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Hosts use altered macronutrient intake to circumvent parasite-induced reduction in fecundity

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ABSTRACT

Explanations for the evolution of pathogen-induced fecundity reduction usually rely on a common principle: the trade-off between host longevity and reproduction. Recent advances in nutritional research have, however, challenged this assumption and shown that longevity and reproduction are not inextricably linked. In this study, we showed that beetles infected by cysticercoids of the tapeworm *Hymenolepis diminuta* increased their total food intake and, more particularly, their carbohydrate consumption compared with uninfected insects. This increased intake was only pronounced during the first 12 days p.i., when the parasite grows and develops into a mature metacestode. Despite consuming more nutrients, infected individuals sustained lower levels of body lipid and were less efficient at converting ingested protein to body protein. However they demonstrated a capacity to compose a diet that sustained high levels of reproductive output unless confined to foods that were nutritionally dilute. We did not find any indication that macronutrient intakes had an effect on host pro-phenoloxidase activity; however, phenoloxidase activity was significantly affected by protein intake. Our results showed that when offered nutritionally complementary diets, infected hosts do not systematically suffer a reduction in fecundity. Thus, in our view, the assumption that a reduction in host reproduction represents an adaptive response by the host or the parasite to divert resources away from reproduction toward other traits should be reassessed.

1. Introduction

A reduction in host fecundity commonly accompanies infection (Hurd, 1993, 2001, 2009), and may be induced by parasites from a wide range of taxa, from microsporidia (e.g. Stirnadel and Ebert, 1997) to mites (e.g. Forbes and Baker, 1991). While fecundity reduction may simply result from tissue damage (e.g. Adamo et al., 1995) or competition for nutrients between the host and its parasites (e.g. Polak, 1996), it has also been considered as an adaptive response by the host (Hurd et al., 2001) or the parasite (Baudoin, 1975; Hurd et al., 2001; Ebert et al., 2004), functioning to divert resources away from host reproduction toward longevity, growth and/or immunity.

Such explanations are usually predicated on the assumption that there is a trade-off between different life-history traits (Stearns, 1992), especially between reproduction and longevity, and that these traits compete for a limiting pool of resource. Recent studies, however, have challenged this assumption by quantifying the nutritional relationship between longevity and reproductive effort (Lee et al., 2008; Maklakov et al., 2008; Grandison et al., 2009). By systematically manipulating dietary composition in relation to key macronutrients (principally proteins, their constituent amino acids and carbohydrates), such studies have shown that although the diet compositions at which longevity and reproductive effort are maximised differ, there is no evidence for a direct longevity cost associated with reproduction (see also Barnes et al., 2006). These results indicate that a reduction in fecundity does not inevitably lead to an extension of lifespan with consequently increased probability of transmission of the parasite between hosts. Rather, it might be predicted that when offered appropriate foods the host could offset the negative effects of the infection on their reproductive output by adjusting intake to accommodate the extra nutritional demands of infection.

The rat tapeworm *Hymenolepis diminuta* is one of the most important model systems in the study of host–parasite relations. The parasite is transmitted as an embryonated egg from the definitive rodent host to one of a range of insect intermediate hosts,

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including the beetle *Tenebrio molitor* (Arai, 1980). The parasite's life cycle is completed when the beetle is eaten by one of several suitable mammalian hosts (Arai, 1980; Webster and Macdonald, 1995). A major challenge for the parasite is for its host to remain alive long enough to have a high likelihood of transmission to the definitive host. In a classic study, Hurd et al. (2001) found that infection by *H. diminuta* increased beetle survival by 40% and that this was accompanied by a decrease in the rate of hatching of eggs from infected females (Hurd and Arme, 1986). Hurd (2001) even proposed that reduction in fecundity and the associated extended lifespan of the beetle may be in the interests of both the parasite and the host, the former having an increased probability of transmission and the latter benefiting by prolonging its period of reproduction to achieve a similar or greater lifetime reproductive success compared with uninfected hosts.

It has previously been reported for insects that the magnitude of the reduction in host performance (i.e. survival, growth, immunity) following infection with pathogens varied with dietary protein-to-carbohydrate balance (Lee et al., 2006; Povey et al., 2009). In the present study, our aim was to determine whether beetles infected with *H. diminuta* can adjust the amount and/or ratio of protein and carbohydrate ingested to offset the negative effects of parasitism on their reproductive output. We then explored several possible explanations for modifications in the feeding behaviour of infected hosts, including compensation for extra nutritional costs imposed by the growing parasites and self-medication to support activation and maintenance of immune function (Lee et al., 2006; Povey et al., 2009)

2. Materials and methods

2.1. Insects and experimental infections

Stock colonies of *T. molitor* were continuously reared in large breeding boxes on a semi-defined artificial diet containing one part yeast, 10 parts wheat flakes, one part wheat germ and 10 parts oat flakes, with wet cotton wool as a water source. *Tenebrio molitor* pupae were collected from the stock colony and sexed on the basis of differences in their developing genitalia (Bhattacharya et al., 1970). Female pupae were incubated separately at 26 °C until adulthood. All life stages were maintained at 25 °C under a 12:12 h (light:dark) photoregime.

Hymenolepis diminuta adults were removed from infected rats and dissected to collect infective eggs (parasites come from the Nematode Molecular Genetics Group, Research School of Biology, The Australian National University, Canberra, Australia). Eggs were stored in water at 4 °C. Newly emerged beetles were starved for 1 day and then exposed for 2 days to eggs of *H. diminuta* mixed with apple pulp. Uninfected beetles were deprived of food for 1 day and fed with apple pulp for 2 days.

2.2. Synthetic foods

The dry, granular, synthetic foods were based on those described by Simpson and Abisgold (1985). In total, four foods differing in their content of protein (P) and digestible carbohydrate (C) were prepared: P7:C14 (% by dry weight in the diet), P14:C7, P28:C14, P14:C28. The protein content of all diets consisted of a 3:1:1 mix of casein, peptone and albumen, while the digestible carbohydrate contained 1:1 sucrose/white dextrin. Other components of the food were Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%) and 0.2% of a vitamin mix (Dadd, 1961). The remaining part of the food comprised the non-nutritive bulking agent, cellulose. The macronutrient contents of the diets were equivalent in mass, and because carbohydrate and protein have similar caloric value the diets were approximately equal in energy density. Before being used, the four diet mixtures had been passed through a grinder to produce a standard granule size of ~0.5 mm.

2.3. Experiment 1: macronutrient self-selection tests

On the fourth day post-emergence, adult beetles were weighed and placed into separate $7 \times 13.5 \times 13.5$ cm plastic boxes. The bases of these boxes had been filled with a 1.5 cm layer of skin-safe silicone in order to mould the shape of three small (diameter 35 mm) Petri dishes set out in an equilateral triangle with their rims standing at the same level as the silicone floor. Two Petri dishes contained foods with different P:C ratios. The third dish was filled with wet cotton wool as a water source. Petri dishes with food were covered with a lid with six, 3 mm holes to allow beetles to feed without immersing themselves in the food. Twenty-four infected and 24 uninfected beetles (i.e. 48 boxes) were simultaneously presented with two foods with different dietary P:C ratios. These combinations resulted in three treatments (P7:C14 versus P14:C7, P14:C28 versus P28:C14 and P14:C28 versus P14:C7) with eight female beetles in each treatment. The aim in this experiment was to provide different, complementary food pairings to allow an insect to mix a balanced diet and demonstrate whether they have the capacity to regulate intake of both protein and carbohydrate. Convergence to a single P:C intake point, despite being offered different diet pairings, indicates separate regulation of both macronutrients (Simpson et al., 1995).

Each dish of food was dried overnight in a desiccating oven at 32 °C and weighed to the nearest 0.01 mg before being presented to the insect. The fresh mass of an individual dish of food was between 1300 and 2000 mg, an amount that exceeds total daily consumption, but did not present insects with excessive food which would have made estimation of food consumption prone to error (Schmidt and Reese, 1986). Arenas were sealed with a plastic lid once the food and the experimental beetles were inside. Each day, lids of the Petri dishes were turned to give beetles access to fresh food below the holes. Every 3 days, uneaten food was collected and fresh Petri dishes of pre-weighed food were provided. Faeces and eggs were sorted from the uneaten food and the food was dried overnight at 32 °C. Food consumption was calculated as the difference between the initial and the final dry weight of each dish. Protein and carbohydrate intakes were determined from food intake values and the known composition of individual foods. This procedure was repeated every 3 days over 21 days for the 48 beetles in the experiment. Eggs were stored for 3 weeks at 25 °C under a 12:12 h (light:dark) photocycle. The number of larvae was then counted. Female beetles were mated every 3 days throughout the experiment. When the food was changed, we placed females in contact with two males for 3 h.

At the end of the experiment, beetles were killed by brief exposure to a temperature of −80 °C. Sex was confirmed by dissection and parasite load determined. The insect carcasses were then freeze-dried at −80 °C. Dried carcasses were lipid-extracted in three, 24 h changes of chloroform. At the end of the third chloroform wash, insects were dried and reweighed. Lipid content was calculated from their mass change. The lipid-free carcasses were analysed for protein content using the Bradford method (Bradford, 1976).

2.4. Experiment 2: no-choice diet tests

A total of 150 uninfected beetles were provided with a single pre-weighed food of one of 15 artificial diets varying in their P:C composition and dilution. Diets had one of three macronutrient dilutions (14%, 42% and 70%) and one of five P:C ratios (P:C = 1:5,

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1:2, 1:1, 2:1 and 5:1). After emergence, adult female beetles were weighed and kept separately in plastic boxes (7 × 13.5 × 13.5 cm). The experimental boxes were similar to those used in the first experiment. Each chamber housed a Petri dish containing one of the 15 diets, an empty Petri dish and a third dish filled with wet cotton wool as a water source. As previously described, each dish of food was dried overnight in a desiccating oven at 32 °C and weighed to the nearest 0.01 mg before being presented to the insect. Food was changed every 3 days. Beetles were fed during 12 consecutive days.

At the end of the experiment, insects were chilled on ice and 5–10 μl of haemolymph were collected from a wound in the beetle's neck. Haemolymph was diluted (1:20) in a freshly prepared solution made of 2/3 of sodium phosphate buffer (PBS:149.6 mM NaCl; 10 mM Na_2HPO_4; pH 6.5) and 1/3 of ice cold anti-coagulant (98 mM NaOH; 145 mM NaCl; 17 mM EDTA; 41 mM citric acid, 5% sucrose, pH 7.2). The mixture was stored in an Eppendorf tube at −80 °C. Haemolymph samples from the same individual were used for measuring phenoloxidase (PO) and pro-phenoloxidase (proPO) activities. Samples were defrosted on ice water and vortexed. Cell walls were then removed by centrifugation (4 °C, 1500*g*, 30 min). PO activity was monitored spectrophotometrically as the formation of dopachrome (Horowitz and Shen, 1952). Eight microlitre of the supernatant were added to 8 μl of L-DOPA (4 mg/ml in water), 8 μl of PBS and 56 μl of distiled water (Haine et al., 2008). We included a negative control (i.e. 8 μl of L-DOPA, 8 μl of PBS and 64 μl of distiled water). Reactions were carried out in triplicate. During the reaction, the enzyme catalyses the conversion of L-DOPA to dopachrome; dopachrome can then be measured spectrophotometrically. Readings were taken every 12 s, at 27 °C, for 2 h on a spectrophotometer (VERSAMAX tunable microplate reader, Molecular Devices Corporation, Sunnyvale, CA, USA) at 492 nm. Enzyme activity was measured as Vmax (the slope of the reaction curve during the linear phase) because previous studies have established that the concentration of L-DOPA used satisfied Michaelis–Menten kinetics, and the slope of the reaction curve therefore directly correlates with the concentration of PO in the sample (Thompson, J.J.W., 2002. Aspects of melanin production and immunity in the mealworm beetle *Tenebrio molitor*. M. Phil. Thesis, Department of Animal and Plant Sciences, University of Sheffield, UK). ProPO activity was measured after activation using chymotrypsin. Reaction mixtures contained 8 μl of haemolymph supernatant, 5 μl chymotrypsin (5 mg/ml in distilled water), 8 μl PBS and 51 μl distilled water. The mixture was incubated in a 96-well plate for 10 min at room temperature before addition of 8 μl L-DOPA (4 mg/ ml in water) (Haine et al., 2008). Reactions were made in triplicate and incubated at 27 °C. We also used a negative control (i.e. 8 μl L-DOPA, 5 μl chymotrypsin, 8 μl PBS and 59 μl distiled water). Vmax was determined as for PO activity. Sex was then confirmed by dissection. We were unable to get enough haemolymph to conduct immunological tests from three individuals from three different treatments (1:1 14%, 1:1 42% and 1:5 14%).

2.5. Statistical analyses

2.5.1. Experiment 1: macronutrient self-selection tests

Various aspects of food and nutrient consumption and insect performance were analysed using ANOVA. All statistical analyses were checked for violations of model assumptions through standard residual analyses and any violations were ameliorated using variable transformation. Back-transformed values are shown in the plots. All non-significant interaction terms between the variables of the analysis were removed from the model (Engqvist, 2005). All statistical analyses were performed using Systat 12 (Systat Software, Inc., San Jose, California, USA). Analysis of covariance and bi-coordinate utilisation plots were used to explore efficiency of conversion of ingested nutrients into growth (Raubenheimer and Simpson, 1994).

2.5.2. Experiment 2: no-choice diet tests

We conducted a second order surface analysis to test the effects of carbohydrate and protein consumption on proPO and PO activities. The analysis included the main factors (carbohydrate and protein intakes), quadratic effects of the main factors and the interaction between the main effects (see Lee et al., 2008). Results were considered significant at the 5% level. Any non-significant interactions between the main effects were removed from the final model (Engqvist, 2005). When a significant effect of carbohydrate and/or protein intake was detected, surface plots using the FIELDS package in R (v2.10.1) were created (http://www.image.ucar.edu/GSP/Software/Fields/index.shtml). All statistical analyses were performed using Systat 12 (Systat Software, Inc., San Jose, California, USA).

3. Results

3.1. Experiment 1: macronutrient self-selection

The initial weight of beetles was not significantly different between the dietary treatments and between infected and non-infected insects (two-way ANOVA, parasitism: $F_{1,44} = 0.093$, $P = 0.762$, diet choice: $F_{2,44} = 4.275$, $P = 0.988$).

3.1.1. Parasitism, food intake and intake target

Data on the cumulative amounts of carbohydrate and protein ingested by infected and uninfected beetles are presented in Fig. 1. Parasitism and diet choice had significant and independent effects on the total intake of protein (two-way ANOVA, parasitism: $F_{1,44} = 15.956$, $P < 0.001$, diet choice: $F_{2,44} = 6.027$, $P = 0.005$), and

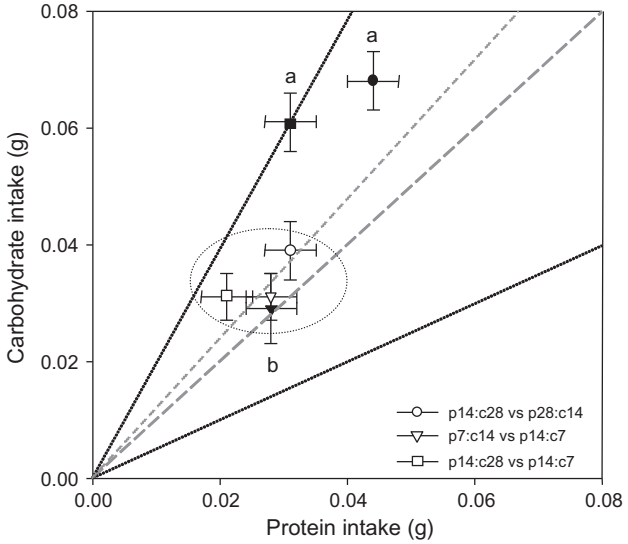

Fig. 1. Bivariate means (±standard error) of protein (P) and carbohydrate (C) intakes by uninfected *Tenebrio molitor* beetles (open symbols) and beetles infected by the tapeworm *Hymenolepis diminuta* (closed symbols). Different letters indicate significant pairwise differences for the P:C ratio selected by infected beetles (honestly significant difference (HSD) post-hoc tests, $P < 0.05$) across the different diet combinations. The grey long-dashed and short-dashed lines indicate the expected protein–carbohydrate intake trajectories on the treatments P14:C28 versus P28:C14 or P7:C14 versus P14:C7 and P14:C28 versus P14:C7, respectively, if feeding had occurred indiscriminately between both foods. The black dotted-dashed lines indicate the intake trajectories if feeding had occurred on only one food of the different food combinations.

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the interaction between parasitism and diet choice had a significant effect on the total intake of carbohydrate (two-way ANOVA, interaction: $F_{2,44} = 3.827$, $P = 0.005$).

Uninfected insects converged on the same bi-coordinate point of nutrient intake (Fig. 1). They achieved a statistically indistinguishable ratio (P:C (natural logarithm (Ln) transformed): one-way ANOVA $F_{2,22} = 0.758$, $P = 0.482$), which was marginally carbohydrate-biased, and ingested similar amounts of both nutrients in the three food pairings (protein intake: one-way ANOVA $F_{2,22} = 1.697$, $P = 0.210$, carbohydrate intake: one-way ANOVA $F_{2,22} = 0.697$, $P = 0.511$). Achieving such a similar intake required uninfected beetles having substantial differences in food consumption across the three diet pairing treatments (see Fig. 2A).

Infected insects showed less convergence of the P:C ratio chosen (P:C (Ln-transformed): one-way ANOVA $F_{2,22} = 7.831$, $P = 0.003$, Fig. 1) and the amount of nutrients ingested (protein intake: one-way ANOVA $F_{2,22} = 5.845$, $P = 0.011$, carbohydrate intake: one-way ANOVA $F_{2,22} = 12.346$, $P < 0.001$). Infected insects on the most diluted food pairing (open triangle symbols in Fig. 1) achieved the same point of nutrient intake as did all three uninfected treatment groups (see also Fig. 2A–C), but those on the most concentrated food pairings ate substantially more nutrients, particularly carbohydrate for which intake nearly doubled (see Fig. 2A–C).

3.1.2. Dynamics of intake

Data presented above were for total intake across the 21 days of the experiment. Fig. 3 illustrates the pattern of nutrient intake during successive 3-day periods throughout these 21 days. Total nutrient intakes (square-root transformed) were different between infected and uninfected beetles only during the first half of the experiment (approximately the first 12 days) and thereafter infected and uninfected insects tended to ingest similar amounts (repeated-measures ANOVA, nutrient intake: food pairing P14:C28

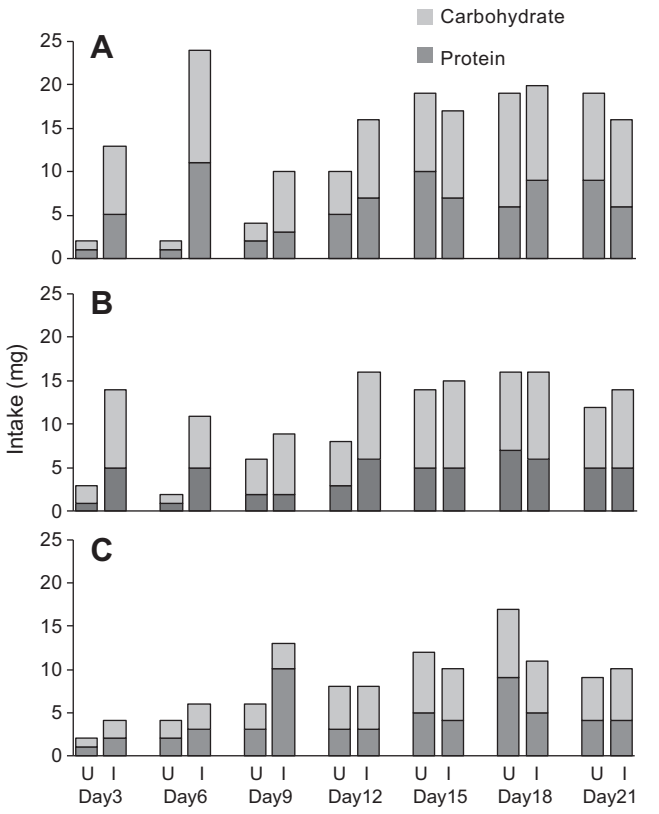

Fig. 3. Intakes (±standard error) of protein (P) and carbohydrate (C) in relation to parasitism over the 21 days of experiment for the three diet choices: P14:C28 versus P28:C14 (A), P14:C28 versus P14:C7 (B) and P7:C14 versus P14:C7 (C) (U = uninfected *Tenebrio molitor* beetles, I = *T. molitor* beetles infected by the tapeworm *Hymenolepis diminuta*).

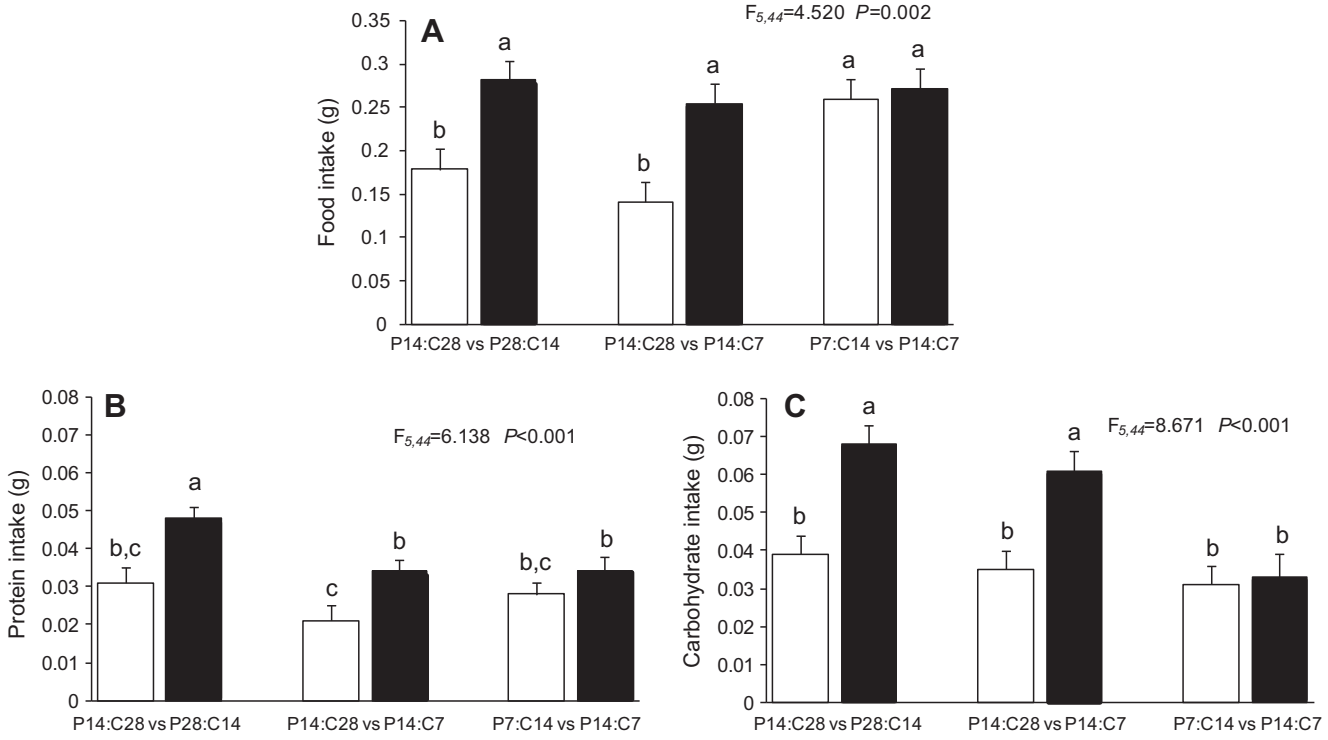

Fig. 2. Intakes (±standard error) of total food (A), protein (B) and carbohydrate (C) in relation to parasitism for the different diet choices for the 27 days of the experiment (white = uninfected *Tenebrio molitor* beetles, black = *T. molitor* beetles infected by the tapeworm *Hymenolepis diminuta*). Different letters indicate significant pairwise differences (honestly significant difference (HSD) post-hoc tests, $P < 0.05$) across the different diet combinations.

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versus P28:C14, time-by-parasitism interaction $F_{1,15} = 4.554$, $P = 0.001$; P14:C28 versus P14:C7, $F_{1,15} = 5.309$, $P < 0.001$; P14:C7 versus P7:C14, $F_{1,13} = 12.566$, $P < 0.001$). The same pattern was observed when we considered protein and carbohydrate intakes separately (repeated-measures ANOVA, protein intake: food pairing P14:C28 versus P28:C14, time-by-pairing interaction $F_{6,15} = 5.344$, $P = 0.013$; P14:C28 versus P14:C7, $F_{6,15} = 9.036$, $P < 0.001$; P7:C14 versus P14:C7, $F_{6,13} = 18.268$, $P < 0.001$; carbohydrate intake: food pairing P14:C28 versus P28:C14, time-by-pairing interaction $F_{6,15} = 4.556$, $P = 0.001$; P14:C28 versus P14:C7, $F_{6,15} = 3.559$, $P = 0.004$; P7:C14 versus P14:C7, $F_{6,15} = 2.223$, $P = 0.049$).

3.1.3. Nutrient utilisation

Bi-coordinate plots for body protein and body lipid contents are presented in Fig. 4. Lipid content (Ln-transformed) of infected beetles' carcasses was significantly lower than that of uninfected beetles, while food pairing and the interaction between pairing and parasitism did not significantly influence lipid content (two-way ANOVA, parasitism: $F_{1,41} = 33.01$, $P < 0.001$, food pairing: $F_{1,41} = 1.355$, $P = 0.271$). Protein content (Ln-transformed) was significantly influenced by the interaction between food pairing and parasitism (two-way ANOVA, interaction: $F_{2,43} = 4.657$, $P = 0.016$). This result was driven by the low protein content of infected beetles on the food pairing P14:C28 versus P28:C14; otherwise body protein contents were similar across the remaining five treatments (one-way ANOVA $F_{4,35} = 0.742$, $P = 0.571$). Among infected beetles, the parasite load (Ln-transformed) did not significantly influence protein content ($r^2 = 0.0003$, $n = 21$, $P = 0.936$) or lipid content ($r^2 = 0.0013$, $n = 20$, $P = 0.88$).

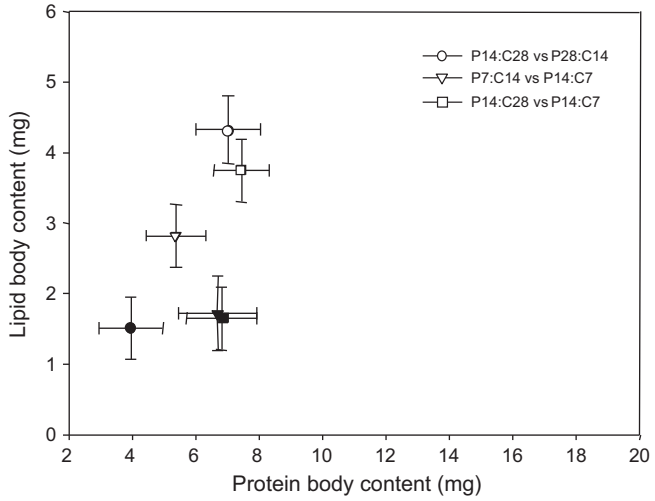

Fig. 4. Bivariate means (±standard error) of body protein and lipid content for uninfected *Tenebrio molitor* beetles (open symbols) and *T. molitor* beetles infected by the tapeworm *Hymenolepis diminuta* (closed symbols). Protein (P); Carbohydrate (C).

The analysis of covariance for protein content (Ln-transformed) of beetles' carcasses with protein intake as the covariate indicated that there were significant covariate by factor interactions ($F_{1,43} = 10.836$, $P = 0.002$). Thus, the slopes described by the utilisation plots for protein were different between infected and uninfected beetles (Fig. 5A). It is apparent that the more protein eaten the greater the protein mass grown by uninfected beetles, whereas infected beetles showed the opposite trend (Fig. 5A). For lipid growth, there was no evidence of an effect of carbohydrate intake on lipid body content (i.e. covariate by factor interaction was non-significant, $F_{1,41} = 0.819$, $P = 0.371$). Rather, infected beetles maintained a consistently lower body lipid content than uninfected beetles, irrespective of intake (Fig. 5B).

3.1.4. Reproductive output

The reproductive output (number of larvae after 21 days) was affected interactively by infection status and food pairing (two-way ANOVA, interaction: $F_{2,44} = 3.599$, $P = 0.037$, Fig. 6). Whereas uninfected beetles produced a consistent number of larvae across food pairing treatments, infected insects produced substantially more larvae on the most concentrated food pairing and fewer on the least concentrated pairing (Fig. 6). Analysis of covariance for the number of larvae (corrected for beetle weight) with protein and carbohydrate intakes as covariates and infection status as the independent factor indicated that there was no significant covariate by factor interactions for either protein intake ($F_{1,44} = 0.586$, $P = 0.449$) or carbohydrate intake ($F_{1,44} = 2.053$, $P = 0.160$). However, it is apparent from Fig. 7 that the more nutrients eaten, the greater the number of larvae produced by infected and uninfected beetles (protein intake effect: $F_{1,44} = 6.165$, $P = 0.01$; carbohydrate intake effect: $F_{1,44} = 10.645$, $P = 0.002$).

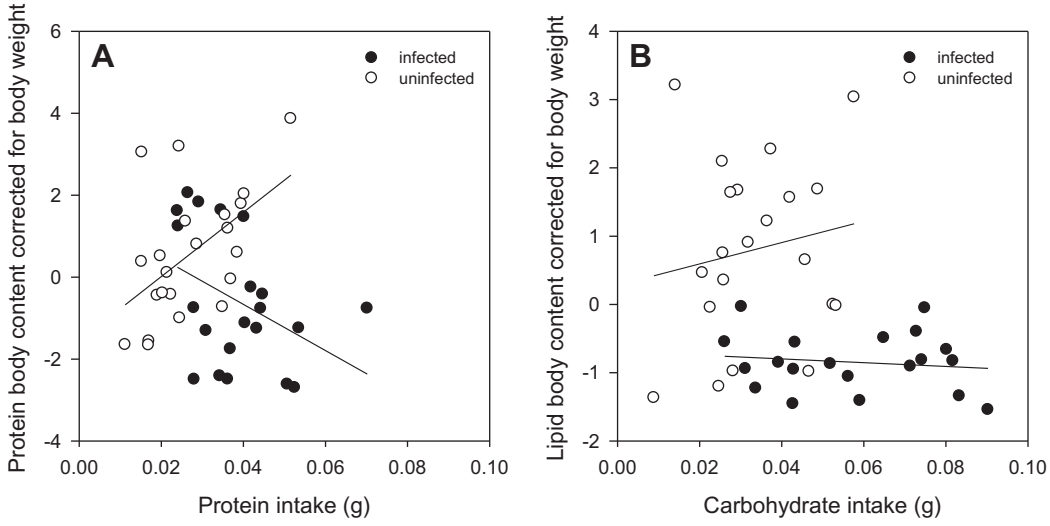

Fig. 5. Utilisation plots (±standard error) exploring the conversion efficiency of ingested nutrients to body content for uninfected *Tenebrio molitor* beetles and *T. molitor* beetles infected by the tapeworm *Hymenolepis diminuta*. Each point indicates an individual insect and in each of these plots simple linear regressions are fitted to demonstrate the conversion efficiencies. Plot (A) shows the efficiency at which ingested carbohydrate is converted to body lipid content. Plot (B) shows the efficiency at which ingested protein is converted to body protein content.

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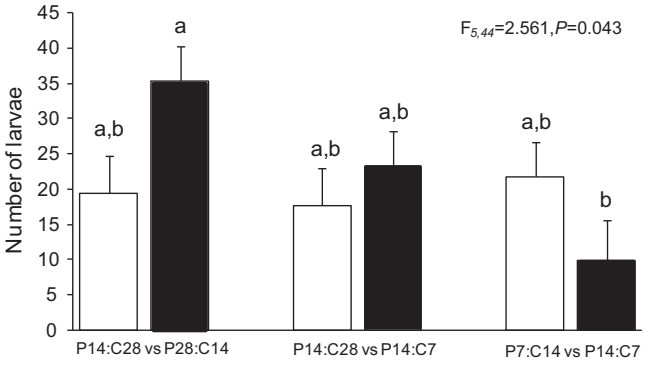

Fig. 6. Number of larvae (±standard error) produced by *Tenebrio molitor* beetles under choice conditions in relation to parasitism status (white = uninfected beetles, black = beetles infected by the tapeworm *Hymenolepis diminuta*). Different letters indicate significant pairwise differences (honestly significant difference (HSD) post-hoc tests, $P < 0.05$) across the different diet combinations. Protein (P); Carbohydrate (C).

3.1.5. Parasitic load and nutrient intake

Parasitic load was not significantly different between the three treatment groups of infected beetles at the end of the experiment (one-way ANOVA $F_{2,23} = 1.252$, $P = 0.309$). The mean parasitic load over all diet choices was 225 ± 41 cysticercoids (mean ± standard error (SE)). Among infected beetles, we did not find any significant effect of protein or carbohydrate intakes on parasitic load (see Supplementary Table S1).

3.2. Experiment 2: no-choice diet tests

The initial weight of beetles was not significantly different between the 15 food treatments (one-way ANOVA, $F_{14,147} = 0.709$, $P = 0.762$). Four individuals were discarded from the analysis because their total food consumptions were extreme outliers from the rest of the data (extreme studentized deviate test).

Data for proPO and PO activities are presented in Supplementary Table S2. We were not able to find any significant effect of protein and carbohydrate intakes on proPO activity (see Supplementary Table S3). However, there was a significant effect of protein intake on PO activity (see Supplementary Table S4). The response surface for PO was fitted over protein–carbohydrate intake arrays. PO activity increased as protein intake fell (Fig. 8).

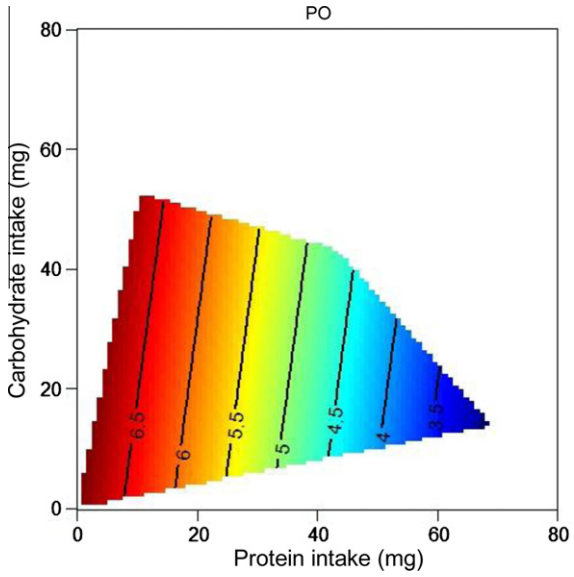

Fig. 8. Response surface for phenoloxidase mapped over protein–carbohydrate intake arrays for uninfected *Tenebrio molitor* beetles.

4. Discussion

The central prediction of this study was that infected hosts would increase intake of nutrients to reduce the negative effects of the infection on their reproductive output. Our data showed that female beetles infected with *H. diminuta* did not suffer any decrease in the number of larvae produced unless they were constrained from increasing nutrient intake, as appeared to occur on the most diluted of the food pairings. They did so as a result of increasing food consumption during the first 12 days p.i. Hence, infection does not inevitably lead to reduced reproductive output, at least when hosts are able to adjust their nutrient intake. Although it remains to be seen whether infected beetles would have lived longer than non-infected insects when offered choices between foods, our results contradict the established hypothesis that beetles, when infected, divert their resource from reproduction to longevity (Hurd et al., 2001). More generally, our data indicate that assumptions that parasites (or hosts themselves) cause hosts to shunt resources from reproduction to increased lifespan should be carefully considered (see also Bonds, 2006).

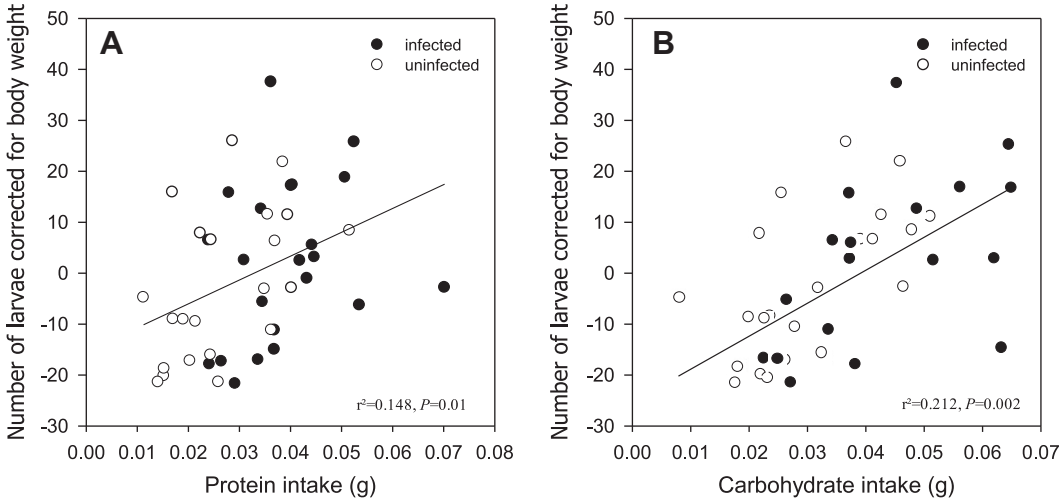

Fig. 7. Plots (±standard error) exploring the relationship between the number of larvae corrected for body weight produced by uninfected *Tenebrio molitor* beetles and *T. molitor* beetles infected by the tapeworm *Hymenolepis diminuta* and the intake of protein (A) or the intake of carbohydrate (B).

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With ad libitum access to three different nutritionally complementary food pairings, uninfected female *T. molitor* regulated intakes of protein and carbohydrate to an intake target (sensu Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). The self-selected ratio was on average 44% protein to 56% carbohydrate. Uninfected beetles maintained an intake target by adjusting the amount eaten to take account of the degree of dilution of the two complementary foods. In the wild, beetles presumably have access to a range of nutritionally different food items and can therefore regulate their intake of macronutrients by selecting between foods. This capacity to regulate the balance of protein and carbohydrate eaten by differentially selecting between complementary foods has been previously demonstrated for other insects such as – locusts (Chambers et al., 1995), caterpillars (Lee et al., 2002, 2003) and flies (Lee et al., 2008; Fanson et al., 2009), as well as for mammals such as mice (Sørensen et al., 2008) and mink (Mayntz et al., 2009).

When infected by the tapeworm *H. diminuta*, female beetles increased their total intake of food compared with uninfected insects. In a previous study, Shea (2005) found similar effects for infected males but did not find any increase in food consumption for infected female beetles. His study differed from ours in using virgin rather than mated females and providing only a single food source, commercial rat chow. Infected female beetles in our experiment were, however, unable to sustain substantially elevated nutrient intakes when confined to the most diluted food treatment (P7:C14 versus P14:C7), in which both foods were diluted with cellulose – presumably due to constraints such as reduced food retention time in the gut or metabolic effects of ingesting excess cellulose (Lee et al., 2004).

The increased consumption of macronutrients was observed only over the first 12 days of the experiment, which is consistent with the fact that the initial period of infection of *H. diminuta* is the most demanding of host resources. During the first 12–15 days p.i., the *H. diminuta* oncosphere grows rapidly and develops into a mature metacestode, while using metabolites circulating in the host haemolymph (Arme and Coates, 1973; Arme et al., 1973; Arme, 1988; Jeffs and Arme, 1982; Phillips and Arme, 1983; Hurd and Arme, 1984; Rosen and Uglem, 1988). The nutritional state of an animal usually determines whether and how much of a given food is eaten. Such a relationship has been demonstrated experimentally for insects, in which nutritional regulatory behaviour results in part from blood-borne feedbacks that directly modulate the responsiveness of external taste receptors to specific nutrients in foods (Simpson and Raubenheimer, 1996). Similar feedbacks could explain our results: infected beetles increased their protein and carbohydrate consumption because haemolymph nutrient titres were kept low due to uptake by parasites and increased demands associated with fighting the parasites, such as damage repair and the energetic and structural demands of activating and maintaining immune responses.

Despite having eaten larger amounts of carbohydrate, infected insects had less body lipid at the end of the experiment; moreover, they were less efficient at converting ingested protein to body mass. These effects suggest that macronutrients were being diverted elsewhere, stimulating enhanced feeding behaviour – most likely to parasite growth and host defence. There is nevertheless no apparent correlation between the parasitic load and lipid and protein contents of infected beetles. This could be because the observed effects are not a simple function of the number of parasites present (Hurd and Arme, 1987) but due to molecules secreted by parasites that have an effect even when small numbers of parasites are present (see for instance Webb and Hurd, 1996, 1999). This situation is certainly very different from the one observed in the association between *Tribolium confusum* and *H. diminuta* in which the parasite is much larger relative to the host (Shostak et al., 2008) and the different effects observed on the host during the infection could be more directly related to the parasitic load (Shostak, 2009).

Previous work has shown that *H. diminuta* metacestodes have a pronounced effect on the reproductive output of tenebrionid beetles, which is most marked during the first 2 weeks of infection (Keymer, 1980; Hurd and Arme, 1986; Maema, 1986). Egg production seems to be constrained by both the host and parasite; they both produce molecules that directly affect insect vitellogenesis (Webb and Hurd, 1996, 1999). In our study, beetles had the capacity to compose a diet that sustained high levels of reproductive output unless confined to foods that were nutritionally diluted. The host can thus overcome the nutritional costs of the infection and its inhibition by any host- and parasite-secreted substances when it accesses balanced foods. It would be interesting to follow vitellogenin synthesis in infected hosts under such circumstances.

We found that protein intake had a significant effect on PO activity in uninfected beetles, with a greater PO activity when the intake of protein was lower. It is therefore difficult to see how increasing macronutrient intake when parasitised would have served to promote PO activity. It is, of course, possible that other components of the immune response were elevated at higher nutrient intakes. Povey et al. (2009) found that PO activity in caterpillars increased as protein intake increased. In that case, caterpillars infected with a bacterium selected a higher protein diet than uninfected larvae (Lee et al., 2006; Povey et al., 2009), which, unlike the present results for beetles, was consistent with nutritional self-medication. The absence of an effect of nutrient intake on proPO activity in the present study suggests that beetles maintain a constant level of proPO in the haemolymph whatever their nutritional state, to be ready to fight any attack. We did not find any effect of carbohydrate or protein consumption on parasitic load. Taken together, the results for PO and parasitic load suggest that changes in feeding behaviour associated with parasitism conferred no benefit in terms of the host's effectiveness at fighting the infection. It has nevertheless been shown that the presence of *H. diminuta* is associated with a modified expression of the proPO 2/3 gene in the flour beetle *T. confusum* (Hitchen et al., 2009). It would be worthwhile to study proPO gene expression and activity in infected *T. molitor* fed with different diets.

Parasites divert host energy and nutrients towards their own growth and cause the host to invest resources in immune function. Infected hosts, however, have the capacity to adjust their dietary intake to recoup the extra nutritional demands of being infected. It was previously predicted that some parasites such as the cysticercoids of the tapeworm *H. diminuta* inhibit host reproduction to lengthen the host's lifespan, thereby increasing opportunities for transmission. However, our results showed that, when offered nutritionally complementary diets, infected hosts do not suffer a fecundity reduction because they increase nutrient intake commensurately with the increased demands of parasitism. Our results suggest that it may not be valid in all cases to view a reduction in host reproduction as an adaptive response by the host or the parasite, functioning to divert resources away from reproduction toward other traits. At best, such a strategy will apply under circumstances where food is limited in quality or quantity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2010.06.007.

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