Parasite-induced changes in the behaviour of cestode-infected beetles: adaptation or simple pathology?

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Abstract: Although the cause is often unclear, many parasites alter the behaviour of their intermediate hosts. The larval form of the rat tapeworm, *Hymenolepis diminuta*, has previously been shown to modify the behaviour of its intermediate host, the flour beetle, *Tribolium confusum*, in a manner that may be adaptive to the parasite. To test this explanation we observed host behaviours including activity, concealment, and the response to and production of pheromones. Infected female beetles examined both 4–5 and 11–12 days post infection were slower moving and slower to conceal themselves than uninfected conspecifics; however, they did not differ from uninfected individuals in staying concealed. Infection of *T. confusum* did not affect the production of pheromones by mated and virgin females or the response of females to male pheromones. A second hypothesis for altered behaviours may be that modified behaviours result from pathology. The survivorship of mated infected female beetles was significantly lower than that of infected virgin beetles and uninfected beetles. Thus, both mated status and infection were important factors in survivorship, but only infection had significant effects on the altered behaviours. In this system, therefore, the hypothesis that behavioural changes are due to adaptive manipulation of the host by the parasite is supported.

Résumé : Bien que la cause en soit souvent obscure, plusieurs parasites entraînent des modifications du comportement de leurs hôtes intermédiaires. La forme larvaire du ver solitaire du rat, *Hymenolepis diminuta*, modifie le comportement de son hôte intermédiaire, le Tribolium brun de la farine, *Tribolium confusum*, de manière à pouvoir créer des avantages évolutifs pour le parasite. Pour éprouver cette hypothèse, nous avons observé le comportement des hôtes, activités, camouflage, réactions aux phéromones et production de phéromones. Des femelles infectées du *Tribolium*, examinées 4–5 jours, puis 11–12 jours après l'infection, se déplaçaient moins rapidement et mettaient plus de temps à se cacher que des femelles conspécifiques non infectées; elles ne différaient cependant pas des individus non infectés dans leur capacité de rester camouflées. L'infection n'affectait pas la production de phéromones chez les femelles vierges ou les femelles accouplées, ni la réaction des femelles aux phéromones mâles. Une autre hypothèse a été envisagée pour expliquer les modifications du comportement, celle de la pathologie. La survie des femelles accouplées infectées était significativement plus faible que celle des femelles vierges infectées ou que celle des insectes non infectés. Le statut d'insecte accouplé et l'infection ont donc une influence importante sur la survie, mais seule l'infection affecte significativement le comportement. Dans ce système, l'hypothèse selon laquelle les changements de comportement de l'hôte sont des adaptations entraînées par la présence du parasite est donc confirmée.
[Traduit par la Rédaction]

Introduction

Alteration of behaviours due to parasitic infection is often attributed to parasite adaptation (Bethel and Holmes 1973; Moore 1984; Hurd and Fogo 1991). In a few studies, however, it is proposed that the host benefits from the behavioural changes by preventing parasitism in kin by eliminating an established parasite (Smith Trail 1980; Poulin 1992) or by adopting different reproductive strategies that limit the effect of parasitism (Poulin et al. 1994). A third possibility, often neglected, is that the altered behaviours are adaptive to neither the parasite nor the host but are only a response to the pathological effects of parasites. However, the behaviour of a sick host could also potentially benefit the parasite. This study examines the consequences of parasitism on host behaviour with respect to benefits to the parasite and to pathological effects.

Adaptive modifications of host behaviour by parasites are most likely to occur in parasites with complex life cycles, where parasites require transmission between different hosts to complete their life cycle. A well-studied example of a complex life cycle involves the parasite *Hymenolepis diminuta* (Cestoda: Hymenolepididae), its intermediate host, the flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae), and its definitive host, the rat (*Rattus* spp.). Within this system there is also evidence of modification of intermediate host behaviours upon infection (Yan et al. 1994). The eggs of *H. diminuta* are released with the rat feces and are ingested when the beetle feeds on the infected feces. Within the beetle's hemocoel, the parasite matures in 8 days to form an infective cysticercoid, an immature cestode (Voge and Heyneman 1957). If a rat ingests an infected beetle, the cysticercoid will develop into a mature cestode to complete the life cycle.

Although it is difficult to assess why the altered host

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behaviours occur, several experiments have been used to determine whether the host or parasite benefits. If altered behaviours are adaptive to the parasite, they will isolate the intermediate host from conspecifics to increase the infected individual's susceptibility to predation (Moore and Gotelli 1990). In support of this explanation, several studies, including some on tenebrionid beetles, have demonstrated that infected intermediate hosts tend to be slower moving (Hurd and Fogo 1991) and less concealed (Hurd and Fogo 1991; Moore 1983; Yan et al. 1994) than uninfected conspecifics. Mating behaviours may also be influenced by the parasite, since egg production, mate acquisition, and mating by the host require energy. It would seem beneficial to the parasite to divert this energy from host reproduction to the parasite for its own use in growth (Moore and Gotelli 1990). As expected if the parasite is to benefit from altered reproductive behaviour, Hurd and Fogo (1991) found that *Tenebrio molitor* infected with *H. diminuta* did not respond to pheromone secreted by uninfected individuals.

The parasite-adaptation hypothesis can also be tested by examining the effect of parasite maturity on behavioural alterations, as it would seem maladaptive for the parasite to initiate changes in the intermediate host before the parasite can develop in the definitive host. In *H. diminuta*, only the fully developed cysticercoid can develop into a mature cestode in the definitive host, and Hurd and Fogo (1991) found that only the fully developed cysticercoid affects the behaviour of *T. molitor*. If the parasite is adaptively modifying the host, beetles with immature cysticercoids should behave similarly to uninfected conspecifics.

Behavioural changes that are adaptive to the host are more difficult to predict for hosts such as *T. confusum*, where the consequences for kin are probably negligible and the options for eliminating parasites are unclear. However, we might expect the host to increase its reproductive effort upon infection because of a shortened expected life-span (Poulin et al. 1994), in contrast to the parasite's interests in minimizing reproduction effort, as discussed above.

While many studies have examined behavioural changes in infected intermediate hosts, the contribution of pathological effects has rarely been considered. Several studies have looked at behaviours induced by pathology of the host (Boorstein and Ewald 1987; Hart 1990) but have failed to examine possibilities of parasite adaptation. Furthermore, pathology has not been considered a reasonable explanation for altered behaviours (Hurd and Fogo 1991; Moore 1983; Yan et al. 1994; Poulin 1992), although we can reasonably expect pathology to be a major, or indeed the sole, contributor to the altered behaviours. Pathological effects should be reflected in survivorship of the host, possibly because of energy consumption by the parasite.

However, some pathological effects may also coincide with the parasite's interests if sick individuals are more likely to be preyed upon. In this case, the behavioural changes are functionally a parasite adaptation (see Reeve and Sherman 1993) mediated by the physiological consequences of pathology. Pure pathology can be distinguished if changes in behaviour benefit neither host nor parasite. Alternatively, if changes seem to benefit the parasite without otherwise affecting the host's viability, we can conclude that the behaviours are adaptive to the parasite and not a result of simple pathology.

Here we test several predictions of the parasite-adaptation hypothesis for *T. confusum* infected with *H. diminuta*, and determine whether altered behaviours are also congruent with the pathology hypothesis. As discussed above, the parasite-adaptation hypothesis predicts that infected beetles will be slower and less concealed and will participate less in reproductive behaviour. However, these results should only apply to beetles with mature cysticercoids. Moreover, because the mated status of the host should be irrelevant to the parasite's interests in terms of increased transmission, there should be no effect of mated status on behaviour. The host-adaptation hypothesis makes no predictions about activity or concealment, but proposes that increased investment in reproduction may be adaptive to the host. The pathology hypothesis predicts that any changes in behaviour are matched by changes in survivorship, assuming that pathology is reflected in increased host mortality. In addition, we might expect that mated females, which have the additional energy demands of egg production, would experience greater pathological effects of parasitism.

Methods

Host and parasite

In this study we examined the behaviour and survivorship of female *T. confusum* only. Female *T. confusum* are more susceptible to infection (Mankau 1977), and differences in the behaviour between sexes have not been found in similar species of beetles (Hurd and Fogo 1991; Yan et al. 1994). As well, a mated female will have the greater stress of egg production in addition to parasitism, thus allowing us to distinguish between the parasite-adaptation and pathology hypotheses. All female beetles were housed together until 4 days before infection and at that time half of them were placed with males of the same age. To keep females separate from males, males were distinguished by marking them with paint. Females exposed to males were assumed to be mated.

Tribolium confusum were kept at 27°C in a standard medium of flour and 5% (by mass) brewer's yeast. Beetle pupae were sexed and female and male beetles were housed separately. Beetles of the same age (4–5 weeks) were used for each experiment, as beetles of this age have been found, upon infection, to have the highest intensity and prevalence of *H. diminuta* (Kelly et al. 1967). Eggs were collected from the combined feces of four male Sprague–Dawley rats infected with *H. diminuta*. Only fresh feces (1 h old) were used, to ensure egg viability.

Beetles were starved in paper-lined petri dishes for 1 week prior to infection. To infect beetles, we prepared a concentrated fecal solution from approximately 2.0 g of rat feces and 50 mL of tap water that was centrifuged for 45 s at 2500 rpm. About 0.05 mL of egg–feces solution, with approximately 18 eggs, was placed in a filter paper lined petri dish and three beetles were allowed to feed for 24 h. The number of eggs could not be held constant, as all eggs were not clearly visible. Over the 24-h period the petri dishes were covered and kept moist to maintain the viability of the eggs. Beetles serving as controls were treated in the same manner but were fed feces from uninfected rats. After experiments were completed, beetles exposed to the *H. diminuta* eggs were dissected to determine the intensity of infection (number of parasites); any uninfected beetles in this group were removed from subsequent analysis.

Experiments on beetles were conducted with immature (4–5 days post infection) and mature (11–12 days post infection) parasites. For the purpose of our experiment we denote an infective fully developed cysticercoid as a mature (11–12 days post infection) parasite. Consequently, there were eight possible treatments based on all possible comparisons of infection (infected or uninfected beetles), mated status (virgin or mated beetles), and maturity (imma-

ture or mature parasites). All experiments were performed at 27°C between 10:00 and 16:00.

Activity

An activity chamber (28 × 28 cm) was coated with flour 0.5 cm deep and marked with a 2 × 2 cm grid of nylon thread suspended above the flour. We placed a single beetle in the center of the grid and recorded the number of squares it visited every 15 s for a 10-min period. The time beetles spent motionless and the time they spent on their backs were also recorded; *T. confusum* flip on their backs often, usually from trying to climb walls (T. Robb and M.L. Reid, personal observation). The flour was changed after each beetle was tested to prevent pheromone contamination. Velocity was calculated as the total distance moved per unit time spent moving (total time minus time with no movement and time spent on the back).

Concealment

To test concealment behaviour, we placed a single beetle in a petri dish on top of a piece of filter paper slightly smaller than the dish and recorded the time it took the beetle to go under the paper. Ten minutes after this initial concealment time, we recorded whether the beetle was on top of the filter paper. New filter paper was used for each trial to prevent pheromone contamination.

Response to aggregation pheromone and production of sex pheromone

Female *T. confusum* secrete a sex pheromone attractive to males only, whereas males secrete an aggregation pheromone attractive to both male and female conspecifics (O'Ceallachain and Ryan 1977). To examine the response of females to male aggregation pheromones, pheromone was collected from 10 uninfected males on a piece of filter paper over a 12-h period (Hurd and Fogo 1991). The filter paper was cut in half and placed in a petri dish lined with a half sheet of clean filter paper. Ten infected or uninfected females were immediately placed in the dish, giving them a choice of an untreated or treated side. After 10 min the number of beetles on the untreated side was recorded. To examine the effects of parasitism on the production of female sex pheromones we used the same method to collect pheromones from 10 uninfected or infected female *T. confusum* and the responses of 10 uninfected male beetles after 10 min were recorded.

Survivorship

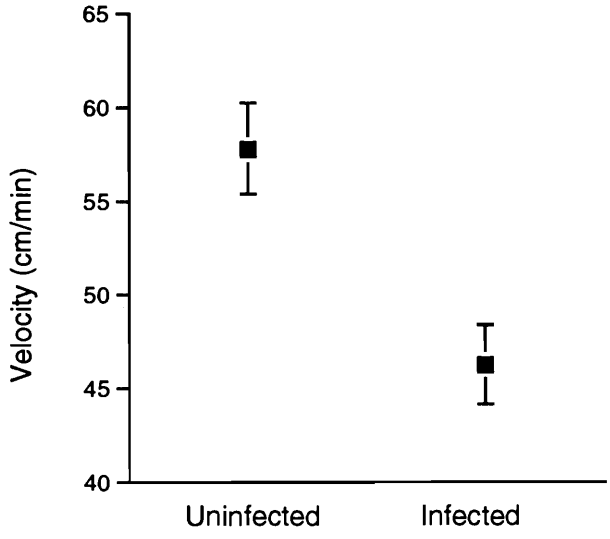
Beetles were randomly placed in three vials of their respective experimental groups: uninfected virgin females, uninfected mated females, infected virgin females, and infected mated females. Each vial contained 25 beetles in 5 g of cellulose, which provided them with a medium comparable to flour while having no nutritional value. Cellulose from each vial was mechanically sifted every day to prevent beetle consumption of eggs. Dead beetles were removed and the body lengths were measured, as body size can influence survivorship (Roff 1992). The sifted cellulose was combined and then divided among the remaining vials. This was repeated daily until no beetles remained alive.

Analyses

Behavioural data (activity, concealment, pheromonal effects) were analyzed using a three-way ANCOVA in which the main effects were infection, mated status, and maturity of the parasite and the covariates were intensity of infection (number of parasites per beetle) and, for pheromone experiments only, prevalence of infection (percentage of beetles infected). We also considered all interactions between effects and any nonsignificant interactions were removed from the analysis. All data were examined for normality and homogeneity of variance, and transformed when required. For the concealment test we also used a goodness-of-fit test for the proportion of beetles on top of the filter paper after 10 min. For each of these experiments we tested whether individuals that were uninfected despite being exposed to parasites differed from a random sample by comparing their behaviours with those of the control beetles by means of *t* tests.

To evaluate the survivorship data the SAS procedure statement LIFETEST was used (SAS Institute Inc. 1989; Fox 1993). This included the main effects of infection and mated status as well as whether exposure to the parasite had an effect on survivorship. A Kruskal–Wallis multiple comparison test was used to determine which survivorship curves differed (Neave and Worthington 1988).

Fig. 1. Mean velocity (±SE) of uninfected (n = 42) and infected (n = 39) *Tribolium confusum*.



Results

Infection

Of the beetles exposed to parasite eggs, approximately two-thirds became infected (activity, 65.52% infected; concealment, 63.87%; survivorship, 66.17%). The number of beetles not infected despite exposure to parasites was not significantly different from that expected from a random process (Poisson: activity, $G = 0.81$, df = 2, $p > 0.05$; concealment, $G = 1.82$, df = 2, $p > 0.05$; survivorship, $G = 0.36$, df = 2, $p > 0.05$). The mean intensities (±SD) for each experiment were as follows: activity, 1.66 ± 0.81 parasites; concealment, 1.57 ± 0.71 parasites; production of pheromones, 1.55 ± 0.71 parasites; response to pheromone, 1.12 ± 0.39 parasites; and survivorship, 1.81 ± 0.39 parasites. Intensities were not significantly different among experiments for activity, concealment, and production of pheromone, but the intensities for survivorship and response to pheromones were significantly different from each other and the rest of the treatments (ANOVA, F = 8.31, df = 4,262, $p \leq 0.0001$; Tukey's test).

Activity

Beetles infected with *H. diminuta* were slower moving than uninfected beetles (Fig. 1). There was no difference in moving velocity between virgin and mated females (Table 1). The intensity and maturity of the parasites interacted to affect the velocity of the beetles (Table 1). At 11–12 days post infection, when the cysticercoids were mature, beetle velocity decreased as intensity of infection increased from 0 to 4 cysticercoids per

Fig. 2. Mean velocity (± SE) of *Tribolium confusum* 4–5 days (○) and 11–12 days (●) post infection. Numbers beside the data points denote sample sizes.

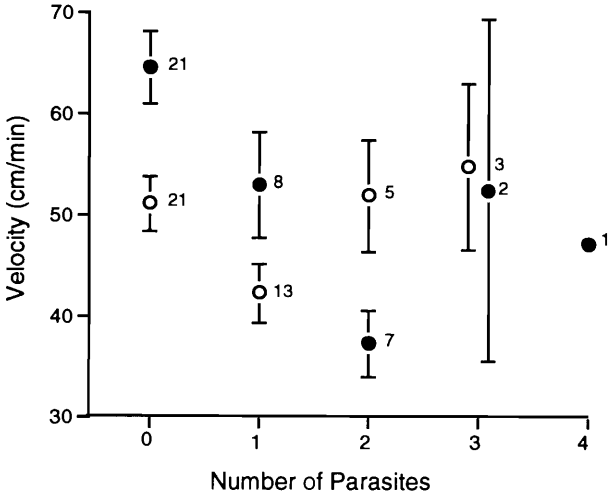


Table 1. Results of a three-way ANOVA on the velocity of *Tribolium confusum* (n = 81).

Source	df	F	p
Infection	1	1.54	0.0105
Mated status	1	6.90	0.2188
Parasite maturity	1	12.39	0.0007
Intensity	1	0.43	0.5129
Intensity × parasite maturity	1	8.57	0.0045

Table 2. Results of a three-way ANOVA on speed of concealment of *Tribolium confusum* (n = 201).

Source	df	F	p
Infection	1	3.94	0.0487
Mated status	1	1.29	0.2580
Parasite maturity	1	0.02	0.8800
Intensity	1	0.96	0.3280

beetle (0 parasites are the control beetles; Fig. 2). However, at 4–5 days post infection, when parasites were immature, there was not a similar effect of intensity on velocity (Fig. 2). The velocity of the individuals exposed to the parasite but not infected was not significantly different from that of the control beetles (t test, t = 0.29, df = 59, p = 0.77).

Concealment

Infected individuals took longer to conceal themselves than their uninfected conspecifics (Fig. 3, Table 2). Neither mated status nor the maturity of the parasite affected the time it took until concealment (Table 2). However, infected and uninfected beetles did not differ significantly in their location 10 min after the initial concealment (G = 0.88, df = 1, p > 0.25) (Fig. 3). The number of parasites did not affect the degree of alteration of behaviours (Table 2). There was no difference in time to concealment between individuals uninfected despite exposure to parasites and uninfected control beetles (t test, t = 0.33, df = 162, p = 0.74).

Fig. 3. Effects of *Hymenolepis diminuta* on the time taken for mated and virgin *Tribolium confusum* to conceal themselves (mean + SE). The numbers above the graph indicate the percentages of beetles on top of the filter paper (unconcealed) 10 min after the initial concealment.

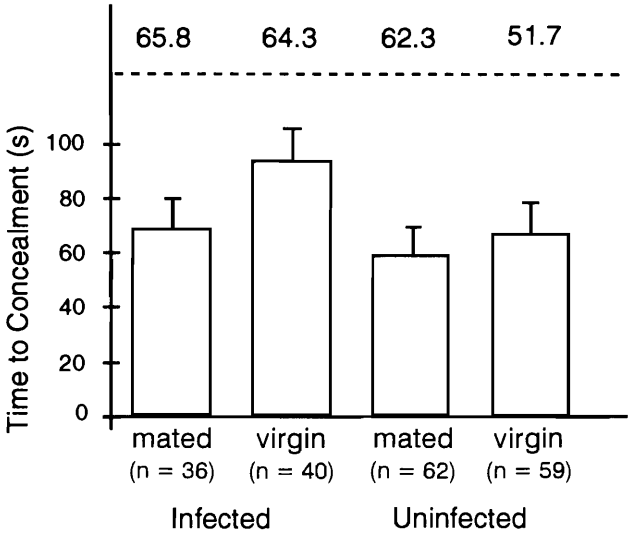


Table 3. Results of a three-way ANOVA on the production of aggregation pheromones and response to sex pheromones in *Tribolium confusum*.

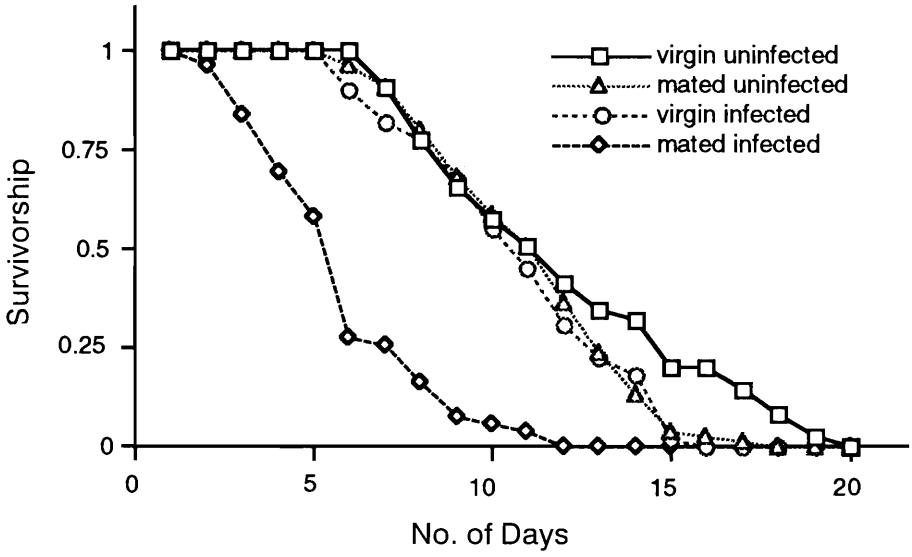
Source	df	F	p
Production (n = 40 groups)			
Infection	1	1.30	0.2622
Mated status	1	1.67	0.2048
Parasite maturity	1	0.67	0.4169
Intensity	1	0.03	0.8607
Prevalence*	1	1.39	0.2471
Response (n = 48 groups)			
Infection	1	1.17	0.2860
Mated status	1	0.11	0.7458
Parasite maturity	1	0.48	0.4909
Intensity	1	1.26	0.2680
Prevalence*	1	0.33	0.5714
Mated status × maturity	1	8.66	0.0053

*Number of infected beetles in the group of 10 tested.

Response to aggregation pheromone and production of sex pheromone

No significant differences were found between infected and uninfected female beetles in their response to the aggregation pheromone secreted by male beetles (Table 3). As well, the prevalence of infection among the responding beetles did not influence the proportion of beetles found on the untreated side (Table 3). However, both mated status and maturity of the beetle interacted to alter the response to aggregation pheromone secreted by male conspecifics. For mated individuals, as the parasite matured (and for uninfected individuals as beetles aged) the response to pheromones increased (from 63% on the treated side at 4–5 days post infection to 80% at 11–12 days post infection). In contrast, for virgin females the opposite occurred (from 77.5% on the treated side at

Fig. 4. Survivorship of virgin and mated female *Tribolium confusum* infected with *Hymenolepis diminuta* and of uninfected controls.



4–5 days post infection to 70% at 11–12 days post infection). Prevalence of infected beetles responding to pheromones was low, as we could not tell whether an individual was infected at the time of the experiment. The mean number of infected beetles ranged from 20% for mated 11–12 days post infection to 35% for mated 4–5 days post infection. Intensity did not alter the degree of behavioural change (Table 3).

Males did not differ in their response to female sex pheromone according to whether pheromones were produced by uninfected or infected females (Table 3). Prevalence of infection among the beetles producing the pheromone was relatively low: 34% for mated immature beetles, 30% for mated mature beetles, 36% for virgin immature beetles, and 36% for virgin mature beetles. Mean intensities did not affect the response of males to the female sex pheromone (Table 3).

Survivorship

There was an overall difference in survivorship of *T. confusum* females according to treatment (Wilcoxon's $\chi^2 = 167.60$, df = 5, $p = 0.0001$; log-rank $\chi^2 = 159.31$, df = 5, $p = 0.0001$). Mated infected individuals differed from both infected and uninfected virgin females and uninfected mated females (Fig. 4). As well, individuals exposed to parasites but not infected did not differ significantly in survivorship from the controls (multiple comparisons test, $p > 0.2$). The average body size of the beetles did not vary among the vials ($F = 2.58$, df = 3,250, $p > 0.1$).

Discussion

Behavioural patterns of the intermediate and definitive hosts in a predator–prey relationship can be used by the parasite to increase its success in transmission (Bethel and Holmes 1973). According to the parasite-adaptation hypothesis, a parasite that does not directly seek out its definitive host might increase the chance that its first host will fall prey to the second, definitive host (Moore 1984). This can be accomplished through behavioural changes of the intermediate host to make it more conspicuous to the predator (definitive host). In this study we found that *T. confusum* infected with *H. diminuta* were slower moving than uninfected conspecifics, and slower to conceal themselves. Similarly, Yan et al. (1994) observed that *T. confusum* infected with *H. diminuta* were more often found on the surface of the flour medium than were uninfected beetles. Slower moving infected beetles and those delaying concealment should be more susceptible to predation, since the normal *T. confusum* escape response is running (T. Robb, personal observation). The more conspicuous behaviours exhibited by infected *T. confusum* are logical indicators of the increase in vulnerability to predation, therefore we can suggest that the behaviours are adaptive to the parasite. Moreover, Moore (1983) concluded from field studies that the alterations in behaviours, similar to those seen in this study but in a different system, made infected individuals more susceptible to predation. Like previous studies, this study suggests that the parasite takes an active role in manipulating the intermediate host for the purposes of its own survival and development by altering activity and concealment behaviours (Dawkins 1982; Moore 1984; Moore and Gotelli 1990).

The parasite-adaptation hypothesis also predicts that alterations of the host's behaviour will only occur when the parasite has matured in the intermediate host. Such an effect was only observed for activity behaviour, in which an increase in intensity of parasitism reduced beetle velocity when parasites were mature (11–12 days) but not when parasites were immature (4–5 days; Fig. 2). This suggests that there is a weak effect of parasite maturity that is only evident when intensity is high. For the remaining behaviours examined (concealment and pheromone production and response), the maturity of the parasite had no effect on the behaviour of the host. Our results are in contrast to those of other studies, where only the mature parasite affected the host behaviour (Hurd and Fogo 1991; Poulin et al. 1992), perhaps because of differences in the intensity of parasitism (cf. Hurd and Fogo 1991).

While activity and concealment behaviours were largely consistent with the parasite-adaptation hypothesis, the mating

behaviour of infected *T. confusum* did not support this hypothesis. From the parasite's perspective we might expect that the energy used by the beetle in mating and egg production would be better used by the parasite for its own development (Moore and Gotelli 1990). However, the response of infected *T. confusum* to aggregation pheromone secreted by male conspecifics did not differ from that of uninfected beetles, and infection of *T. confusum* also did not detectably alter production of sex pheromones in females. Our results contrast with those of Hurd and Fogo (1991), who found that *T. molitor* infected with *H. diminuta* did not respond to pheromones secreted by uninfected individuals. In each of the trials of our experiment, relatively few beetles were infected (the largest percentage being only 35%), and this moderate prevalence may not have been large enough to show an effect. We did observe a small effect of host age on the response to pheromones that differed between virgin and mated females, but this is difficult to explain. This difference in response between beetles 4–5 and 11–12 days post infection is unlikely to be an effect of senescence, as the life-span of *T. confusum* is 1–2 years (Pearl et al. 1941).

The lack of an effect of parasitism on reproductive behaviour is also inconsistent with the host-adaptation hypothesis, according to which parasitized hosts are expected to invest more in reproduction because of reduced future prospects of breeding. Our results may be due to a low prevalence of parasitism as noted above, but Yan et al. (1994) also observed in this system that investment in reproduction (measured by fecundity) did not differ between infected and uninfected beetles.

Altered behaviours that are consistent with the parasite-adaptation hypothesis may result from physiologically damaging (pathological) effects of the parasite, or the pathological and parasite-mediated changes may act independently. For example, damage caused by the larvae of the digenean *Diplostomum spathaceum* during their migration through the tissues of fish increases susceptibility to predation because of behavioural changes, and the alterations vary with the intensity of infection (Brassard et al. 1982). For *T. confusum* infected with *H. diminuta* there is no apparent tissue damage upon infection (Heyneman and Voge 1971) and high intensities do not immediately kill the beetle (H.P. Arai, personal communication; Hurd and Fogo 1991). However, the cysticercoid can be up to one-fourth as long as the beetle, which suggests that parasitism involves a substantial cost to *T. confusum* (Voge and Heyneman 1957).

In our study, pathology of the host was evident in the mated infected female *T. confusum*, which had a significantly lower survivorship than the virgin infected females and uninfected virgin and mated females. It appears that the costs of both parasitism and egg production are needed to have a detectable effect on survivorship. However, in the behaviour tests, mated status did not have a noticeable effect on concealment, activity, or mating. The lack of congruence between changes in survivorship and changes in behaviour suggests that the parasite is manipulating host behaviour independently of pathological effects. This result is significant for studies such as ours in which parasite adaptation is inferred from behavioural changes but enhanced transmission is not directly demonstrated. While parasite adaptation may involve pathological effects as a mechanism, we cannot draw such a conclusion with certainty without showing increased parasite transmission, otherwise the changes may be due solely to pathology. In our study, however, behavioural changes in the host can be attributed to adaptive manipulation by the parasite by unknown mechanisms, while pathological effects that seem to benefit neither the parasite nor the host are evident in the greatly lowered survivorship of mated infected females.

It is notable that we observed effects of parasitism on behaviour and survivorship with intensities of infection that were lower than those in previous studies. While most of the infected beetles in our study had one or two parasites, levels of infection in previous studies have exceeded 200 parasites per host (Hurd and Fogo 1991). This difference can be attributed to the method of infection. We used a feces–egg solution, whereas previous studies have used a pure concentration of eggs (Hurd and Fogo 1991; Yan et al. 1994); the feces–egg solution is likely to be closer to natural conditions. Interestingly, low, subpathological intensities were sufficient to alter activity and concealment behaviours. However, we did observe an effect of intensity on the influence of parasite maturity on host activity (Fig. 2). Thus, further investigation of intensity effects may be a profitable avenue of research into parasite and host adaptations and counteradaptation.

In summary, upon infection by *H. diminuta*, both activity and concealment behaviours of *T. confusum* were altered as predicted by the parasite-adaptation hypothesis. The additional predictions that mating behaviour would be affected by parasitism and that maturity of the parasite would influence behavioural change were not significantly supported. No support was found for the host-adaptation hypothesis. Pathological effects of parasitism were evident in the greatly reduced survivorship of infected mated females, but this was not mirrored in the pattern of behavioural changes. Therefore, we conclude that the changes we observed in the behaviour of parasitized females can be best explained by the parasite-adaptation hypothesis, although their consequences for increased parasite transmission remain to be determined directly.

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