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A parasite that increases host lifespan

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Tenebrio molitor is an intermediate host for the rat tapeworm, *Hymenolepis diminuta*. Parasite oncospheres hatch in the beetle midgut and burrow through into the haemocoel, where they rapidly grow and mature into metacestodes. Repair of damage incurred during invasion and the nutritional demands of the parasites are likely to impose costs on the host. Despite these costs, there is an overall very highly significant difference in survival time ($p < 0.001$) between infected and control populations of beetles, with a hazard ratio of 2.35 (control versus infected). Infected females showed a 40% increase in survival time to 50% mortality and males showed a 25% increase in survival time to 50% mortality. This parasite-induced increase in host longevity is discussed in the light of changes in resource allocation that may occur in infected beetles. Previous findings have demonstrated that reproductive success is significantly reduced in infected females. The outcome of changes in the reproductive effort made by male beetles is less clear. We suggest that the optimum trade-off between reproduction and longevity may be altered to favour longer host survivorship, which is likely to enhance parasite transmission.

Keywords: *Tenebrio molitor*; *Hymenolepis diminuta*; host longevity; resource allocation; hazard ratios; parasite-induced fecundity reduction

1. INTRODUCTION

The essence of a parasitic relationship is its exploitative nature; parasites have a negative impact on host fitness. However, in general, the level of virulence will be determined by strategies that maximize parasite transmission (Combes 1997). Thus, a host that is rapidly killed by an infection may not provide the parasite with resources for long enough for it to complete the current phase of its life cycle or to maximize its chances of transmission to the next host.

Exploitation of the costs of host reproduction is a common, and perhaps ideal, strategy for reducing the effect of infection on host survival, whilst still using host resources. By inducing a flow of nutrients from gametes to soma, resources will be diverted away from a non-essential process to support the basic metabolic requirements of the parasite and its host (Obrebski 1975). Thus, host-fecundity reduction may stop the parasite adversely affecting host longevity (Forbes 1993). This concept has been extended to encompass total host reproductive effort (Perrin 1996).

Life-history theory predicts that a trade-off exists between reproduction and survival, such that greater reproductive activity results in a decreased lifespan (Price 1980; Stearns 1989, 1992; Williams 1966). We could thus predict that some parasites manipulate patterns of host-resource allocation to reproduction to such an extent that they extend host lifespan, thereby increasing opportunities for transmission. Few studies have specifically examined host longevity in conjunction with fecundity reduction; however, the host gigantism observed in some trematode–snail associations has been viewed as a strategy that prolongs host life (Minchella 1985), and a bivalve–trematode association has demonstrated that hosts with a longer lifespan contain a higher proportion of parasites located in the gonads, thereby destroying reproductive tissue rather than other essential organs (Taskinen *et al.* 1997). The aim of this study was to test this hypothesis, using a cestode–beetle intermediate-host association as a model.

The rat (dwarf) tapeworm, *Hymenolepis diminuta*, has a two-host life cycle and is transmitted, as an embryonated egg, from the definitive host to one of a range of insects, including the beetle *Tenebrio molitor* (Arai 1980). Metacestodes mature within the haemocoel, where they remain as long as the insect survives (Arai 1980). The parasite's life cycle is completed when the beetle is predated by one of several suitable mammalian hosts, including *Rattus rattus* and *Rattus norvegicus* (Arai 1980; Webster & Macdonald 1995). Opportunities for successful transmission are therefore dictated by, among other things, beetle longevity.

H. diminuta metacestodes have a pronounced effect on the reproductive output of the tenebrionid beetles *Tribolium confusum* and *T. molitor*, which is most marked during the first 2 weeks of infection (Keymer 1980; Hurd & Arme 1986; Maema 1986). Egg production is constrained at two physiological points. In the fat body of *T. molitor* the synthesis of yolk protein is down-regulated by a parasite-derived modulator molecule (Webb & Hurd 1999), and in the ovary yolk uptake is inhibited by a factor that is probably host derived (Major *et al.* 1997). In addition, many of the follicles of infected beetles undergo resorption during their development (Hurd & Arme 1987). This reduction in reproductive effort is likely to result in fewer resources being devoted to egg production. For instance, there is a 75% reduction in the production of the yolk protein precursor, vitellogenin, in the fat bodies of parasitized beetles 24 days after infection (Webb & Hurd 1996).

Male insects devote fewer metabolic resources to producing gametes, thus the potential for resource management in infected males may be less. We have been unable to detect an adverse effect on spermatogenesis (Carver 1997). The size and trehalose activity of the bean-shaped accessory glands and the protein content and trehalose activity of spermatophores actually increase in infected beetles (Carver & Hurd 1998; Carver *et al.*

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1999). However, infected male *T. molitor* are less responsive to female sex pheromones (Hurd & Parry 1991). Worden *et al.* (2000) reported that the reproductive success of infected male *T. molitor* older than 20 days is reduced when they are infected with more than 257 parasites.

The *H. diminuta–T. molitor* association provides us with a model in which the sexes are equal in terms of prevalence and intensity of infection (Hurd & Arme 1987) but in which the effect of the tapeworm on reproductive output differs. We have used this association to demonstrate that infection increases host lifespan, and that the effect is greater in female beetles than in male beetles.

2. MATERIAL AND METHODS

(a) *Parasite and host*

Eggs of *H. diminuta* (ARME strain) were collected from the faeces of male Wistar rats by salt floatation (Hurd & Arme 1984) and stored in tap water at 4 °C for a maximum of 3 weeks. *T. molitor* pupae were collected from a stock colony, sexed and maintained at 26 °C until pupation (Hurd & Arme 1984). Newly emerged beetles were starved for 2 days and then exposed for 24 h to eggs of *H. diminuta*, mixed with apple pulp to make them more palatable. Control beetles were exposed to apple pulp alone. Male beetles were marked with a spot of Liquid Paper correction fluid on the elytra. Previous investigations have shown that such markings do not affect mating potential or longevity (H. Hurd, unpublished data).

(b) *Experimental protocol*

Beetles were removed 24 h after exposure to the tapeworm eggs, and eight populations of 13 male and 13 female beetles were set up in paper-lined Petri dishes. Eight populations of uninfected beetles were set up in the same manner. All populations were fed wheat bran *ad libitum*, supplemented every third day with an apple slice. Beetle deaths were recorded every second day and dead beetles were removed from the population.

On the seventh day after infection, a subset of three male and three female beetles was removed at random from each population, chilled on ice for 30 min then heads removed prior to being dissected to check for infection.

This experiment was repeated at a later date, using a different collection of eggs. The number of populations and their compositions were identical to the first experiment.

(c) *Statistical analysis*

Survival data from the 16 populations in the first experiment were checked for homogeneity using the log-rank test and were found to be homogeneous within the eight control groups ($\chi^2 = 12.85$, $p > 0.05$) but significantly heterogeneous within the eight infected groups ($\chi^2 = 47.51$, $p < 0.001$). As the populations were sampled from a large stock colony, it is not surprising that some heterogeneity was present. This only appeared in the infected group, where it is expected that the response of individual beetles would be variable. As the control groups were homogeneous, it was decided to combine the data for subsequent analyses. In the second experiment, both the control and the infected groups were heterogeneous, although not markedly so ($\chi^2 = 24.9$, $p < 0.01$ and $\chi^2 = 25.37$, $p < 0.001$, respectively), and it was decided to combine the data.

Survival was analysed using the log-rank test on the combined data together with Cox's proportional hazard regression (Christensen 1987; Selvin 1996), with the indicators for control versus infected and sex as the predictors. All the analyses were carried out using the statistical package Stata (Stata Corporation 2000).

3. RESULTS

All presumed-infected beetles dissected 7 days after exposure to infection contained metacestodes. The intensity of infection was not determined exactly, but the parasite burden was usually over 50.

During the first 8 days after infection, when metacestodes were developing, no beetles died. Mortality increased steadily from 12 days after emergence and there was a marked increase in survival of infected female and male beetles compared with the controls (figure 1). There was an overall very highly significant difference ($z = 3.67$, $p < 0.001$) between infected and control groups, with a hazard ratio (HR, a measure of relative risk) of 2.35 (control versus infected). There was also an overall very highly significant difference ($z = 3.30$, $p < 0.001$) between male and female beetles (HR = 2.13 male versus female).

There was a very highly significant increase in the survival of infected female beetles relative to the controls ($\chi^2 = 51.91$, $p < 0.001$; HR = 4.80, $p < 0.001$ (control versus infected)) (figure 1*b*), whereas there was only a significant increase in the survival of infected male beetles relative to the controls ($\chi^2 = 8.28$, $p < 0.01$; HR = 1.47, $p < 0.05$ (control versus infected)) (figure 1*a*). For infected female beetles this represented an increase of 8 days in the median survival time to 50% mortality; for infected male beetles this represented an increase of 4 days.

Within the infected group, there was a very highly significant difference between males and females ($\chi^2 = 48.52$, $p < 0.001$); the females showed the greater longevity, with a greater median survival time to 50% mortality of 8 days. Within the control group, there was no significant difference between males and females ($\chi^2 = 3.61$, $p > 0.05$).

In the second experiment the lifespan was less for all categories of beetle, but very highly significant overall differences between infected and control (HR = 1.44, $p < 0.001$ (control versus infected)) and between male and female (HR = 1.45, $p < 0.001$ (male versus female)) beetles were again found. Again, there was a very highly significant increase in survival in infected females ($\chi^2 = 17.22$, $p < 0.001$; HR = 1.87, $p < 0.001$ (control versus infected)) but there was no increase in infected males ($\chi^2 = 0.93$, $p > 0.05$; HR = 1.13, $p > 0.05$)). However, caution should be used in interpreting the level of significance here as combined data were used despite heterogeneity (see § 2c).

4. DISCUSSION

Overall, infection significantly increased beetle survival. The effect was much more pronounced in female than in male beetles, such that there was a 40% increase in survival to 50% mortality in infected females but only a 25% increase in survival to 50% mortality in infected males.

H. diminuta does not multiply in the intermediate host; thus, the initial period of infection will be the most demanding of host resources. During these 12–15 days the

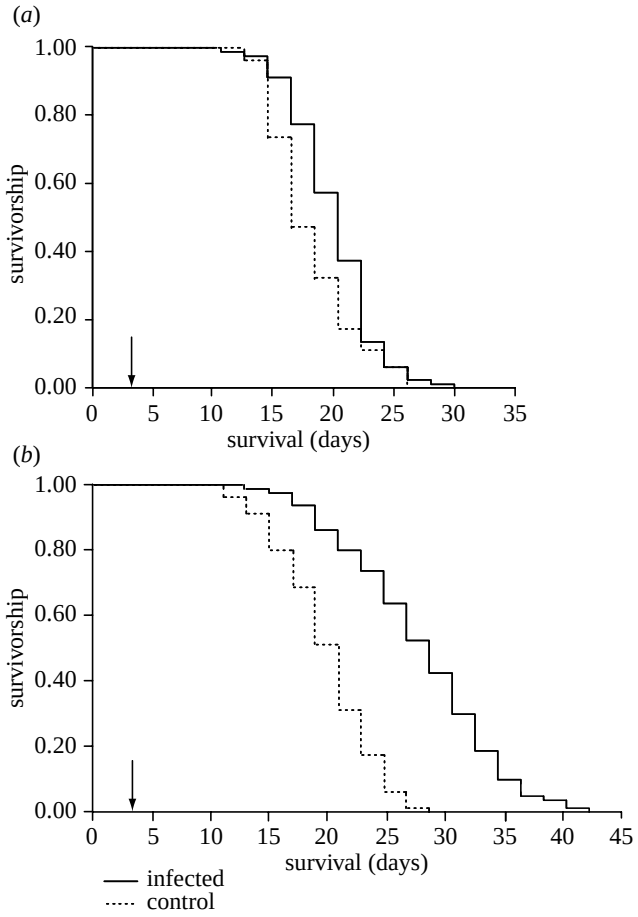

Figure 1. Relationships between cumulative survival probability (survivorship) and post-emergence survival times in infected (solid lines) and uninfected (dotted lines) *Tenebrio molitor*; (*a*) male beetles and (*b*) female beetles. Arrows indicate time of infection with *Hymenolepis diminuta*.

oncosphere grows rapidly and develops into a mature metacestode, while using metabolites circulating in the haemolymph (Arme 1988). At the same time, the host will need to repair damage done to the midgut epithelium by oncospheres burrowing through from the gut (Lethbridge 1971). Our findings suggest that these initial metabolic demands do not adversely affect host survivorship; indeed in female hosts longevity is considerably increased and in male hosts it is marginally so.

Parasite-induced changes in host life-history traits may be the result of adaptive manipulation, the extended phenotype theory or just an accidental by-product of infection (Dawkins 1990). The idea that pathology results in an extension of host lifespan is counter-intuitive, whereas the fitness benefits that might accrue from increased survivorship are suggestive of manipulation, albeit indirectly as a by-product of fecundity reduction (Poulin 1995). But which partner is manipulative, the parasite or the host (Hurd 1998)?

Evolutionary theory predicts that fitness will be maximized at the point of most-beneficial trade-offs between fitness components. Thus, a shift to a reduction in reproductive success per unit time, concomitant with a longer lifespan during which to reproduce, may represent the optimum strategy for infected females. If a female survives a period of stress by reducing reproductive output in some way, she may gain a lifetime advantage (Ohgushi 1996). Data from our physiological studies support the view that the host is able, via a female-derived molecule that inhibits the uptake of yolk into the ovaries, to make this adaptive change in the pattern of resource allocation to reproduction (Major *et al*. 1997). This molecule is produced in response to the early stages of *H. diminuta* metacestodes, and may be responsible for inhibiting the binding of juvenile hormone to receptors on the follicular epithelium (reviewed in Hurd & Webb 1997). The infected female continues to reproduce but at a slower rate; thus, an extended lifespan represents an extension of the reproductive period, and lifetime reproductive fitness may eventually equal that of an uninfected beetle. We have yet to investigate this hypothesis.

There is no difference between the lifespans of uninfected male and female *T. molitor*, but it is likely that male beetles invest fewer metabolic resources in reproduction than females. Does the parasite-induced change in resource partitioning that we see clearly in females also occur in males? Although we are beginning to develop a detailed understanding of the extent of, and the mechanisms underlying, metacestode-induced reduction of egg development (reviewed by Hurd 1998), our understanding of the effect of this parasite on male beetle reproduction is still rudimentary. *T. molitor* males respond to female sex pheromone and perform characteristic courtship behaviour prior to mating (Happ 1970; August 1971). Both male response to the female copulatory release pheromone and the production of sex pheromone by males are reduced by infection (Hurd & Parry 1991). In addition, males take longer to mate and are less successful in competition for females (F. J. Carver, personal communication). Mating occurs at regular intervals during which a spermatophore, composed of secretions from the accessory glands, is transferred to the female (Gadzama & Happ 1974). Interestingly, the bag-shaped accessory glands of *H. diminuta*-infected males grow significantly larger and produce spermatophores that contain more protein that those from non-infected males (Carver & Hurd 1998; Carver *et al*. 1999). Although Worden *et al*. (2000) reported that females that mate with old male beetles produce fewer offspring than those that mate with uninfected males, we have observed the opposite effect of infection in younger males also infected with mature metacestodes (H. Hurd and R. Ardin, unpublished data) and suggest that material passed to the female from infected-male spermatophores enhances egg production. At the present time, the results from studies of reproductive behaviour and physiology do not enable us to predict whether there is a net diversion of resources away from reproduction in infected male beetles. However, the slight but significant increase in infected-male longevity that we observed in the first experiment suggests that this might be so.

We are, of course, unable to assess what effect the parasite might have had on host survival if fecundity reduction had not occurred. Thus, we have no ideal control with which to assess whether this host-mediated response is adaptive. Likewise, it is very difficult to demonstrate a causal relationship between fecundity reduction and increased longevity in this model. Alternative hypotheses are that infected beetles consume more food or that the

parasite causes changes in tissue metabolic levels (Thompson 1983). We have no data with which to evaluate these possibilities, but assessment of the effect of metacestodes on stored and circulating carbohydrates in *T. molitor* leads us to think that this is unlikely (Kearns *et al.* 1994). We therefore believe that the findings presented here indicate that some benefit accrues to the host by curtailing reproductive success at the time of infection, rather than continuing to produce eggs at the same rate as uninfected females.

So far we have considered the host, but what about the parasite? A good measure of parasite fitness in the intermediate host is transmission success (Hurd 1998). *H. diminuta* is trophically transmitted; adult worms develop in the intestines of mammalian hosts that have fed upon infected insects. Successful establishment in the definitive host cannot occur until the metacestode is mature (Arai 1980). Thus, any parasite manipulation that increases the chances of host survival during this time will be advantageous. In addition, the extension of the host's lifespan will provide more opportunities for transmission. Thus, the parasite may gain a fitness benefit from manipulating host resources in favour of longevity rather than fecundity. The initial period of infection coincides with a significant reduction in female host fecundity (Hurd 1993) and a reduction in the reproductive behaviour of males (Hurd & Parry 1991). If decreased reproductive output does result in increased host longevity, we can assume that the parasite actively contributes to its enhanced chance of transmission success. This is because developing metacestodes directly manipulate host reproduction via the production of a molecule that inhibits yolk production in the fat body (Webb & Hurd 1999).

Our findings suggest that, due to their increased longevity when infected, female beetles may provide more chances for transmission of *H. diminuta* than males. A higher incidence and burden of *H. diminuta* infection was detected in female *T. confusum* and *Tribolium castaneum* (Mankau 1977) but no such difference was detected in *Tribolium brevicornis* infections (Mankau *et al.* 1971). We were unable to detect differences in parasite prevalence between the sexes when *T. molitor* acted as host, and male beetles actually had a higher incidence of infection (Hurd & Arme 1987). The explanation may lie in the passive nature of transmission to the beetle, as it is unlikely that any evolutionary pressure could affect the distribution of the parasite between the host sexes, and the intensity and burden of infection are more likely to be affected by host feeding patterns.

It is difficult to predict the contribution, if any, that increased longevity will make to *H. diminuta* transmission in the wild, as additional factors will come into play that have been excluded from our laboratory model. Prime amongst these are the needs to forage for food and to escape from predators. These activities will have energy requirements that may differ between infected and uninfected beetles (Hurd & Fogo 1990). Although beetles harbouring mature infections undergo several behavioural changes, we have not been able to demonstrate that these lead to enhanced predation by the rat definitive host in a semi-natural situation (Webster *et al.* 2000); but, infected beetles may be more vulnerable to predation by other predators and this could be a counter-influence to the increased lifespan that we have recorded in the laboratory.

Despite the fact that this system has been in our laboratory for more than 20 years with no opportunity for natural selection to operate or for the parasite and host to co-evolve, we still observe significant effects of infection that may be adaptive in nature (Hurd & Webb 1997; Hurd 2001). However, other strains of parasite or species of host may not exhibit the same effect of infection (e.g. Robb & Reid 1996).

In conclusion, we suggest that these findings add weight to our argument that, in the *H. diminuta–T. molitor* association, fecundity reduction may be an adaptive strategy, selected as a result of the trade-off that exists between reproduction and longevity (Hurd 1998, 2001). Furthermore, other physiological evidence demonstrates that both host and parasite produce molecules that regulate female egg production, supporting the view that there are evolutionary pressures on both host and parasite to manipulate reproduction.

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