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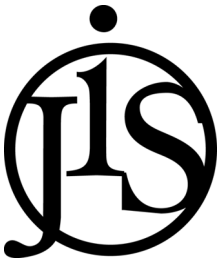

Sex differences in frass production and weight change in *Tenebrio molitor* (Coleoptera) infected with cysticercoids of the tapeworm *Hymenolepis diminuta* (Cestoda)

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Abstract

In their intermediate host, parasites alter aspects of host physiology including waste production and body weight. Further, this alteration may differ between female and male hosts. To study this, a beetle (*Tenebrio molitor*)-tapeworm (*Hymenolepis diminuta*) system was used. Infected and uninfected male and female beetles were individually housed in vials without food. Each beetle's weight change and frass production were measured over 24 h periods at 3, 7, 12 and 16 days post-infection. Treatment (infection) had no effect on weight change, but males lost more weight than females. Further, infected females produced more frass than control females. Males on the day of infection had a higher food intake than females. These results suggest that males will be more exposed to infection than females and could explain why males had a higher median cysticercoid infection level.

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Introduction

Parasitism may result in physiological changes in infected intermediate hosts (Ormerod, 1967; Bentley and Hurd, 1993) the extent of which may differ by sex. For example, the well-studied tapeworm, *Hymenolepis diminuta*, has a grain beetle, *Tenebrio molitor*, as its intermediate host which facilitates transmission to the rat definitive host. Changes in amino acid concentrations of female *T. molitor* grain beetles infected with the tapeworm, *H. diminuta* differ from the changes observed in males (Hurd and Arme, 1984). In females, these changes include increased concentrations of isoleucine, leucine, arginine, serine and threonine and decreased concentrations of tyrosine, phenylalanine, proline and alanine/citrulline in the hemolymph. In males, concentrations of threonine and glycine increase while concentrations of histidine and arginine are lowered. Kearns et al. (1994) found that after infestation of *T. molitor* by cysticercoids of *H. diminuta,* fat body reserves were depleted by three days in males, but by five days in females.

Changes in body weight and waste production, as well as fecundity, basal oxygen consumption and food intake can indicate changes in an organism's metabolism (Downer, 1981; Eckert et al., 1988). For example, under stressful conditions such as starvation, organisms cope by either storing more energy or by lowering their metabolism (Marron et al., 2003). Thus, stress due to parasitism is expected to result in changes in body weight and waste production and could indicate metabolic change. Such stress contributes to the selective pressure the parasite exerts on its host, the extant of which may differ by the sex of the host. Some of these selective pressures include lowered male response to pheromones produced by uninfected females (Hurd and Parry, 1991), and fewer defense compounds found in the defensive glands of infected beetles (Blankespoor et al., 1997). It also includes more direct fitness costs such as reduced host fecundity (Hurd and Arme, 1986), infected males being less attractive to females, and females mated with infected males producing fewer offspring than females mated with uninfected males (Worden et al., 2000). Changes in body weight and waste production may be one additional selective pressure the parasite exerts on the host and could help explain other pressures. Reduced body weight, for example, could explain reduced fecundity. If selective pressure differs between male and female hosts, then the response of the host to parasites could also differ. The aim of this study is to determine the effect of *H. diminuta* cysticercoid infection on frass production and weight change of female and male *T. molitor*.

Materials and Methods

The "OSU Strain" (Pappas and Leiby, 1986) of *H. diminuta* was maintained in the grain beetle *T. molitor*, and male Sprague-Dawley rats. Beetles were maintained on wheat bran to which small pieces of potato were added to the cultures on a regular basis. Pupae were removed from the cultures, and male and female pupae (Bhattacharya et al., 1970) were placed in separate dishes containing wheat bran. Male and female beetles that emerged during a 24 h period were collected (13 beetles of each sex per day), maintained at 26°C until they were 9 days old, by which time they had attained reproductive maturity, and randomly assigned in either control or experimental groups. All beetles were marked individually with latex based paint, starved for 24 hours and then weighed. Unisex groups of 13 experimental beetles were allowed to feed for 24 hours (= day 1) on 1.5 g of air dried apple scrapings mixed with a 0.05 ml solution of water and tapeworm eggs. Unisex groups of 13 control beetles were allowed to feed for 24 hours on 1.5 g of air-dried apple scrapings mixed with a 0.05 ml solution of distilled water.

Each beetle was weighed after feeding on day 1 and placed in a separate glass vial, without food or water (no attempt was made to prevent coprophagy), and maintained at 26°C and constant humidity (90% RH). Three days after infection (= day 3), each beetle was weighed, and the frass present in the vial was removed and counted in a manner that accounted for frass varying in size. A plot of frass weight versus frass number verified this method. On day 4, each beetle was weighed again, and the frass present in the vial was removed and counted. This process was repeated on days 7, 12, and 16. Cysticercoids require 12-16 days to become fully infective, so host changes should be most noticeable during this time (Hurd and Arme, 1987; Voge and Heyneman, 1957). All infected beetles were dissected, and the numbers of cysticercoids were recorded.

Statistical Analysis

Differences in weight and frass count for every beetle after each 24 hour period were calculated

Table 1. Average beetle weights (mg), average total proportional weight change (Day 17 - Day 1 / Day 1), and average frass count for female and male beetles. Standard errors are in parentheses. Six beetles were dropped from the analysis, one beetle exposed to tapeworm eggs was uninfected, and a total of 131 beetles survived until Day 17.

	Female Control	Female Infected	Male Control	Male Infected
N	36	32	32	31
Day 0	124.4 (2.71)	111.4 (3.44)	111.7 (3.9)	119.4 (4.07)
Day 1	132.2 (2.8)	118.6 (3.34)	125.8 (4.08)	131.7 (3.96)
Day 17	107.6 (2.22)	97.1 (3.05)	98.3 (2.94)	103.8 (2.94)
Proportional weight change	-0.18 (0.007)	-0.18 (0.008)	-0.21 (0.007)	-0.21 (0.005)
Frass count	20.8 (1.05)	27.7 (1.66)	25.4 (1.92)	26.1 (1.64)

and analyzed for statistical differences between control and infected beetles. Data for proportional total weight change were transformed with the arcsine square root. The general linear model (GLM) function in Minitab 13.1 (Minitab Inc., PA, USA) measured effects of treatment, sex, and the interaction between sex and treatment. If the treatment effect was significant, the repeated measures analysis program of SAS v. 8 (SAS/STAT®, 2001) was used to determine the source of the difference with the additional factor of time (days). An ante-dependence co-variance model (which allows for unequal variances, correlations and covariances such that correlations decrease through time) was fitted to the data to determine significant differences in the least squares means of the 16 relevant combinations of sex, infection status and day effects, and significance was re-adjusted to 0.003 according to the *post hoc* Bonferroni adjustment. The coefficient of linear correlation (*r*) was used to determine the correlations of weight change and frass count with the number of cysticercoids. The Fligner-Policello procedure (Hollander and Wolfe, 1999) tested for differences in the medians between male and female cysticercoid loads without assuming equal variances.

Results

Out of 208 beetles, 27 control beetles and 43 infected beetles died before the end of the experiment. However, there was no treatment effect on mortality (2-tailed Fisher's exact test, $p > 0.05$) so only beetles that survived the entire experiment were included in the analysis. Due to experimental error, six beetles were not included in the analysis, and one beetle exposed to tapeworm eggs was not infected and so was also not included in the analysis.

Despite the random selection of beetles, control females (124.4 mg ± 2.8, n = 36) weighed more than infected females (111.4 mg ± 3.4, n = 32) and control males (111.7 mg ± 3.9, n = 32) at the start of the experiment (Table 1; GLM, $p < 0.001$ with Bonferroni *post hoc* comparison), but did not differ from infected males (119.4 mg ± 4.1, n = 31). Males gained significantly more weight than females during the day 1 feeding period independent of infection status (Table 2). While there was no effect of infection on total proportional weight loss from day 1 to day 17, males lost significantly more weight than females during the course of the experiment (Table 3).

Table 2. Results of a general linear model of treatment on weight gain (mg) on day one in beetles for the two sexes (data from Table 1).

Source	d.f.	Mean Square	F	p
Sex	1	1067.99	32.4	<0.001*
Treatment	1	51.17	1.55	0.215
Sex x Treatment	1	9.31	0.28	0.596
Error	127	32.97		

*males gained significantly more weight on the day of infection

Table 3. Results of a general linear model of treatment on proportional total weight loss (after an arcsine square root transformation) in beetles for the two sexes (data from Table 1).

Source	d.f.	Mean Square	F	p
Sex	1	0.049462	19.29	<0.001*
Treatment	1	0.000751	0.29	0.589
Sex x Treatment	1	0.000003	0	0.975
Error	127	0.002564		

* males lost significantly more weight than females

Infected females produced significantly more total frass (days 1-17) than control females (Table 4), and infected females passed significantly more frass on days 13-16 than uninfected controls (Fig. 1). Frass production was unrelated to the number of cysticercoids recovered from infected males ($r = 0.219$, $p = 0.237$) and females ($r = -0.137$, $p = 0.456$; data not shown).

Figure 1. Average frass count of females with standard errors and sample sizes for control (empty bars) and infected (shaded bars) beetles for each indicated day. Asterisks indicate a significant difference between control and treatment (repeated measures ANOVA testing the effects of sex, infection status, day and their interactions on frass production). The overall effects of sex, infection status and day were significant at $p < 0.001$. On days 13-16, infected females produced more frass than control females (d.f. = 141, t-value = -3.29, adjusted $p < 0.003$ for Bonferroni *post hoc* comparisons).

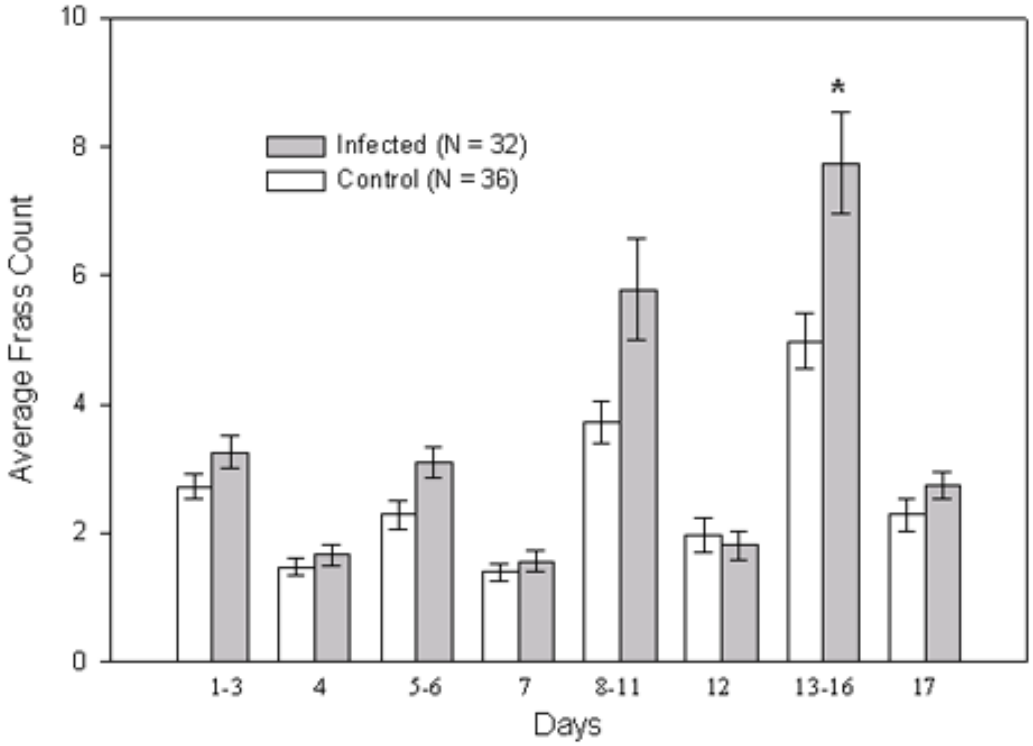

Table 4. Results of a General Linear model of treatment on frass production in beetles for the two sexes (data from Table 1).

Source	d.f.	Mean Square	*F*	*p*
Sex	1	77.09	0.95	0.33
Treatment	1	468.09	5.8	0.017*
Sex x Treatment	1	306.45	3.8	0.054
Error	127	80.72		

* Infected females produced more frass than control females (Tukey multiple comparison, $p = 0.0112$)

All beetles exposed to tapeworm eggs bore a mean of 57 cysticercoids (range 0-223, median = 42.5, IQR = 67.5 n = 64). Infected male beetles had significantly more cysticercoids (median = 61, IQR = 83) than females (= 37.5, IQR = 47; p = 0.0085). Further, weight gain after day one was independent of the number of cysticercoids in both males (r = 0.169, p = 0.363, n = 31) and females (r = 0.239, p = 0.189, n = 32; data not shown).

Discussion

Infected females did not produce more frass than controls until days 13-16 indicating that the effect of infection developed over time. If assimilation efficiencies were equal then this suggests that infection increased the female host's metabolic rate. Given that control females had higher initial weights, one might assume that they would produce more frass, but they did not. The higher frass production on days 13-16 coincided with the time of cysticercoid maturation (Hurd and Arme, 1987; Voge and Heyneman, 1957). It also coincided with behavioral changes observed by Hurd and Fogo (1991), and with the observed reduction in vitellogenin proteins in the ovaries of infected females and the corresponding increase of these proteins in the hemolymph at 12 days post-infection (Hurd and Arme, 1986; Webb and Hurd, 1996; Hurd and Webb, 1997; Hurd, 1998). Virgin female beetles may be able to divert reproductive resources in response to parasitism because they have a store of egg nutrients not

available to males. This may explain why infected and control males did not differ in frass production.

If high metabolism is associated with biochemical constraints that make it difficult for males to respond physiologically to parasitism then it may also explain why infected males, unlike females, did not show changes in frass production. Two observations suggest that male beetles have a higher metabolic rate than females. First, the larger weight gain in males on the day of infection suggests that males had a higher feeding rate. Second, males lost more weight during the course of the experiment. This suggests that males have a higher metabolic rate, store fewer energetic resources than females or both. A higher metabolic rate in male *T. molitor* is consistent with literature indicating that other male invertebrates, such as *Daphnia* (MacArthur and Baillie, 1926), houseflies (Edwards, 1946) and eucalyptus-boring beetles (Rogowitz and Chappel, 2000) also have higher metabolic rates.

The higher feeding activity in males predicts a greater exposure to infection in males: the more males feed, the greater are their odds of ingesting infective tapeworm eggs. In addition, the statistically significant higher intensity of cysticercoids in males could either indicate that males are more susceptible to, or more exposed to, infection than females. Males that eat more apple scrapings with tapeworm eggs increase their exposure to and probability of acquiring more cysticercoids. But this is not supported by the data since no correlation exists between weight gain on the day of infection and number of cysticercoids. Thus, these experimentally infected males may simply be more susceptible to infection than females, which is consistent with other reports of male infection bias (Pappas et al., 1995; Gray, 1998; Wedekind and Jakobsen, 1998). Future studies need to tease out the differences between susceptibility to and exposure to infection.

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