error	26	0.5567			

Model parameter estimates

Variable	Standardized regression coefficient	Standard error of coefficient
Trehalose	0.0115	0.0052
Nitrogen	0.0087	0.0036
Intercept	–1.5716	0.3479

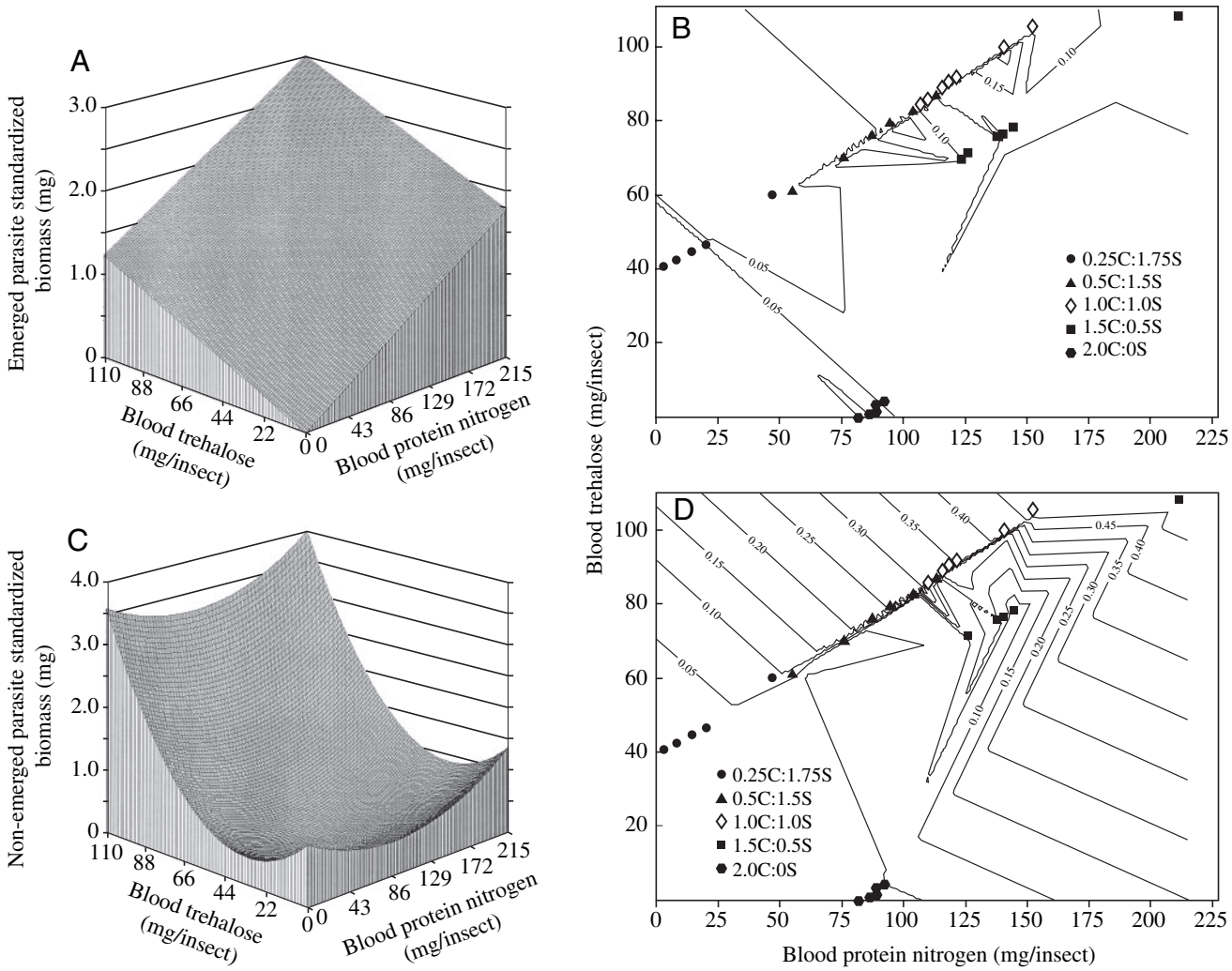

Fig. 3. Multidimensional profiles illustrating the effects of blood protein nitrogen trehalose levels (mg per insect) on biomass of *C. congregata*, emerged and non-emerged from *M. sexta* larvae maintained over the fourth and fifth stadia on a chemically defined artificial diet having varying ratios of casein (C) and sucrose (S). (A,C) Three-dimensional models for emerged and non-emerged parasite biomass, respectively. See text for model specifications and details. (B,D) Contour maps show actual emerged and non-emerged biomass, respectively. Blood metabolite levels are indicated for individual larvae on the various diets.

trehalose and between 60 and 200 mg per insect protein nitrogen.

Discussion

The present study demonstrates that development of *C. congregata* depends on host nutrition, specifically the effects of nutrient intake on the levels of host blood metabolites. As described above, parasite larvae feed principally on blood with blood metabolites providing nourishment for parasite growth and development. Although *Cotesia* spp. are thought to consume nutrients by oral ingestion, possibly aided by a stomodaeal pump (Quickie, 1997; Nakamatsu and Tanaka, 2002), first and particularly second instar *C. congregata* larvae have both unsclerotized cuticle and well developed anal vesicles (Fulton, 1940). The later form by eversion of the rectum such that the absorptive epithelium is in direct contact with the external medium. Studies with another braconid species, *Microplitis croceipes*, have demonstrated that the anal vesicle actively absorbs amino acids and sugars, including trehalose (Edson and Vinson, 1977). In addition to oral uptake, therefore, direct absorption of nutrients probably plays an important role in nourishment of *C. congregata* during larval development. The anal vesicle of *C. congregata* is withdrawn toward the end of the second stadium and the mandibles become sclerotized in preparation for incising the host integument followed by emergence.

The relationships between blood protein nitrogen and trehalose levels in normal and parasitized *M. sexta* larvae on the various diets are strikingly similar to the nutrient intake arrays demonstrating the relationships between protein and carbohydrate consumption (Thompson et al., 2005). Alterations of nutrient intake as a result of parasitism, therefore, may be an adaptive strategy by *C. congregata,* where

Table 5. *ANOVA summary for models estimating the relationship between blood protein nitrogen and trehalose levels (mg per insect) and non-emerged parasite burden and biomass*

Parasite burden					
Source	d.f.	Mean square	*F* value	Probability	r^2
Model	4	5.0169	15.17	<0.0001	71.66
Error	24	0.3306			

Model parameter estimates

Variable	Standardized regression coefficient	Standard error of coefficient
Trehalose	–0.0222	0.0147
Nitrogen	–0.0087	0.0069
Trehalose^2	0.0005	0.0002
Nitrogen^2	0.0001	0.4760
Intercept	–0.4779	

Parasite biomass					
Source	d.f.	Mean square	*F* value	Probability	r^2
	4	5.4369	20.87	<0.0001	77.67
Model	24	0.2605			
Error					

Model parameter estimates

Variable	Standardized regression coefficient	Standard error of coefficient
Trehalose	–0.0348	0.0131
Nitrogen	–0.0102	0.0062
Trehalose^2	0.0006	0.0002
Nitrogen^2	0.0001	0.0001
Intercept	–0.2013	0.4425

resultant blood metabolite levels are closest to optimal for supporting parasite growth and development on specific diets. The host blood metabolite array, the relationship between blood protein nitrogen and trehalose levels, may represent a rule of compromise for the parasite, somewhat analogous to the rules of compromise apparent from nutrient intake arrays. Rules of compromise define how animals regulate diet consumption, post ingestive responses and development time to accommodate imbalances in dietary nutrient composition (Raubenheimer and Simpson, 1999; Thompson et al., 2005). Here, the rule of compromise involves the manner in which parasitism affects host feeding and physiology to influence blood metabolite levels. Although the host blood metabolite array for parasitized larvae reflects nutrient availability rather than nutrient uptake by parasites, it is similar to the nutrient intake arrays typically observed during development of specialist feeders. In these cases, insects will suffer a large shortfall of a deficient nutrient to avoid consuming a small surplus of an excessive nutrient, as the nutrient ratio shifts away from the most optimal ratio. *C congregata* is relatively host specific, having been reported from several host species, all restricted to the family Sphingidae (Gilmore, 1938a). Host specificity is, in part, defined by the ability of *C. congregata* to modify the feeding and physiology of its host, *M. sexta*, resulting in the most suitable milieu for supporting successful development of the immature parasitic stage. Other parasitoids may bring about different effects on the feeding and physiology of *M. sexta*, or other host species, likewise producing suitable nutritional environments but reflecting different developmental strategies and host specificity.

Parasites developing in *M. sexta* larvae include those that successfully emerge from hosts to pupate and complete development and those that fail to emerge. Over a wide range of final host size there is a maximum number and total biomass of parasites that emerge from individual host larvae. In our study, these were approximately 100 parasites and 100 mg total biomass. This burden of parasites is considerably lower than the approximate 200 maximum number reported by Alleyne and Beckage (1997) for *M. sexta* larvae similarly parasitized by *C. congregata* but maintained on the wheat germ-based rearing diet. Larvae purportedly grow as well on the chemically defined diet as on the rearing diet (Ahmad et al.,

1989), but specific differences in nutrition may account for the different results for emerged parasites. Depending on diet, the number and total biomass of non-emerged parasites may exceed those of parasites that successfully emerge. This phenomenon, a large number of parasites failing to emerge, has been described in both field collected (Fulton, 1940; Thurston and Fox, 1972) and laboratory reared *M. sexta* larvae (Barbosa et al., 1991; Alleyne and Beckage, 1997).

Hosts reared on three diets, 0.5C:1.5S, 1.0C:1.0S and 0.5C:1.5S, supported maximal and similar numbers and biomass of emerged parasites. Hosts on the 1.0C:1.0S supported the largest parasite numbers and biomass of parasites, including both those that emerged and those that failed to emerge. The same diet supports the greatest mass gain by parasitized larvae (Thompson et al., 2005), and has the ratio of protein and carbohydrate selected by parasitized larvae when offered a choice of a high protein and a high carbohydrate diet (Thompson et al., 2001).

The effects of dietary nutrient ratio on parasite burden and biomass are supported by data showing the influence of blood nutrient levels on parasite parameters. Different responses of emerged and non-emerged parasites to blood nutrient levels were readily apparent from the models and contour maps illustrating these relationships. However, the specific nutrient levels supporting the greatest parasite burden and biomass reflect protein:carbohydrate ratios varying between approximately 1 and 1.5, within the range of the dietary nutrient ratios supporting the greatest parasite burden and biomass, whether emerged or non-emerged. Future investigations will examine how host protein and carbohydrate intake are partitioned into host and parasite growth.

Our studies estimate nutrient concentrations and levels at approximately half way through the fifth stadium only and may not be reflective of nutrient availability throughout the entire period of parasite development. The concentrations of various blood metabolites in parasitized larvae may change over time (Vinson and Iwantch, 1980). Studies by others demonstrate that in *M. sexta* larvae parasitized early in the fourth stadium, blood protein concentration continuously increases during the fifth stadium, albeit at a lower rate than in normal larvae, and reaches approximately 11 mg ml^{-1} when feeding stops (Beckage et al., 1989; Beckage and Kanost, 1993). The protein concentration then declines to approximately 7 mg ml^{-1} when parasites emerge. In those studies, the protein concentration at mid-fifth stadium, approximately 9 mg ml^{-1}, is similar to the concentration we observed at the same point of development in parasitized larvae on the 0.5C:1.5S diet (Thompson et al., 2005), although the latter diet contains about 20% less protein than the wheat germ diet on which larvae were fed in the above investigation.

Some investigators have suggested that parasite emergence is limited because *M. sexta* host larvae become exhausted of blood and fat body, concluding that parasites failing to emerge simply have not consumed sufficient nutrients (Bentz and Barbosa, 1990; Alleyne and Beckage, 1997). Some of our results appear to support this conclusion. The observation, for example, that the burden, biomass and proportion of non-emerged parasites to total parasites are highest in the largest hosts fed the 1.0C:1.0S diet, that also have the greatest proportion of total parasite biomass to final host mass. Our earlier results demonstrating that parasitized larvae feeding on this diet significantly increase diet consumption (Thompson et al., 2005) would suggest that larvae accommodate any increased demand for nutrients. The present finding that non-emerged parasite burden increases along with increased protein consumption would also argue against this. If nutrition were limiting, we would predict that hosts maintained the 1.0C:1.0S diet, but with low parasite burden, would have fewer and a lower proportion of parasites failing to emerge. To test this, we exposed *M. sexta* larvae individually to *C. congregata* females and removed hosts following a single oviposition encounter. After completing development, the numbers of emerged and non-emerged parasites were determined. Parasite burdens generally ranged from 50 to 200, much lower than the burdens for superparasitized hosts maintained on the better experimental diets. The wide range of parasite burden may in part be due to the amount of time females spend during a single oviposition, which varies from one to several seconds. Regardless of the parasite burden, however, in every case at least half of the parasites failed to emerge. This is consistent with, although somewhat higher than, the 25% average non-emerged parasites reported by Thurston and Fox (1972) for field collected host larvae with parasite burdens of 50–100.

Persuasive evidence against the hypothesis that nutrition limits parasite emergence is the observation that many non-emerged parasites are mature second instar larvae and appear morphologically indistinguishable from larvae that do emerge. The similar mass of emerged and non-emerged parasites found in the present study strongly suggests they were similarly nourished. In contrast to results of some others (Beckage and Riddiford, 1982; Alleyne and Beckage, 1997), we did not observe any significant decrease in the size of individual parasites with increased parasite burdens. In our studies, however, differences in parasite burden were achieved by maintaining hosts on different diets, while in the studies of Alleyne and Bekage (1997) were found highly variable parasite burdens and individual parasite mass in hosts maintained on the same diet.

Additional study is necessary to assess the potential role of nutrient depletion in parasite failure to emerge. Experiments, for example, involving administration of supplemental nutrients through injection into hosts after cessation of host feeding may prove useful for indicating the potential of nutrition to alter parasite success. Alternately, parasitized hosts might be manipulated in a manner that extends the feeding period and total nutrient consumption. At this time, however, we believe that other factors are probably involved in determining parasite emergence, or failure to emerge.

Host nutrition may mediate parasite development and success through effects on host endocrinology, which parasitism disrupts. Abnormally elevated blood concentration of juvenile hormone and lower than normal 20-

hydroxyecdysone concentration occur during parasitism of many insects, including *M. sexta* (Beckage and Gelman, 2001; Cole et al., 2002). Elevated juvenile hormone explains the decreased growth and delayed development of parasitized host larvae and ultimately their failure to pupate. Furthermore, juvenile hormone probably influences parasite development, as application of juvenile hormone or methoprene, a juvenile hormone agonist, to the cuticle of intact parasitized larvae prevents parasite emergence (Beckage and Riddiford, 1982). Host larvae probably display varying patterns of developmental hormone concentrations depending upon nutritional status, as reflected by the effects of nutrition on host size (Nijhout, 1994) as well as parasite burden. In normal larvae, such responses ensure that moulting and metamophosis occur at appropriate times under specific nutritional conditions. Under suboptimal nutritional conditions, juvenile hormone concentrations may remain high for longer periods, delaying development while larvae grow to an adequate size. *M. sexta* larvae fed only sucrose during the first 3 days of the fifth stadium display delayed development and metamorphosis in response to a slower than normal decrease in juvenile hormone synthesis (de la Garza et al., 1991). In parasitized insects, particularly in host larvae on suboptimal diets, altered hormone concentrations may desynchronize the development of the parasite. In the case of *M. sexta* parasitized by *C. congregata* juvenile hormone concentration begins to decrease a few days before parasite emergence. If juvenile hormone is too high at the time parasites are ready to begin emergence and moulting, parasites may become unresponsive and their further development permanently delayed. Thus, the distribution of emerged and non-emerged parasites may in part reflect differential response among parasites to hormone levels in hosts on the various diets.

A significant loss of parasite resources appears to characterize the nutritional and developmental interactions between *C. congregata* and *M. sexta*. First, a large number of parasite eggs, or larvae, probably early instars that are no longer apparent upon dissection, fail to develop. We assume because all host larvae were reared under the same nutritional conditions, and were of the same developmental stage and size at the time of oviposition, that all are of comparable nutritional quality. Furthermore, because all hosts were parasitized in like manner, we assume that similar numbers of parasite eggs are deposited in most host larvae. Although the initial number of eggs was not determined, the difference in total parasite burden between the best and poorest diets suggests that if the above assumptions are accurate a large portion of eggs fail to develop. Although ovicide, the destruction during superparasitization of one parasitoid's eggs by another parasitoid, usually through use of the ovipositor, might provide an explanation, ovicide is unknown in *Cotesia* spp. or other gregarious endoparasites. Second, of the eggs that develop into mature larvae, a large portion fail to emerge. All of this suggests that *C. congregata* exhibits a high degree of imprecision in determining an optimal number of eggs to deposit in host larvae. This may in part be because *C. congregata* oviposits eggs quickly, and may therefore be unable to allocate eggs precisely. Also, assessing the optimal egg number required to efficiently realize the greatest reproductive potential or rate of gain of fitness is often compromised by fitness penalties for depositing too few eggs (Godfrey, 1994).

Fitness penalties for ovipositing more eggs than successfully develop relate to the parasite's egg number, or capacity to produce eggs, and the scarcity of host individuals to parasitize (Weisser and Houston, 1993). *C. congregata*, a pro-ovigenic koinobiont (Godfrey, 1994; Quickie, 1997) eclosing to the adult stage with very large numbers of eggs, so that the fitness penalties for ovipositing too many eggs may be minimal if hosts are scarce. *C. congregata* parasitizes a variety of Sphingid moth larvae (Gilmore, 1938a), which generally display wide dispersal patterns. *M. sexta* deposit eggs in patches, but patches can be widely distributed and individual gravid female moths may fly continuously over an entire night (Gilmore, 1938b). In cultivated cotton, moths lay one to five eggs on individual plants and females have as many as 2000 eggs. In the case of wild host plants, principally solanaceous species, patches would be even more widely distributed. Because over a rather wide variation in nutritional conditions the number and biomass of emerged parasites is nearly constant, deposition of excess eggs by *C. congregata* may not be adaptive, but neither may it be detrimental. The potential adaptiveness of superparasitism may differ depending on whether superparasitism is due to multiple parasitization by an individual parasitoid, self-superparasitism, or is due to conspecific superparasitism, parasitization by more than one parasitoid (van Alphen and Visser, 1990). Our casual observation during the present study is that superparasitism was principally conspecific. In either case, however, superparasitism may be adaptive for an individual parasitoid competing for scarce hosts.

References

Ahmad, I. M., Waldbauer, G. P. and Friedman, S. (1989). A defined artificial diet for the larvae of *Manduca sexta*. *Entomol. Exp. Appl.* **53**, 189-192.

Alleyne, M. (1995). Alterations in host growth and metabolism of *Manduca sexta* larvae parasitized by *Cotesia congregata*. MS thesis, University of California, Riverside, CA, USA.

Alleyne, M. and Beckage, N. E. (1997). Parasitism-induced effects on host growth and metabolic efficiency in tobacco hornworm larvae parasitized by *Cotesia congregata*. *J. Insect Physiol.* **43**, 407-424.

Baker, F. C., Tsai, L. W., Reuter, C. C. and Schooley, D. A. (1987). *In vivo* fluctuations of JH, JH acid, and ecdysteroid titer, and JH esterase activity, during development of fifth stadium *Manduca sexta*. *Insect Biochem.* **17**, 989-996.

Barbosa, P., Gross, P. and Kemper, J. (1991). Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecol.* **72**, 1567-1575.

Beckage, N. E. and Gelman, D. B. (2001). Parasitism of *Manduca sexta* by *Cotesia congregata*: a multitude of disruptive endocrine effects. In *Endocrine Interactions of Insect Parasites and Pathogens* (ed. J. P. Edwards and R. J Weaver), pp. 59-81. Oxford: BIOS Scientific.

Beckage, N. E. and Kanost, M. R. (1993). Effects of parasitism by the braconid wasp *Cotesia congregata* on host hemolymph proteins of the tobacco hornworm, *Manduca sexta*. *Insect Biochem. Mol. Biol.* **23**, 643-653.

Beckage, N. E. and Riddiford, L. M. (1982). Effects of methoprene and

juvenile hormone on larval ecdysis, emergence, and metamorphosis of the endoparasitic wasp, *Apanteles congregatus*. *J. Insect Physiol.* **28**, 329-334.

Beckage, N. E. and Riddiford, L. M. (1983). Growth and development of the endoparasitic wasp *Apanteles congregatus*: dependence on host nutritional status and parasite load. *Physiol. Entomol.* **8**, 231-241.

Beckage, N. E., Nesbit, D. J., Nielsen, B. D., Spence, K. D. and Barman, M. A. E. (1989). Alteration of hemolymph polypeptides in *Manduca sexta* larvae parasitized by *Cotesia congregata*: A two dimensional electrophoretic analysis and comparison with major bacteria-induced proteins. *Arch. Insect Biochem. Physiol.* **10**, 29-45.

Beckage, N. E., Tan, F. F., Schleifer, K. W., Lane, R. D. and Cherubin, L. L. (1994). Characterization and biological effects of *Cotesia congregata* polydnavirus on host larave of the tobacco hornworm, *Manduca sexta*. *Arch. Insect Biochem. Physiol*. **26**, 165-195.

Bentz, J.-A. and Barbosa, P. (1990). Effects of dietary nicotine (0.1%) and parasitism by *Cotesia congregata* on the growth and food consumption and utilization of the tobacco hornworm, *Manduca sexta*. *Entomol. Exp. Appl*. **57**, 1-8.

Chapman, R. F. (1998). *The Insects: Structure and Function*, p. 106. Cambridge, UK: Cambridge University Press.

Cole, T. J., Beckage, N. E., Tan, F. F., Srinivasan, A. and Ramaswamy, S. B. (2002). Parasitoid-host endocrine relations: self-reliance or co-optation. *Insect. Biochem. Mol. Biol.* **32**, 1673-1679.

Dahlman, D. L. and Vinson, S. B. (1993). Teratocytes: developmental and biochemical characteristics. In *Parasites and Pathogens of Insects*, Vol. 1 (ed. N. E. Beckage, S. N. Thompson and B. A. Federici), pp. 145-165. New York: Academic Press.

De la Garza, L. M., Bhaskaran, G., Barrera, P. and Dahm, K. H. (1991). Effects of starvation and sucrose feeding on corpora allata of last instar larvae of *Manduca sexta*: in vitro activity, size, and cell number. *Physiol. Entomol*. **16**, 163-172.

Edson, K. M. and Vinson, S. B. (1977). Nutrient absorption by the anal vesicle of the braconid wasp, *Microplitis croceipes*. *J. Insect Physiol.* **23**, 5-8.

Ewald, P. W. (1995). The evolution of virulence: a unifying link between parasitology and ecology. *J. Parasitol.* **81**, 659-669.

Freund, R. J. and Littell, R. C. (2000). *SAS System for Regression*, pp. 134-137. Cary: SAS Institute.

Fulton, B. B. (1940). The hornworm parasite, *Apanteles congregatus* (Say) and the hyperparasite, *Hypopteromalus tabacum* (Fitch). *Ann. Entomol. Soc. Amer.* **33**, 231-243.

Gilmore, J. U. (1938a). Notes on *Apanteles congregatus* (Say) as a parasite of tobacco hornworms. *J. Econ. Entomol.* **31**, 712-715.

Gilmore, J. U. (1938b). Observations on the hornwroms attacking tobacco in Tennessee and Kentucky. *J. Econ. Entomol.* **31**, 706-712.

Godfray, H. C. J. (1994). *Parasitoids: Behavioral and Evolutionary Ecology*, pp. 99-106. Princeton: Princeton University Press.

Lawrence, P. O. and Lanzrein, B. (1993). Hormonal interactions between insect endoparasites and their host insects. In *Parasites and Pathogens of Insects*, Vol. 1 (ed. N. E. Beckage, S. N. Thompson and B. A. Federici), pp. 59-86. New York: Academic Press.

Mackauer, M. and Sequeira, R. (1993). Patterns of development in insect parasites. In *Parasites and Pathogens of Insects*, Vol. 1 (ed. N. E. Beckage, S. N. Thompson and B. A. Federici), pp. 1-23. New York: Academic Press.

Nakamatsu, Y. and Tanaka, T. (2003). Venom of ectoparasitoid, *Euplectrus* sp. near *plathypenae* (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. *J. Insect Physiol*. **49**, 149-159.

Nakamatsu, Y., Gyotoku, Y. and Tanaka, T. (2001). The endoparasitoid *Cotesia kariyai* (Ck) regulates the growth and metabolic efficiency of *Pseudaletia separata* larvae by venom and Ck polydnavirus. *J. Insect Physiol.* **47**, 573-584.

Nakamatsu, Y., Fujii, S. and Tanaka, T. (2002). Larvae of an endoparasitoid, *Cotesia kariyai* (Braconidae: Hymenoptera), feed on the host fat body directly in the second stadium with the help of teratocytes. *J. Insect Physiol*. **48**, 1041-1052.

Nijhout, H. F. (1994). *Insect Hormones*, pp. 84-88. Princeton: Princeton University Press.

Quickie, D. L. J. (1997). *Parasitic Wasps*, pp. 221-255. New York: Chapman and Hall.

Raubenheimer, D. and Simpson, S. J. (1999). Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.* **91**, 67-82.

Roberts, L. S. and Janovy, J. (2000). *Gerald D. Schmidt and Larry S. Roberts' Foundations of Parasitology*, p. 6. Boston, USA:McGraw Hill.

Schmidt, O., Theopold, U. and Strand, M. R. (2000). Innate immunity and evasion by insect parasitoids. *BioEssays* **23**, 344-351.

Slansky, F. (1978). Utilization of energy and nitrogen by larvae of the imported cabbageworm, *Pieris rapae*, as affected by parasitism by *Apanteles glomeratus*. *Environ. Entomol*. **7**, 179-185.

Smith, C. L. and Smilowitz, Z. (1976). Growth and development of *Pieris rapae* larvae parastized by *Apanteles glomeratus*. *Exp. Appl.* **19**, 189-195.

Smyth, J. D. (1976). Parasitism. In *Introduction to Animal Parasitology*, Chapter 1, pp. 1-7. Aylesbury: Hodder and Stoughton.

Tanaka, T., Yagi, S. and Nakamatu, Y. (1992). Regulation of parasitioid sex allocation and host growth by *Cotesia (Apanteles) kariyae* (Hymenoptera: Bracondidae). *Ann. Entomol. Soc. Amer.* **85**, 310-316.

Thompson, S. N. (1985). Metabolic integration during the host associations of multicellular animal endoparasites. *Comp. Biochem. Physiol.* **81**, 21-42.

Thompson, S. N. (1993). Redirection of host metabolism and effects on parasite nutrition. In *Parasites and Pathogens of Insects*, Vol. 1 (ed. N. E. Beckage, S. N. Thompson and B. A. Federici), pp. 125-144. New York: Academic Press.

Thompson, S. N. (2001). Parasitism enhances the induction of glucogenesis by the insect *Manduca sexta* L. *Int. J. Biochem. Cell Biol.* **33**, 163-173.

Thompson, S. N., Redak, R. A. and Wang, L.-W. (2001). Altered dietary nutrient intake maintains metabolic homeostasis in parasitized larvae of the insect *Manduca sexta* L. *J. Exp. Biol.* **204**, 4065-4080.

Thompson, S. N., Redak, R. A. and Wang, L.-W. (2005). Nutrition interacts with parasitism to influence growth and physiology of the insect *Manduca sexta* L. *J. Exp. Biol*. **208**, 611-623.

Thurston, R. and Fox, P. M. (1972). Inhibition by nicotine of emergence of *Apanteles congregatus* from its host, the tobacco hornworm. *Ann. Entomol. Soc. Amer.* **65**, 547-550.

van Alphen, J. J. M. and Visser, M. E. (1990). Superparasitism as an adaptive strategy for insect parasitioids. *Annu. Rev. Entomol.* **35**, 59-79.

Vinson, S. B. and Iwantsch, G. F. (1980). Host regulation by insect parasitoids. *Q. Rev. Biol*. **55**, 143-165.

Weisser, W. W. and Houston, A. I. (1993). Host discrimination in parasitic wasps: when is it advantageous? *Funct. Ecol.* **7**, 27-39.

Zhang, D., Dahlman, D. L. and Jarlfors, U. E. (1997). Effects of *Microplitis croceipes* teratocytes on host haemolymph protein content and fat body proliferation. *J. Insect Physiol.* **43**, 577-585.