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The effect of the acanthocephalan parasite *Pomphorhynchus laevis* on the lipid and glycogen content of its intermediate host *Gammarus pulex*

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Abstract

Besides conspicuous changes in behaviour, manipulative parasites may also induce subtle physiological effects in the host that may also be favourable to the parasite. In particular, parasites may be able to influence the re-allocation of resources in their own favour. We studied the association between the presence of the acanthocephalan parasite, *Pomphorhynchus laevis*, and inter-individual variation in the lipid and glycogen content of its crustacean host, *Gammarus pulex* (Amphipoda). Infected gravid females had significantly lower lipid contents than uninfected females, but there was no difference in the lipid contents of non-gravid females and males that were infected with *P. laevis*. In contrast, we found that all individuals that were parasitised by *P. laevis* had significantly increased glycogen contents, independent of their sex and reproductive status. We discuss our results in relation to sex-related reproductive strategies of hosts, and the influence they may have on the level of conflict over energy allocation between the host and the parasite.

Keywords: Acanthocephalan parasite; *Gammarus pulex*; Host manipulation; Glycogen; Lipid; *Pomphorhynchus laevis*; Sex

1. Introduction

The influence of parasites on host fitness is a central problem in evolutionary biology and parasitology (Toft et al., 1991; Esch and Fernández, 1993). The effects of a parasite on its host may be numerous, as is the case for some parasites with complex life cycles that are known to drastically alter their intermediate host's phenotype (Poulin, 1998). Although some effects are massive and obviously deleterious to the host, others may be indirect and more subtle. However, even subtle costs may alter some life-history traits, and hence, have an influence on demographic parameters of host populations (see Thompson and Kavaliers, 1994). One of the most striking effects of so-called 'manipulative parasites' is a change in host activity and behaviour that results in increased trophic transmission toward the parasite's final hosts (Lafferty, 1999). Although the ultimate nature of such changes have been described in detail and quantified in various host–parasite systems (Bethel and Holmes, 1973; Camp and Huizinga, 1979; Hechtel et al., 1993; Maynard et al., 1998; Cézilly et al., 2000), the proximate underlying physiological mechanisms remain to be documented in most cases (Thompson and Kavaliers, 1994). Besides conspicuous changes in behaviour, manipulative parasites may also induce more subtle physiological effects in the host that may also be favourable to the parasite (Minchella, 1985). In particular, parasites may be able to influence the re-allocation of resources in their own favour. For example, the total or partial castration of hosts by parasites has been documented in numerous parasite host systems (Thompson, 1983; Hurd, 1990; Horton and Moore, 1993), and may lead to a re-allocation of resources from reproduction to growth and maintenance (Stark, 1965; Pearre, 1976; Mueller, 1980; Minchella, 1985). There have been few tests of how parasites benefit from re-allocated resources, and whether such a phenomenon occurs at all in males, whose reproductive investment is totally different to that of females (Zuk, 1990). Sex-specific differences in the prevalence and intensity of parasite infections have previously been found in vertebrates (Zuk, 1990), the immunosuppressive effect of gonadal steroids often making males more susceptible (Zuk, 1992; Folstad and Karter, 1992). However, there is much less known about the effects of host sex on parasite prevalence and parasite-manipulation strategies in invertebrates, although prevalence differences have previously been documented (Hurd, 1990, 1993). Since male and female hosts are likely to face

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different trade-offs, particularly for those concerning the allocation of energy to reproduction, there may be sex-specific differences in the physiology or metabolism that influence the success of parasite-mediated manipulations of the host phenotype.

One way to directly compare the relative costs and effects of parasite infection in male and female invertebrate hosts is to compare the effect of parasites on a common currency such as lipid and glycogen contents. Lipids constitute one of the most important metabolic substances of invertebrate organisms, as they produce twice as much metabolic energy per unit weight as carbohydrates and proteins (Hadley, 1985). High lipid content is an indicator of good physiological condition in invertebrates. Lipids are stored as fat bodies and serve as nutrient reserves that are utilised during starvation (Lemcke and Lampert, 1975) and reproduction (Cargill et al., 1985), suggesting that they have a central role in mediating life-history trade-offs (Downer and Matthews, 1976). In contrast, the levels of glycogen are representative of the energy available for current activities (see Sparkes et al., 1996). Short-term physiological costs in hosts are thus more likely to affect glycogen levels, while more prolonged physiological effects, such as those linked to a re-allocation of energy, are likely to influence lipid reserves.

Here, we study the association between the prevalence of the acanthocephalan parasite, *Pomphorhynchus laevis*, and inter-individual variation in the lipid content of its crustacean host, *Gammarus pulex* (Amphipoda). In addition to modified behaviour and appearance (Kennedy et al., 1978; Bakker et al., 1997; Cézilly et al., 2000), cystacanths of *P. laevis* are known to induce some physiological changes in their intermediate host's phenotype. Infected gammarids show reduced O_2 consumption (Rumpus and Kennedy, 1974), and increased haemocyanin concentration (Bentley and Hurd, 1993, 1996). However, the effect of acanthocephalan parasites on host lipid and glycogen content, and how this varies with host sex, remains unknown.

2. Materials and methods

2.1. Study site

All animals used in the study were collected between 29th June and 17th July from a site on the River Ouche, Burgundy, France, using kick-sampling and a hand net (Hynes, 1954). On each day that animals were collected, they were immediately taken to the laboratory and placed in well-aerated aquaria containing water from the collection site. Individuals were sexed from the shape and size of segment 6 (propodus) of gnathopods 1 and 2, and the presence or absence of eggs or embryos in the brood pouch. Females with eggs or embryos in the brood pouch were classified as 'gravid', while females whose brood pouch was empty were termed 'non-gravid'. Size was determined by linear dimensions (body height at the level of the fourth coxal plate basis; see Brun, 1971) using a Nikon SMZ-10A stereoscopic microscope (Mag × 3) connected to a VT0 232 video-measure system (Linkam Scientific Instruments Ltd.). This method of measurement was highly repeatable (linear regression; $r^2 = 0.986$, $F_{1,232} = 16255.037$, $P < 0.0001$). We determined the presence or absence of acanthocephala, parasite intensity, and parasite species by carefully dissecting animals in an Eppendorf tube. Any parasites that were found were recorded and then removed. All individuals were classified as either 'infected with *P. laevis*' or 'not infected with *P. laevis*'. At the time of the year at which the animals were sampled, all acanthocepha have reached the cystocanth stage at this location (L. Bollache, personal communication). Any individuals found to be infected with the sympatric acanthocephalan species, *Polymorphus minutus*, were discarded from the study. All individuals were then stored in either 0.2 ml of 2% sodium sulphate solution for 1 day for glycogen analysis; or for 3 days in 0.5 ml of a 1:1 chloroform/methanol solution for fat analysis. All animals were processed on the same day that they had been collected.

2.2. Fat analysis

Fat analysis was based on the techniques used by Van Handel (1985a). All samples were crushed in their Eppendorf using an Eppendorf pestle. The supernatants were then transferred to clean, 16 × 100 mm culture tubes. The tubes were placed inside a fume cupboard in a water bath at 95°C to enable any remaining solvent to evaporate. Two hundred microlitres of concentrated (95%) sulphuric acid was then added to each tube and left for a total of 10 min. The tubes were then removed from the water bath and left to cool prior to adding 5 ml of a Vanillin–phosphoric acid reagent (see Van Handel, 1985a). All tubes were vortexed and left for 5 min to enable the colour to develop. Following this, a 0.3 μl sub-sample of the solution in each tube was pipetted into a separate well of a 96 well micro-plate. The optical density in each well was read directly at 490 nm using a DYNEX MRX plate-reader with REVELATION software. The lipid content in each sample was determined from a calibration curve constructed using 50, 200, 400, 1000 and 2000 μg samples of commercial vegetable oil (linear regression; $r^2 = 0.962$, $F_{1,14} = 452.816$, $P < 0.0001$).

2.3. Glycogen analysis

Glycogen analysis was based on the techniques used by Van Handel (1985b). All samples were crushed in their Eppendorfs using an Eppendorf pestle. One millilitre of methanol was then added to each Eppendorf before vortexing them for 30 s, and then centrifuging them at 2000 × *g* for a further 2 min. The supernatant (containing sugars) from each tube was decanted into a separate 16 × 100 mm culture tube. The remaining *G. pulex* tissue and the glycogen (adsorbed onto the precipitated sodium sulphate) in the tubes were then washed into clean 16 × 100 mm culture

tubes using 1 ml of Anthrone reagent (see Van Handel, 1985b for details). A further 4 ml of Anthrone reagent was then added to the tube before being placed into a water bath at 95°C for 17 min. The tubes were removed from the water bath, vortexed for 20 s and left to cool. Following this, a 0.3 μl sub-sample of the solution in each tube was pipetted into a well of a 96 well micro-plate. The optical density in each well was then read at 630 nm using a DYNEX MRX plate-reader with REVELATION software. The glycogen content of each sample was determined from a calibration curve, constructed using 25, 50, 100, 150, 200 and 400 μg concentrations of a standard glucose solution (see Van Handel, 1985b; linear regression; $r^2 = 0.948$, $F_{1,17} = 294.364$, $P < 0.0001$).

2.4. Statistical analysis

Deviations from normality were tested using the Kolmogorov Smirnov (Liliefors test) test and homogeneity of variance was tested using *F*-max tests (Sokal and Rohlf, 1997). In cases where the data deviated from normality and would not fit a normal distribution following transformation, non-parametric statistics were used. In all cases, the tests were two-tailed and where multiple tests were necessary, a Bonferroni correction was used to eliminate the possibility of type 1 statistical errors (Sokal and Rohlf, 1997).

3. Results

3.1. Fat content

Fat content was skewed to the right, so a log-transformation was necessary to normalise the data prior to analysis. Fat content (log) was positively correlated with body size (see Table 1). There was a significant difference in the total fat content of the three types (mean ± SE: males, 2.649 ± 0.014; gravid females, 2.695 ± 0.022; non-gravid females, 2.583 ± 0.036; see Table 1), as well as how fat content (log) scaled with body size (see Table 1). The effect of the parasite infection on host fat content was therefore tested separately for males, gravid females, and non-gravid females.

Table 1
ANCOVAs for fat and glycogen content[a]

Source	*df*	Mean square	*F* ratio	*P*
Fat (log)				
Type	2	0.148	5.941	0.0031
Body size	1	0.808	32.349	< 0.0001
Interaction	2	0.122	4.873	0.0085
Error	227	0.025		
Glycogen (log)				
Type	2	0.078	2.422	0.0930
Body size	1	0.185	5.761	0.0179
Interaction	2	0.052	1.624	0.2014
Error	123	0.032		

[a] The factor 'type' represents males, gravid females, and non-gravid females. 'Body size' was included into the model as a covariate factor.

Infection with *P. laevis* did not affect the slope of the relationship between fat content (log) and body size in males (ANCOVA: 'infection status' by 'body size' interaction, $F_{1,137} = 1.573$, $P = 0.2120$); gravid females (ANCOVA: 'infection status' by 'body size' interaction, $F_{1,59} = 3.548$, $P = 0.0646$); or non-gravid females (ANCOVA: 'infection status' by 'body size' interaction, $F_{1,25} = 0.496$, $P = 0.4879$). However, gravid females that were infected with *P. laevis* had significantly decreased fat contents (ANCOVA: 'infection status', $F_{1,60} = 11.763$, $P = 0.0011$; see Fig. 1a). There was no difference between the fat contents of infected and uninfected males (ANCOVA: 'infection status', $F_{1,138} = 0.481$, $P = 0.4890$; see Fig. 1b) or non-gravid females (ANCOVA: 'infection status', $F_{1,25} = 0.907$, $P = 0.3497$; see Fig. 1c).

3.2. Glycogen content

Glycogen content was also skewed to the right and was therefore log-transformed prior to analysis. Glycogen content (log) was positively correlated with body size (Table 1). There was no difference in the way that glycogen scaled with body size in males, gravid females, and non-gravid females (Table 1). After removal of this non-significant interaction term, there was a significant difference in the glycogen content of males, gravid females, and non-gravid females (ANCOVA: 'type', $F_{2,125} = 4.815$, $P = 0.0097$). Males had significantly more glycogen than gravid females (Fisher's PLSD, $P < 0.0001$) and non-gravid females (Fisher's PLSD, $P < 0.0001$), but there was no difference in the glycogen content of gravid and non-gravid females (Fisher's PLSD, $P = 0.9915$). Since there was no difference in the homogeneity of slopes, the data for males, gravid females, and non-gravid females (Table 1) were pooled to compare the effect of *P. laevis* infection on host glycogen reserves. Infected individuals had significantly higher glycogen contents compared with uninfected individuals (ANCOVA: 'infection status'; $F_{1,127} = 7.859$, $P = 0.0059$; see Fig. 2).

4. Discussion

Size-corrected levels of lipid and glycogen reserves in *G. pulex* varied between the sexes. Females had higher lipid contents, while glycogen levels were higher in males. The results concur with previous studies of isopods (Clarke, 1984; Wägele, 1992), in which sex differences were suggested to be the result of the different reproductive roles of each sex. Females use lipids in the synthesis of egg yolk (vitellin) (Sutcliffe, 1993); while male reproductive investment depends more on traits involving activity, such as mate-searching, competition for possession of

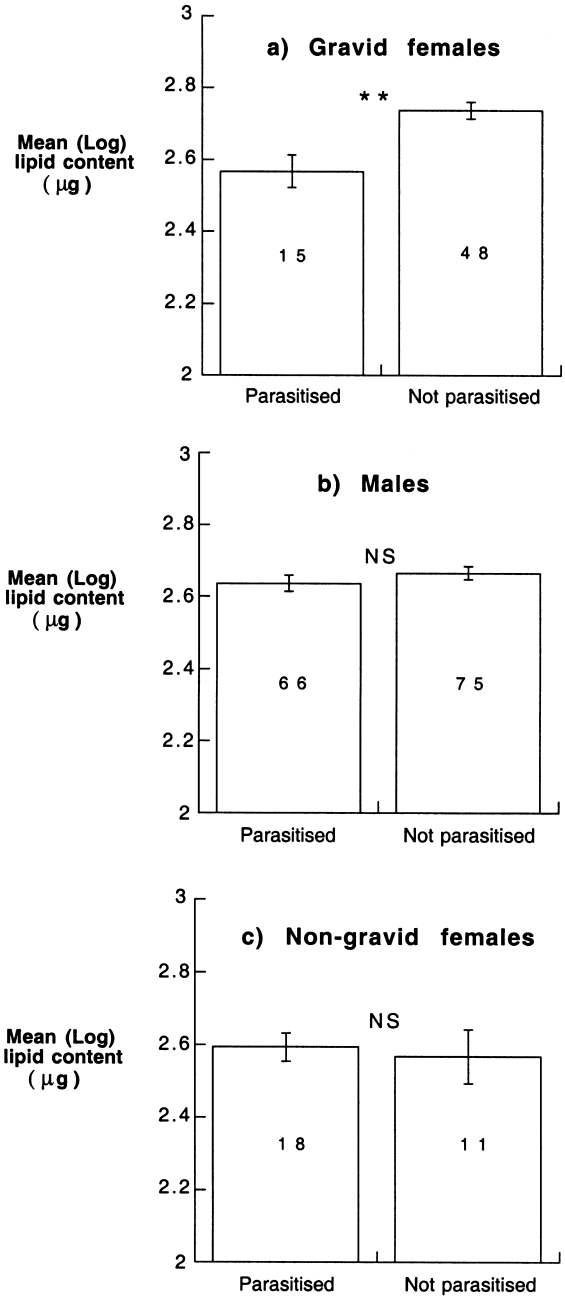

Fig. 1. Difference in the mean ± SE lipid content (log) of individuals infected with *Pomphorhynchus laevis* and individuals not infected with *P. laevis* for: (a), gravid females; (b), males; and (c), non-gravid females. All numbers in the columns refer to sample sizes in each group.

females, and mate-guarding; behaviours for which glycogen is likely to be the main energy source (Sparkes et al., 1996). The different reproductive investments of males and females may explain why the effect of *P. laevis* on host fat reserves was also sex-specific.

Infected gravid females had significantly lower lipid contents than uninfected females, but there was no difference in the lipid contents of non-gravid females and males that were infected with *P. laevis*. The effect of the parasite on gravid females is almost certainly the result of a partial castration of the female by *P. laevis* (Poulton and Thompson, 1987). Females normally accumulate a large store of precursory proteins, carbohydrates, and fats prior to laying a clutch of eggs (Sutcliffe, 1993). One explanation for the sex and female reproductive status differences in *P. laevis* infected host lipid reserves is that males and non-gravid females are not accumulating the large stores of lipid that the parasite can manipulate. In the case of females, this may have been because they were currently in between broods, or alternatively, they may have already begun to enter an autumn 'resting stage' in which ovulation ceases for 2–3 months (see Sutcliffe, 1993). The conflict of interest over energy allocation between host and parasite may be less in males who invest more in growth (Ward, 1988), and possibly also maintenance. This idea is supported by studies that have shown that female *G. pulex* may be more susceptible to stress than males (McCahon and Pascoe, 1988). In this sense, the manipulation of female investment in eggs probably functions as a way of increasing the survival chances of the female, subsequently increasing the probability of the parasite reaching its final host (Baudoin, 1975; Minchella, 1985). A second, but not mutually exclusive, explanation for our results is that the mechanism that the parasite uses to manipulate lipid reserves is specific to female processes, such as vittellogenesis and or egg production. Such sex-specific effects might arise from a differential effect of manipulating endocrine function in male and female hosts (see Thompson and Kavaliers, 1994 for refs).

In contrast to the sex-specific effect of *P. laevis* on stored lipid reserves, we found that all individuals that were parasitised by *P. laevis* had significantly increased glycogen contents, independent of their sex and reproductive status. Why infected gammarids should have increased glycogen reserves is not abundantly clear, although similar results have previously been found in parasitised brine shrimps,

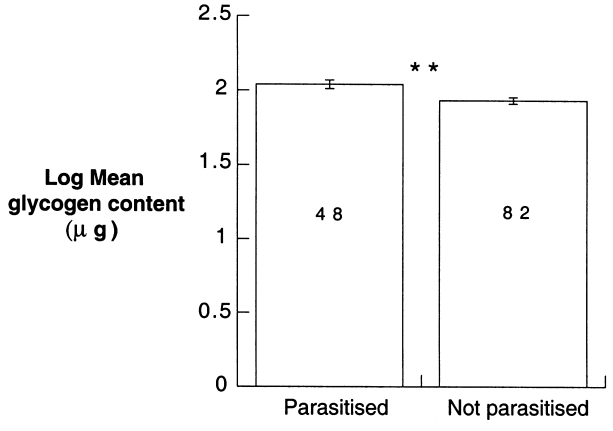

Fig. 2. Differences in the mean ± SE glycogen content (log) of individuals infected with *Pomphorhynchus laevis* and individuals not infected with *P. laevis* for all individuals (see Section 3 for further explanation). All numbers in the columns refer to sample sizes in each group.

Artemia spp. (Amat et al., 1991). The parasite-modified behaviour of gammarids may increase behavioural costs directly, by increasing the activity of the host (Maynard et al., 1998), or indirectly, by increasing the chance of infected hosts ending up in the 'drift' (Kennedy et al., 1978; Brown and Thompson, 1986; McCahon et al., 1991). It may be that the observed increase in glycogen content is an indirect consequence of the increasing energetic demands experienced by infected *G. pulex*. Alternatively, higher glycogen contents in infected *G. pulex* may be the result of an adaptive manipulation of the host's energy reserves that either causes modified behaviour, or alternatively, furnishes the increased energetic demands of parasite-modified behaviour. Previous studies have shown that the infection of *G. pulex* with *P. laevis* results in the mobilisation of carbohydrate sources from storage glands to the haemolymph (Bentley and Hurd, 1996), as well as increases in the haemolymph protein, haemocyanain, which may increase the O_2 carrying capacity of infected gammarids (Bentley and Hurd, 1993, 1995). If parasites are only able to manipulate lipid pathways, increased glycogen reserves in parasitised hosts might have evolved as a counter-adaptation to the parasite. Further work will be necessary to separate whether the changes in glycogen reserves are the result of an adaptive modification of the parasite, an energetic cost of parasite-modified behaviour, or a host counter-adaptation to parasite infection.

Host investment in reproduction is costly, and therefore, detrimental to the well being of the host. For parasites in intermediate hosts, any detriment to the host will also affect the parasite, since it reduces the likelihood of successful transmission to the final host. Thus, it is normally assumed that parasites will be favoured if they can reduce host investment in reproduction (Baudoin, 1975; Minchella, 1985; Amat et al., 1991). The results of this study suggest that the partial castration of females by *P. laevis* is associated with a manipulation of a female's ability to accumulate lipids, and is sex-specific, since there is no comparable change in lipids within infected male *G. pulex*. The result suggests that the different reproductive strategies of males and females may influence the level of conflict over energy allocation between the hosts and the parasite, and raises further questions concerning the generality and utility of parasite-mediated changes in the physiology of hosts of different sexes.

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