Impairment of the chemical defence of the beetle, *Tenebrio molitor*, by metacestodes (cysticercoids) of the tapeworm, *Hymenolepis diminuta*

C. L. BLANKESPOOR[1]*, P. W. PAPPAS[2] *and* T. EISNER[3]

[1] *Department of Biology, Calvin College, Grand Rapids, MI 49546, USA*
[2] *Department of Zoology, The Ohio State University, Columbus, OH 43210, USA*
[3] *Section of Neurobiology and Behaviour, Mudd Hall, Cornell University, Ithaca, NY 14853, USA*

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SUMMARY

The defensive glands of beetles, *Tenebrio molitor*, infected with metacestodes (cysticercoids) of *Hymenolepis diminuta* are everted less frequently upon stimulation, and contain less toluquinone (methylbenzoquinone) and *m*-cresol, than glands of uninfected controls. These differences, as shown in predation trials with wild rats, increase the likelihood that both cysticercoids and beetles will be ingested by the tapeworm's definitive host. This is the first documented case of a parasite inhibiting the chemical defence of an intermediate host, and one of only a few reports of parasite-induced manipulation of host biology supported by empirical evidence implicating facilitated parasite transmission between host species.

Key words: *Hymenolepis diminuta*, *Tenebrio molitor*, chemical defence, parasite transmission, parasite–host interaction, predation.

INTRODUCTION

Many parasites have complex life-cycles that require ingestion of an infected intermediate host by a suitable definitive host. Confronted with the selective pressures of a host that is 'trying' to avoid being eaten, several of these predation-dependent parasites have evolved ways of altering host biology in a manner that is believed to facilitate transmission to the subsequent host (reviewed by Holmes & Bethel, 1972; Curio, 1988; Hurd, 1990; Moore & Gotelli, 1990; Horton & Moore, 1993). Such strategies include manipulating host physiology (Vinson, 1975; Vinson & Iwantsch, 1980; Edson *et al*. 1981; Thompson, 1983), altering host morphology (Carney, 1969; Mueller, 1974), and impairing general host–predator defence behaviours such as phototaxis, photokinesis, activity patterns, and flight responses (Goodchild & Frankenberg, 1962; Giles, 1983; Moore, 1983*a*, *b*). Instances of a parasite with a predation-dependent life-cycle inhibiting a more species-specific host behaviour–for example, a chemical defence–although predicted (LaMunyon & Eisner, 1990), have not been reported.

Tenebrio molitor, a common intermediate host of the tapeworm, *Hymenolepis diminuta*, possesses such a defence mechanism–a pair of abdominal glands that are everted when the beetle is attacked (Tschinkel, 1975*a*, *b*). These glands secrete a mixture of toluquinone and *m*-cresol (Attygalle *et al*. 1991), compounds that, as constituents of the defensive secretion of other beetles, have been shown to have anti-predator potency (Eisner, 1966; Peschke & Eisner, 1987).

We here report that the metacestode of *H. diminuta* impairs the chemical defence of its beetle intermediate host, *T. molitor*. Moreover, this report provides experimental evidence that such impairment renders *T. molitor* more suspectible to ingestion by rats, thereby facilitating transmission of the parasite to its definitive host.

MATERIALS AND METHODS

Source, maintenance, and infection of experimental animals

Adult *H. diminuta* (OSU strain) were maintained in young (8 to 12-week-old) male Sprague-Dawley rats. Late instar larvae of *T. molitor* were obtained commercially (Rainbow Mealworms Incorporated, Compton, CA, USA) and maintained on rolled oats, Gerber's® dry baby cereal (mixed), and fresh carrots. Pupae were removed daily from the colony, sexed (following the method of Bhattacharya, Ameel & Waldbauer, 1970), and incubated separately by sex. Cages containing the pupae were examined daily, and cohorts of newly emerged beetles were removed. All life-cycle stages were maintained in an environmental room under a long-day photoperiod (LD = 16:8) at 27±3 °C and 70–80% humidity.

Newly emerged *T. molitor* were paired based on mass and sex, caged individually, and starved for

* Corresponding author: Department of Biology, Calvin College, Grand Rapids, MI 49546, USA. Tel: +616 957 6498. Fax: +616 957 6501. E-mail: CBLANKES@CALVIN.EDU.

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48 h. One individual within each pair was randomly assigned as an experimental, and the other beetle served as its control. *H. diminuta* eggs were recovered from faeces of infected rats using a gradient centrifugation technique (Lethbridge, 1971), pipetted on apple slices, and fed to experimental beetles on day 2 and 3 post-emergence. Dissections showed that 99·5 % of the experimental beetles contained mature cysticercoids (Voge & Heyneman, 1957) 12–13 days after infection. Control beetles were given apple slices without eggs. On day 4 post-emergence the apple slices were removed, and the beetles were maintained on a diet of rolled oats and fresh carrots. The beetles were housed separately by sex and by treatment in groups of 10–20 beetles/Petri® dish (80 mm × 30 mm) until just prior to being used in an experiment, at which time 10 infected and 10 uninfected beetles of the same age and sex were mixed.

When possible, all beetles were dissected after each experiment to determine which individuals were infected and to determine individual parasite burdens.

Experiment 1 : Effect of H. diminuta *on gland eversion*

Individual beetles (17–20 days old) were held gently between the thumb and index finger so as not to induce gland eversion, and were subjected to one of two simulated predatory attacks (pinching of a leg with forceps or squeezing of the thorax). A positive response was recorded if gland eversion occurred within 5 sec. The proportions of control and experimental beetles everting their glands were compared using the *G*-test of independence with Williams' correction for continuity applied (Sokal & Rohlf, 1981).

Experiment 2 : Effect of H. diminuta *on production of toluquinone and* m-*cresol*

Glands from beetles immobilized by chilling (−10 °C for 8 min) were dissected intact and placed in individual vials containing a known amount (up to 150 mg) of dichloromethane, plus 2,6-dimethyl-*p*-benzoquinone (28 μg/ml) as an internal standard. The glands were ruptured with a glass rod to extract the secretory components, and the samples were analysed using capillary gas chromatography (Baldwin, Dusenbury & Eisner, 1990).

Data were transformed logarithmically to stabilize variance and a three-way ANOVA (Wilkinson, 1992) was performed to determine the effects of infection, age and sex on the amounts of toluquinone and *m*-cresol produced. Post-hoc statistical analyses were conducted to determine differences in secretory output at specific ages (pairwise comparisons of means) and dose-dependent effects of parasite burden (Pearson correlations).

Experiment 3 : Predation trials

Predation trials were conducted to determine if infected beetles were more vulnerable to predation by rats. To ensure that the rats had no previous experience with *T. molitor*, 10 pups (*Rattus norvegicus*) were raised from litters of 2 wild-caught females. After 8 weeks, the 10 rats were individually trained to feed on live food (*T. molitor* larvae), which were placed in their cages daily; predation trials with adult beetles were begun in week 9. Because of their higher secretory output (Figs 2 and 3), only female beetles were used in these trials.

Twenty-four hours before each trial, individual infected and control beetles (17 days post-emergence) were labelled with small, numbered plastic tags (affixed to the beetle's thorax with Canada balsam) and assigned randomly to 1 of 3 treatment groups: untreated; glands removed; or sham-operated. Beetles in all 3 groups were immobilized by brief refrigeration (−2 °C). The glands of beetles in 1 group were removed by dissection (i.e. the portion of the last abdominal sternite to which the glands are attached was excised); glands in the sham-operated group were left intact, but the penultimate abdominal sternite of each beetle was removed (as evidenced by tests with individuals not used in the trials, such sham-operated beetles readily evert their glands when manually stimulated).

Each trial was conducted during scotophase and consisted of placing 20 beetles (10 infected and 10 uninfected) of 1 treatment group in a cage (20 × 24 × 48 cm) containing a 2·5 cm layer of cracked corn. After 1 h a rat, deprived of food but not of water for the previous 4 h, was introduced into the cage. Interactions between rat and beetles were monitored under a 25 watt red light, and the trial was terminated after a total of 10 different beetles had been attacked. Predation sequence (i.e. which beetles were attacked and in what order) and attack outcome (i.e. beetle killed, killed and eaten, or survived) were recorded. Rats were subjected to a second and third trial (7 and 14 days later, respectively), using beetles in the remaining 2 treatment groups. To control for the effects of previous experiences on the second and third trials, the presentation order of the 3 treatment groups was varied deliberately among the rats.

Results were grouped by treatment and analysed using either the *G*-test of independence with Williams' correction for continuity or the Student's *t*-test (Sokal & Rohlf, 1981).

RESULTS

Experiment 1 : Effect of H. diminuta *on gland eversion*

When subjected to either simulated attack, infected

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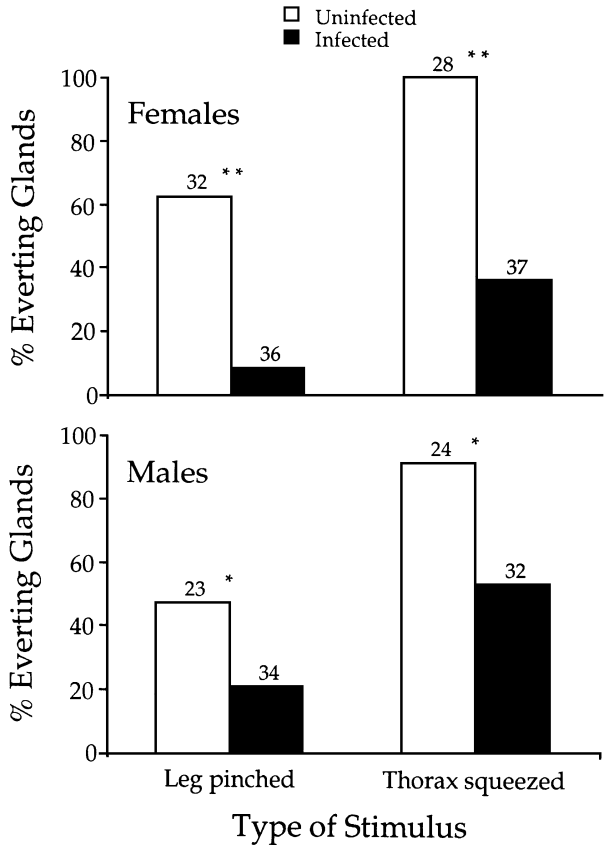

Fig. 1. Percentage of infected (■) and uninfected (□) 17 to 20-day-old *Tenebrio molitor* everting defensive glands after a leg was pinched or the thorax was squeezed. The numbers above columns indicate sample size; symbols indicate level of statistical difference between infected and uninfected beetles (*G*-test with William's continuity correction applied: * $P < 0{\cdot}01$; ** $P < 0{\cdot}001$).

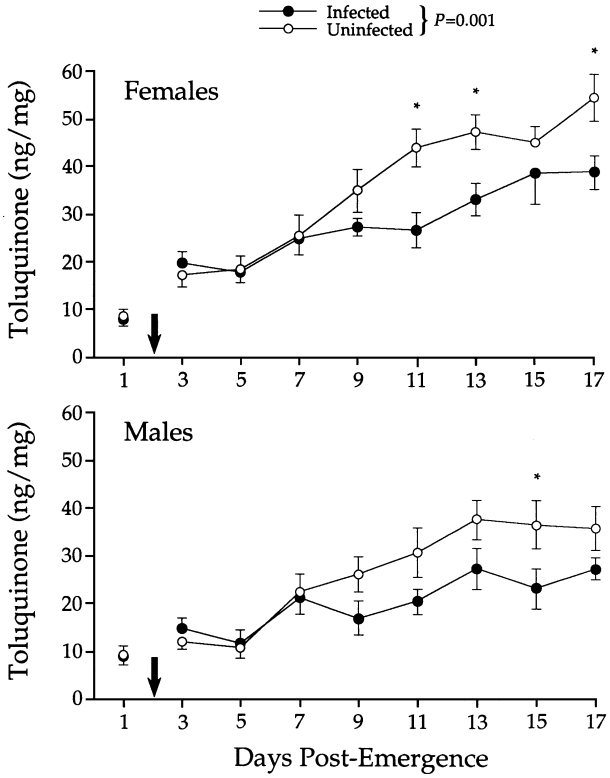

Fig. 2. Glandular toluquinone content (ng/mg body weight) of infected (●) and uninfected (○) beetles at various times after adult emergence (*P*-value calculated using ANOVA). Each data point represents the mean (±S.E.) of 10 beetles. The arrow indicates the date of infection; asterisk indicates statistical differences between infected and uninfected beetles of that particular age (post-hoc pairwise comparison of means, $P < 0{\cdot}01$).

beetles everted their glands significantly less frequently than uninfected controls (Fig. 1). Infected females ($n = 73$) were found to harbour 64 ± 5 (mean ± S.E.) cysticercoids (range = 8–161) and infected males ($n = 66$) had 69 ± 6 (mean ± S.E.) cysticercoids (range = 5–176). This difference was not significant ($P > 0{\cdot}60$).

Experiment 2 : Effect of H. diminuta *on production of secretion components*

As is evident from Fig. 2, the glands of infected beetles contained less toluquinone than those of controls, with differences first becoming significant 9 days post-infection. The amount of toluquinone in the glands of beetles also increased with age ($P < 0{\cdot}0001$) and differed significantly between the sexes, with females outproducing males ($P < 0{\cdot}0001$). This latter observation cannot be attributed to differences in infection levels between males and females since mean numbers of cysticercoids in infected female and male beetles ($n = 80$ for each sex) were 72 ± 7 (S.E.) (range = 8–197) and 75 ± 6 (S.E.) (range = 12–204) respectively, and were not significantly different ($P > 0{\cdot}56$).

Similarly, the glandular content of *m*-cresol was significantly affected by both infection ($P = 0{\cdot}02$) and age ($P < 0{\cdot}0001$) (Fig. 3). However, with this secretion component gender differences were not present ($P > 0{\cdot}99$).

With both compounds, no significant correlation existed between parasite burden and secretory output ($P > 0{\cdot}141$).

Experiment 3 : Predation trials

The numbers of infected and control beetles attacked by rats did not differ significantly, regardless of whether the beetles' defensive glands were intact or had been removed (Table 1). Beetles that were attacked by rats were always killed, but they were not always eaten (Table 2). Untreated, infected beetles, like their sham-operated controls, were ingested more often than untreated, uninfected beetles. In contrast, all uninfected and infected gland-less individuals were eaten.

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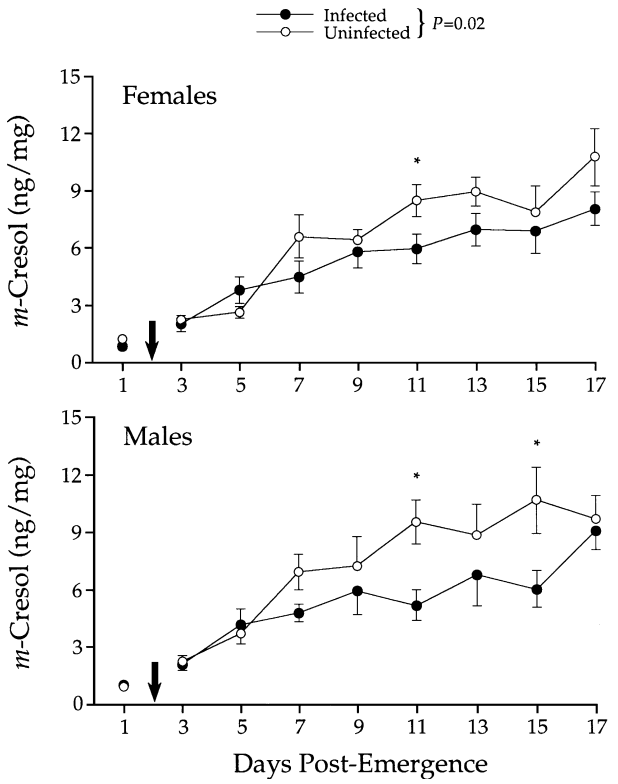

Fig. 3. Glandular *m*-cresol content (ng/mg body weight) of infected (●) and uninfected (○) beetles at various times after adult emergence (*P*-value calculated using ANOVA). Each data point represents the mean (±S.E.) of 10 beetles. The arrow indicates the date of infection; asterisk indicates statistical difference between infected and uninfected beetles of that particular age (post-hoc pairwise comparison of means, $P < 0{\cdot}01$).

Table 1. Effect of *Hymenolepis diminuta* infection on the number of adult *Tenebrio molitor* attacked by rats in predation trials

(Results were grouped by treatment. See text for details.)

	Number of beetles attacked		
Treatment group	Infected	Uninfected	*P**
Untreated	53	47	0·398
Glands removed	51	49	0·778
Sham-operated	53	47	0·398

* *P*-values calculated using the *G*-statistic with William's continuity correction applied.

Table 2. Effect of *Hymenolepis diminuta* infection on the fate of adult *Tenebrio molitor* prey in predation trials with wild rats

(Results were grouped by treatment. See text for details.)

	Fate of beetles attacked		
Treatment group	Infected	Uninfected	*P**
Untreated			0·003
Survived	0	0	
Killed, not eaten	2	11	
Killed, eaten	51	36	
Glands removed			0·999
Survived	0	0	
Killed, not eaten	0	0	
Killed, eaten	51	49	
Sham-operated			0·004
Survived	0	0	
Killed, not eaten	1	9	
Killed, eaten	52	38	

* *P*-values calculated using the *G*-statistic with William's continuity correction applied. Because zero values exist in all three contingency tables, 1 was added to all the observed values before calculating this statistic.

DISCUSSION

There can be little doubt that metacestodes of *H. diminuta* inhibit the chemical defence of *T. molitor*. Compared to uninfected controls, the defensive glands of infected beetles are everted less frequently after a simulated attack, and they contain less toluquinone and *m*-cresol. The tapeworm's effect on gland eversion is more pronounced in female beetles, but the reason for this is not clear. It is interesting to note that the effect of *H. diminuta* metacestodes on the amino acid and protein concentrations in the beetle's haemolymph are also more pronounced in females (Hurd & Arme, 1984), but additional experiments are needed to determine whether these gender-biased effects are related.

The underlying cause for the reduction of toluquinone and *m*-cresol in the glands of infected beetles also remains unclear. It is easy to envision, however, how the parasite might compete with the host for utilization of compounds (for instance, methylsalicylic acid (Athula Attygalle, personal communication), acetate, and tyrosine (Meinwald, Koch & Eisner, 1966)) that potentially serve as precursors for biosynthesis of the beetle's secretory components. Indeed, *in vitro* studies have shown that *H. diminuta* cysticercoids actively absorb low molecular weight compounds such as sodium acetate (Arme, Middleton & Scott, 1973) and various amino acids (Arme & Coates, 1971, 1973). An alternative explanation is that the parasite inhibits the beetle's capacity to produce secretion. Whatever the mechanism, it is interesting to note that the inhibitory effect on secretory production becomes statistically significant 9 days after infection (11 days post-emergence) when the majority of metacestodes first become mature, stage 5 cysticercoids (Voge & Heyneman, 1957). An earlier onset of inhibition would be maladaptive for the parasite, since the metacestodes would not as yet be infective to rats.

The adaptive significance of this parasite-induced impairment appears clear. Results of the predation trials show that *T. molitor* is ordinarily rendered distasteful by its glands. When the effectiveness of

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these glands is impaired, as by cysticercoid infection, the susceptibility of the beetle to ingestion by rats is increased. So too is the likelihood of parasite transmission to the definitive host. This adaptive benefit could, however, be offset by a cost. Infection by *H. diminuta* could also render *T. molitor* increasingly vulnerable to ingestion by predators (e.g. spiders, ants, centipedes) which are not suitable definitive hosts for the parasite.

Countless arthropods have chemical defences, and this example of a parasite inhibiting an insect's chemical defence may not be unique. On the other hand, arthropods are commonly protected by defences more robust than those of *T. molitor*, in that their glands are both larger and secretorily more productive (Eisner, 1970; Bettini, 1978; Blum, 1981), and parasites may be unable to impair such defences to the point of increasing their hosts' vulnerability to predation. There may, therefore, be good reason why so few chemically protected arthropods serve as intermediate hosts to predation-dependent parasites. Thus, additional host–parasite systems need to be studied to determine the uniqueness of this phenomenon and its significance in the evolution of parasites with predation-dependent life-cycles.

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