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Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar

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Mounting effective resistance against pathogens is costly in terms of energy and nutrients. However, it remains unexplored whether hosts can offset such costs by adjusting their dietary intake so as to recoup the specific resources involved. We test this possibility by experimentally challenging caterpillars (*Spodoptera littoralis*) with a highly virulent entomopathogen (nucleopolyhedrovirus), under dietary regimes varying in the content of protein and digestible carbohydrate. We found that dietary protein influenced both resistance to pathogen attack and constitutive immune function to a greater extent than did dietary carbohydrate, indicating higher protein costs of resistance than energy costs. Moreover, when allowed to self-compose their diet, insects surviving viral challenge increased their relative intake of protein compared with controls and those larvae dying of infection, thus demonstrating compensation for protein costs associated with resistance. These results suggest that the change in the host's nutritional demands to fight infection induces a compensatory shift in feeding behaviour.

Keywords: costs of resistance; feeding behaviour; immunity; nutrition; pathogen infection

1. INTRODUCTION

Infection by pathogens imposes significant fitness costs on hosts, reducing survival and/or the rate of reproductive output (Moore 2002). Apart from their direct impacts on hosts, pathogen infections are thought to impose significant resource costs associated with the maintenance and activation of resistance mechanisms, which may conflict with other life-history traits (Sheldon & Verhulst 1996; Zuk & Stoehr 2002; Rolff & Siva-Jothy 2003; Schmid-Hempel 2005).

The ability of the host to fight and withstand infection depends on its nutritional state (Chandra 1996; Lochmiller & Deerenberg 2000; Coop & Kyriazakis 2001). Previous studies have demonstrated that starvation of the host increases parasite virulence (Brown *et al.* 2000), exacerbates the costs of immune activation elicited by non-malignant agents (Moret & Schmid-Hempel 2000) and leads to reduced immune responsiveness (Feder *et al.* 1997; Siva-Jothy & Thompson 2002; Rolff *et al.* 2004). However, starvation or food shortage is a non-specific nutritional assault. Evidence from insect herbivores (Lee *et al.* 2002; Simpson *et al.* 2004), and more recently from predatory invertebrates (Mayntz *et al.* 2005), has shown that animals balance their intake of multiple essential nutrients (notably protein and non-protein energy), rather than maximizing (subject to constraints) food input or the acquisition of a single pre-eminent resource. It thus remains to be established what the relative importance is of specific nutritional resources for resisting pathogen infection (Lochmiller & Deerenberg 2000), and whether animals can selectively ingest foods rich in the required resource(s).

It has been reported on several occasions for insects that the likelihood of host survival under parasite infection is improved as a result of selecting foods containing antiparasitic chemicals (Karban & English-Loeb 1997; Christe *et al.* 2003; Singer *et al.* 2004). It remains, however, to be determined whether animals are able to resist pathogen infection or ameliorate its effects by selecting particular nutrients or nutrient ratios. Accordingly, we made the following predictions: (i) if mounting resistance to infection (e.g. activating and maintaining the immune system) demands greater investment of one type of resource than others, then enrichment of the diet with that nutrient will enhance aspects of the host's ability to fight disease and (ii) the adaptive responses of hosts will include the preferential selection of foods rich in the required nutrient to compensate for its deployment in the resistance mechanisms.

To test these predictions, we used a generalist leaf-feeding caterpillar, *Spodoptera littoralis* (Boisduval) and its

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microparasite, a nucleopolyhedrovirus (NPV), as a model system. NPVs are double-stranded DNA viruses that primarily infect larval lepidopterans. Host-to-host transmission occurs mainly horizontally via environmental contamination by the infectious form of the virus, the occlusion body (OB; Cory & Myers 2003). NPVs are highly virulent pathogens, with infections usually resulting in the death of the host within a week. The caterpillars' defensive responses to NPVs include sloughing of the virally infected midgut tissues (Hoover *et al.* 2000), encapsulation of infected cells (Washburn *et al.* 1996), and activation of the phenoloxidase (PO) cascade (Trudeau *et al.* 2001). A number of studies have investigated the effect of plant secondary metabolites and single nutrients on NPV pathogenicity at the point of ingestion (Duffey *et al.* 1995). However, our study focuses on the effects of the balance between the two key macronutrients, protein and digestible carbohydrate, after the initial pathogen challenge. Intake of both these nutrients is known to be regulated by *S. littoralis*, with carbohydrate providing the major source of energy and protein being used for growth (Lee *et al.* 2002).

To test prediction (i), we monitored the performance consequences of pathogen challenge in virally infected caterpillars that were confined to feed on one of a range of foods varying only in their protein to carbohydrate (P : C) ratios (Experiment 1). We then examined whether diet-related differences in resistance to NPV infection correlated with cellular and humoral immune responses (Experiment 2). Finally, to test prediction (ii), we compared nutrient selection by virally challenged and control animals when presented with complementary foods differing in protein to carbohydrate ratio (Experiment 3).

2. MATERIAL AND METHODS

(a) *Insect and virus*

Caterpillars of *S. littoralis* came from a laboratory culture maintained at the NERC Centre for Ecology and Hydrology, Oxford, for more than 20 years. The strain of NPV (*Sl* NPV) was a mixed genotype isolate originating in Egypt. This NPV strain was amplified in *S. littoralis* larvae, and purified on a 50–60% w/w discontinuous sucrose gradient at 30 000 *g* for 90 min, following manual homogenization, filtration through muslin on ice and a low speed spin (400 *g*) to remove larval debris. Purified virus was then washed and pelleted twice, using Milli-Q water and centrifugation at 10 000 *g* for 30 min. Purified occlusion bodies (OBs) were stored at −20 °C. The concentration of OBs was estimated using a Neubauer haemocytometer with replicated samples taken at two dilutions.

(b) *Synthetic foods*

Foods contained one of five ratios of protein (a 3 : 1 : 1 mixture of casein, peptone and albumen; see Lee *et al.* 2002) to digestible carbohydrate (sucrose): 35% protein with 7% digestible carbohydrate (35 : 7), 28 : 14, 21 : 21, 14 : 28, and 7 : 35 (on a dry mass basis). Other nutrients included 4% of micronutrients (Wesson's salt, vitamins, linoleic acid and cholesterol). The remaining part of the food was filled with the non-nutritive bulking agent, cellulose. Dry ingredients were presented to the experimental insects suspended at a 1 : 6 ratio in 1% agar solution.

(c) *Administration of NPV*

During the pre-experimental periods, caterpillars were reared on a wheat-germ based, semi-artificial diet. Upon moulting to the final (sixth) larval stadium, these insects were placed individually in a 25-well tissue culture plate, where they received a diet plug (weighing *ca* 4–5 mg) inoculated either with 1 µl of NPV suspension in distilled water (800 OBs per aliquot) or with the equivalent volume of distilled water (control). The dose of 800 OBs per aliquot was chosen from pilot experiments to achieve an intermediate level of mortality (*ca* 30–40%), such that neither all caterpillars died nor all survived. The diet plug contained 21% protein and 18% carbohydrate, an optimal diet for uninfected larvae, as determined from earlier experiments (Lee *et al.* 2002). After 24 h, only caterpillars that had completely consumed the plugs were weighed to the nearest 0.1 mg and each was placed individually in its own Petri dish (9 cm diameter) that had five holes in the upper lid to allow ventilation.

(d) *Experiment 1: performance consequences in no-choice diet tests*

A total of 150 caterpillars (75 controls and 75 NPV-challenged) were provided with a single pre-weighed block of one of five artificial diets varying in their P : C composition (see above). Within the first two days, three insects were excluded from the experiment because they failed to accept the diet and another eight (all NPV-challenged) were discarded because they underwent an extra-moult. Throughout the no-choice experiment, fresh diet blocks were replaced every day until insects had ceased to feed at the premoult stage. When insects pupated, the duration of their final stadium was recorded to the nearest day. Pupae were dried to constant mass in a desiccating oven at 50 °C. Dried carcasses were weighed to the nearest 0.1 mg and then lipid-extracted in three, 24 h changes of chloroform before being re-dried and re-weighed. The lipid-free carcasses were then analysed for nitrogen content using the micro-Kjeldahl procedure (AOAC 1980). Dead animals were inspected for the presence of OBs using Giemsa staining technique under a phase contrast light microscope at ×1000 with an oil immersion lens. All but two cadavers contained OBs. The two without OBs were excluded from the analysis.

(e) *Experiment 2: effect of dietary P : C ratio on immune function*

Four physiological traits that are routinely used to determine insect immune function were measured for individual insects: total haemocyte count (THC), phenoloxidase (PO) activity, lysozyme-like antimicrobial activity and encapsulation response (see Wilson *et al.* 2001, 2002 for their detailed relevance to the immune system). One hundred caterpillars were allocated to one of the same five P : C diet treatments that were used in Experiment 1, immediately after ecdysis to the final larval stadium. Three days later (72 h), haemolymph was collected from individual insects by piercing the final proleg with a sterile needle. Immediately after the blood collection, we added 8 µl of haemolymph to 400 µl of ice-cold PBS (pH 7.4; for PO activity assay) and 10 µl to 10 µl of EDTA/glycerol anticoagulant mixture (for THC) in a plastic Eppendorf tube, respectively. These samples were frozen immediately in a −85 °C freezer until THC, lysozyme-like antimicrobial, PO activity and the amount of protein were measured following the standard protocol designed for this study organism (see the detailed procedures in Wilson *et al.*

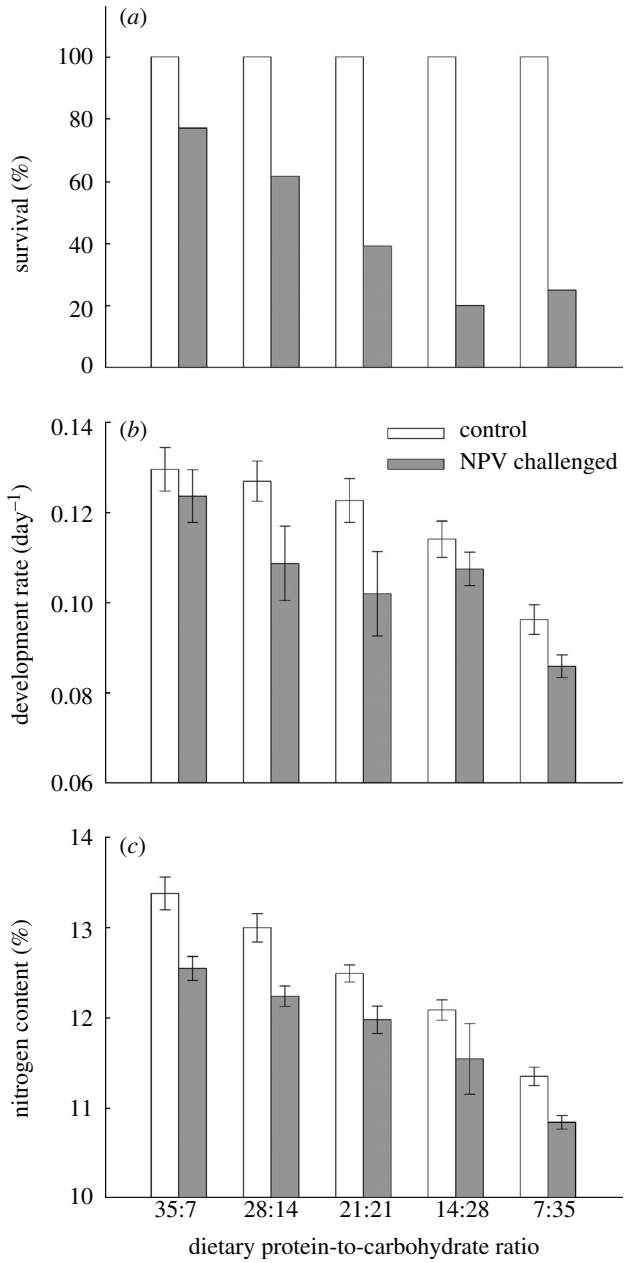

Figure 1. Effects of NPV challenge in relation to dietary protein-to-carbohydrate ratio (P : C) on (*a*) survival, (*b*) development rate (for the reciprocal of stadium duration), and (*c*) percent nitrogen content of lipid-free dry pupae of caterpillars in the no-choice experiment. In (*b*) and (*c*), the bar indicates the mean value (±1 s.e.m.).

2001; Cotter *et al.* 2004). PO activity is expressed as PO units per 1 µl haemolymph, in which one unit represents the amount of enzyme required to increase the absorbance by 0.001 min^{-1}. The absorbance was measured at 492 nm on a VERSAmax tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) after 20 min. of incubation at 25 °C with L-dopa as a substrate (Wilson *et al.* 2001). Haemolymph protein levels were determined by Bradford's method (Bradford 1976), using the plate reader with a standard curve created from a BSA standard. A lytic zone assay against the bacterium *Micrococcus lysodeikticus* at 33 °C for 24 h was conducted to determine the lysozyme-like antimicrobial activity of 1 µl of undiluted haemolymph (Cotter *et al.* 2004). The standard curves were obtained from a serial dilution of 'hen egg white lysozyme equivalents' (ranging 0.01–2 µg ml^{-1}). The number of circulating haemocytes per 1 µl of haemolymph was estimated using a Neubauer haemocytometer. To measure the encapsulation response, a piece of nylon monofilament (approximately 2 mm long) was inserted into the caterpillar's body cavity through the hole made after blood collection. After 24 h, the nylon implant was dissected out, mounted on a slide, and photographed using a Polaroid DMC digital camera (Polaroid Co., Cambridge, MA, USA). The encapsulation response was quantified by measuring the degree of melanization of the implant (scaled from 0, i.e. black, to 255, i.e. white) using Image Pro-Plus software (Media Cybernetics, Silver Spring, MD, USA). A piece of nylon (which was not inserted into the insect) was used as the control, from which we calculated the relative darkness of each implant (% melanization).

(f) *Experiment 3: nutrient self-selection*

A total of 240 caterpillars (two of which were excluded because they failed to accept the food) were allowed to mix their diet between two nutritionally complementary diet blocks, one with an equal P : C ratio (21 : 21) and another with five times more protein than carbohydrate (35 : 7). Eighty caterpillars served as controls, while 160 animals were NPV-challenged. Similar to the no-choice food regime (Experiment 1), diet blocks were replaced every day and any food remaining uneaten was dried to constant mass in a desiccating oven at 50 °C. This procedure continued until insects had ceased to feed at the premoult stage. Consumption was calculated as the difference between initial and final dry masses of the food blocks. Initial dry mass was estimated by regression analysis, using control blocks that were wet-weighed then dried to constant mass and reweighed. The dry mass of food consumed provided a measure of protein and carbohydrate consumption. Our cadaver inspection showed that all 46 dead animals had OBs. Throughout the experiment, insects were kept at a temperature of 27 °C under a 12L : 12D photo regime.

(g) *Statistical analyses*

Analyses of nutrient consumption, body chemistry, and immune variables were performed using General Linear Model procedures in SAS v. 8.2 (SAS Institute, Cary, NC, USA). Multivariate analysis of variance (MANOVA, using Pillai's trace statistic) was used for analysing the overall differences in immune variables since these multiple, correlated measurements were taken from the same insects. The data were checked for conformity to the assumptions of these parametric tests with Kolmogorov–Smirnov tests and inspection of residuals. Where necessary, the data were transformed to meet underlying assumptions. Those data that failed to be normalized even after the transformation (e.g. protein level of haemolymph) were analysed with the non-parametric Kruskal–Wallis ANOVA. Any effects of experimental factors on larval development (i.e. stadium duration) and on survival were analysed using the accelerated failure-time and Cox proportional hazards regression models, respectively (Fox 2001).

3. RESULTS

(a) *Experiment 1: performance consequences in no-choice diet tests*

While control insects on all diets showed 100% survival, for those challenged with virus there was an obvious pattern of mortality in relation to dietary P : C balance (figure 1*a*).

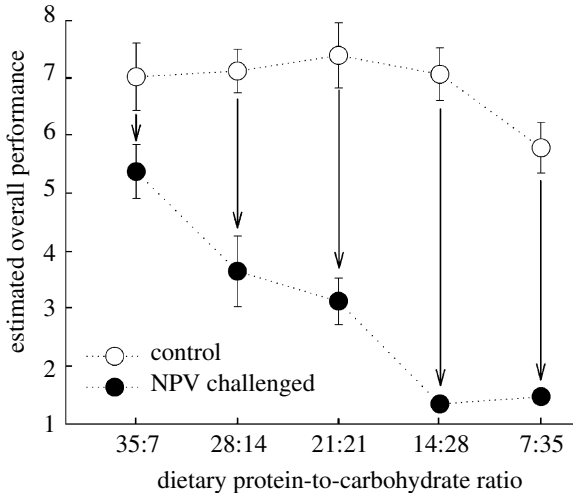

Figure 2. Composite performance estimate of NPV challenged and control caterpillars in the no-choice diet experiment. These values (means ± 1 s.e.m.) are calculated by multiplying survival by average biomass gain per day (mg day^{-1}). Each descending arrow indicates pathogen-induced performance loss of insects feeding on each P : C diet.

On extremely protein-biased food, 77% of NPV-challenged caterpillars completed their development to pupation. However, as the food became increasingly carbohydrate-biased, mortality prior to pupation rose markedly ($\chi^2_1 = 20.70$, $p < 0.001$), with less than 25% survival on the most carbohydrate-rich diets. The increase in dietary carbohydrate content was also associated with delayed larval development (figure 1*b*; $\chi^2_4 = 45.58$, $p < 0.001$). The effect of virus challenge was to decrease the rate of larval development ($\chi^2_1 = 5.50$, $p = 0.019$). The nitrogen content (%) of pupae was reduced gradually with decreasing P : C ratio (figure 1*c*; $F_{4,85} = 32.57$, $p < 0.001$). The pupae of those caterpillars that survived NPV-challenge accumulated 4–6% less body nitrogen content than the unchallenged controls ($F_{1,85} = 30.47$, $p < 0.001$). This effect was consistent across the five P : C diets, as indicated by a lack of significant two-way interaction ($F_{4,85} = 0.42$, $p = 0.792$).

When a compound measure of performance was estimated as a product of growth rate (body mass gain divided by development time) and survival (figure 2), there were significant effects due to the dietary P : C ratio ($F_{4,85} = 4.69$, $p = 0.002$), virus-challenge ($F_{1,85} = 84.92$, $p < 0.001$), and the interaction between the two ($F_{4,85} = 3.02$, $p = 0.022$).

(b) *Experiment 2: effect of dietary P : C ratio on immune function*

MANOVA results indicated that insects on high-protein diets had significantly higher constitutive immune function than those on low-protein diets ($F_{20,352} = 4.65$, $p < 0.001$). Subsequent univariate ANOVAs performed for each response variable showed that the influence of dietary P : C ratio was most pronounced for lysozyme-like antimicrobial activity (figure 3*a*; $F_{4,89} = 22.81$, $p < 0.001$), encapsulation response (figure 3*b*; $F_{4,89} = 7.13$, $p < 0.001$) and, marginally, for PO activity (figure 3*c*; $F_{4,89} = 2.42$, $p = 0.054$). Although THC did not differ significantly across the five P : C diets (figure 3*d*; $F_{4,89} = 0.52$, $p = 0.724$), the number of haemocytes was lowest for caterpillars reared on the extremely protein-poor diet (7 : 35). In addition, we found that the haemolymph protein pool declined with low P : C ratio ($H = 34.84$, d.f. = 4, $p < 0.001$).

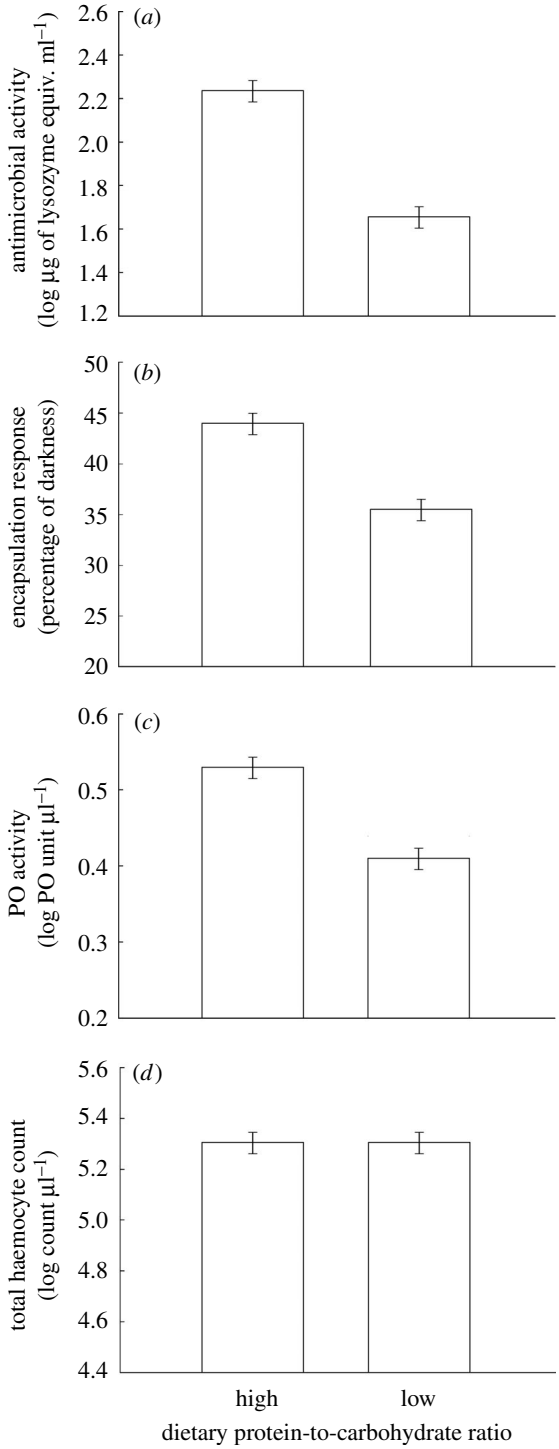

Figure 3. Dietary influence on constitutive immune function variables (means ± 1 s.e.m.): (*a*) lysozyme-like antimicrobial activity, (*b*) encapsulation response, (*c*) phenoloxidase (PO) activity, and (*d*) total haemocyte count (THC). Data from the two highest (35 : 7 and 28 : 14) and two lowest (14 : 28 and 7 : 35) P : C ratios were pooled for illustrative clarity.

(c) *Experiment 3: nutrient self-selection*

Nutrient compositions of the diets self-selected by control and NPV-challenged caterpillars were compared. Approximately 71% of NPV-challenged larvae survived the infection, compared to 100% for the control insects.

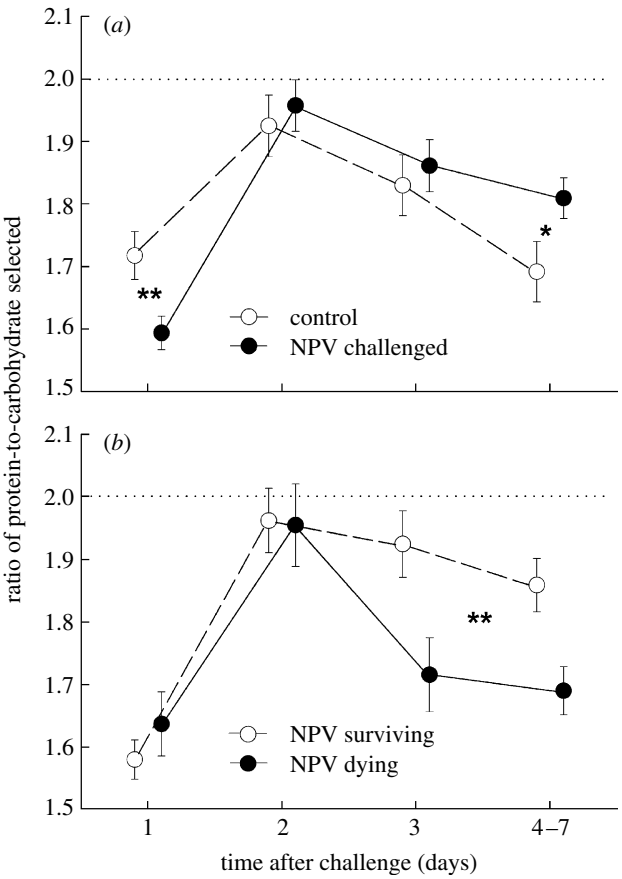

Figure 4. Proportions of protein and carbohydrate (P : C ratio) selected by caterpillars over the days post-infection in the choice diet experiment (means ± 1 s.e.m.). The daily selection ratios were compared between (*a*) NPV challenged and control insects, and between (*b*) surviving and dying insects challenged with NPV ($^{*}0.01 < p < 0.05$, $^{**}0.001 < p < 0.01$). The dotted lines indicate the selected P : C ratio of 2 : 1 if feeding had occurred randomly between the two choice diets (35 : 7 and 21 : 21).

Compared to the unchallenged controls, NPV-challenged caterpillars (both those surviving and succumbing to infection) consumed less nutrients during the first 3 days post-infection, but by the time the insects had ceased to feed at the premoult stage they had eaten similar amounts of food (Student's *t*-test: $t = -1.06$, d.f. = 236, $p = 0.289$). This pattern resulted from the fact that, before dying, caterpillars succumbing to NPV infection continued to feed for approximately 2 or 3 days longer than those surviving and control animals ($\chi^2_2 = 191.39$, $p < 0.001$).

The daily P : C consumption ratios differed between the control and NPV-challenged caterpillars (both surviving and lethally infected; figure 4*a*), with the selected P : C ratio of NPV-challenged animals being significantly lower on day 1 post-infection and higher from day 4 onwards, relative to the controls (repeated-measures ANOVA: time-by-treatment interaction, $F_{3,693} = 3.62$, $p = 0.013$). Within this NPV-challenged treatment group, differences in the developmental progress of nutrient selection were observed between virus-resisting and lethally infected insects (figure 4*b*; time-by-treatment interaction, $F_{3,471} = 2.88$, $p = 0.036$): from day 3 post-infection, those caterpillars that were to survive viral infection chose diets that were significantly more protein-biased (higher P : C) than those chosen by caterpillars that were to succumb to infection (data from day 3 and 4–7 were pooled, $t = 3.24$, d.f. = 317, $p = 0.001$). The selection pattern of the control larvae was similar to that of those that died of infection (time-by-treatment interaction, $F_{3,354} = 0.97$, $p = 0.407$) but differed significantly from the survivors ($F_{3,555} = 4.96$, $p = 0.002$).

All groups of caterpillars (control, surviving or death due to infection) showed evidence of non-random food selection. Were they to have fed between the two foods indiscriminately, the expected P : C ratio would have been 2 : 1 (figure 4). The only point at which nutrient intake approached this was on day 2 of the larval stadium.

4. DISCUSSION

Our results showed that the magnitude of the reduction in host performance following virus challenge varied with dietary P : C balance, with caterpillars reared on low-P : C diets (14 : 28 and 7 : 35) suffering a threefold greater performance loss compared to those on the extremely high-protein food (35 : 7). This was mainly due to lower survival on low-P : C diets. In contrast, the performance of control insects peaked on the 21 : 21 diet, but decreased to some extent as the food became either protein- or carbohydrate-biased. In the face of virus challenge, it is likely that the benefits in terms of increased survival of eating a protein-biased diet outweighed the disadvantage of metabolically transforming excess protein to carbohydrate in order to supplement energy deficiencies (Thompson & Redak 2000; Lee *et al.* 2002; Simpson *et al.* 2004).

Thus, the results from the no-choice feeding tests suggest that protein is more important than energy as a resource promoting host resistance against NPV infection. Protein is the major substrate for producing immunological components used for resisting viral infection (Washburn *et al.* 1996; Trudeau *et al.* 2001). Our measurement of immune responses demonstrated that, even at baseline levels, lysozyme-like antimicrobial activity, encapsulation response and PO activity were higher for those caterpillars reared on the diet high in protein (35 : 7 and 28 : 14) than on low protein diets, particularly 7 : 35. These differences were positively correlated with a significant decline in the haemolymph protein pool with decreasing dietary P : C ratio, suggesting that the constitutive immune responses were constrained by protein reserves.

Besides causing mortality, NPV challenge also negatively affected those caterpillars surviving the challenge. The development of larvae that survived NPV challenge was prolonged by 1–3 days relative to the controls, reflecting either an indirect trade-off between mounting resistance and development rate or a direct effect of sublethal virus infection (Rothman & Myers 1996; Cooper *et al.* 2003; Cory & Myers 2003). In addition, the pupae of these surviving caterpillars accumulated less body nitrogen than the unchallenged animals, indicating protein cost of resisting the viral infection.

What might account for this reduction in nitrogen accumulation? There are three possibilities. First, resisting viral challenge accelerated the breakdown of tissue protein to match the energetic demand of immune reactions (Lochmiller & Deerenberg 2000). While this might apply for those larvae suffering carbohydrate deficiency on the high P : C treatments (35 : 7 and 28 : 14), it is unlikely to explain the decline in nitrogen balance observed in the surviving insects reared on carbohydrate-rich diets

(14 : 28 and 7 : 35), in which energy was not limiting (carbohydrate being the preferred energy substrate in these animals). Second, reduced body nitrogen may have resulted from a decreased conversion efficiency of ingested protein to body mass, influenced by midgut damage incurred during the initial stage of virus infection (Washburn *et al.* 1996). However, another measure of nutrient utilization, lipid deposition, that might also have been expected to be influenced by gut damage, did not differ between infected and control insects ($F_{1,85}=0.16$, $p=0.686$), thus this possibility also seems unlikely. Finally, and in our view most likely, some of the ingested protein was used for fighting infection (e.g. via induction of the PO cascade or haemocyte production), and was thus unavailable for incorporation in protein growth.

The first two experiments indicated protein costs associated with surviving NPV challenge and investing in immune defences, and supported the hypothesis that such costs may be mitigated by an increase in ingested protein. To test the second hypothesis that hosts actively modulate their feeding to meet such protein costs, we performed an *ad libitum* food choice assay, allowing insects to self-select their preferred nutrient mixture. On the first day post-infection, NPV-challenged caterpillars (both surviving and lethally infected) selected diets that were *lower* in protein, relative to the controls, possibly reflecting responses to the damage inflicted by NPV in the midgut. A similar response has been reported for *Manduca sexta* caterpillars attacked by endoparasitoids (Thompson *et al.* 2001). By day 2, both control and virally challenged caterpillars had selected a higher but similar P : C ratio, presumably reflecting the rapid increase in growth over this period (Lee *et al.* 2002). Thereafter, the P : C ratio consumed fell, with virus-treated caterpillars selecting a significantly higher P : C ratio than controls.

When patterns of diet selection were compared within the NPV-treated group, we found a striking difference in the nutrient ratio selected by virus-resisting as compared to lethally infected insects, with the survivors choosing diets that were significantly more protein-biased (higher P : C) than those chosen by caterpillars that were to succumb to infection and by control insects. This difference was apparent from day 3 post-infection onwards. An important aspect of these data is the fact that in none of our treatments did caterpillars select indiscriminately between the two offered foods. This rules out the possibility that the shift in selected P : C ratio seen with viral challenge was the result of illness or side-effects causing caterpillars to lose their ability to regulate their nutrient intake, as reported in parasitized *M. sexta* larvae (Thompson & Redak 2005), suggesting that the response to viral challenge is truly active. Thus, our data confirm the prediction that caterpillars showed active modulation of food choice in response to NPV challenge.

Resistance to NPV is determined, in part at least, by haemocytes encapsulating virally infected tracheal tissues and eliminating virus from the haemolymph, in conjunction with the melanization response catalysed by the PO cascade (Washburn *et al.* 1996; Trudeau *et al.* 2001). Previous studies have indicated that activation of these responses becomes obvious for surviving caterpillars 2–3 days after viral administration (Washburn *et al.* 1996; Trudeau *et al.* 2001), which corresponds well with the timing of the elevated protein intake observed for the survivors in our present study. Since PO is an enzyme (a protein) and protein is the major constituent of haemocytes, it is likely that the selection of high protein diets indicates a compensatory response to the protein costs of immune activation. It is also likely that enhanced protein intake counteracts protein costs associated with repairing virally damaged midgut epithelial cells.

A mechanism that could underpin a compensatory response for the extra protein demand involved in fighting infection is the increased sensitivity of mouthpart taste receptors governing protein appetite through blood–borne nutrient feedbacks (Simpson & Raubenheimer 1996). An equivalent mechanism has also recently been reported for non-nutrient food compounds: increased responsiveness of taste receptors to protective plant toxins was found in parasitized arctiid caterpillars (Bernays & Singer 2005).

Other studies have indicated that infection can lead to changes in the host's nutritional physiology and feeding behaviour that favour parasite success (Vinson & Iwantsch 1980; Thompson *et al.* 2001, 2005). In this case, there was no evidence for viral manipulation in terms of diet selection as those larvae that died selected their diet as if uninfected. However, the only suggestion of such 'manipulation' of the host by the virus in the present experiment was that lethally infected hosts extended the feeding period within the stadium compared to control insects and those that survived the virus challenge. This could be the outcome of NPV-induced interruption of host moulting which would, in turn, allow more time for the virus to replicate, increasing viral fitness via increased yield of OBs and the chance of successful transmission (O'Reilly & Miller 1989; Cory & Myers 2003).

Our study provides the first experimental evidence that pathogen infection induces a compensatory shift in diet selection to supplement the specific nutrients required to fight infection. An intriguing question is whether a persistent challenge from a pathogen might act on the host species as a selective pressure affecting the evolution of nutrient regulatory systems.

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