Tapeworm (*Hymenolepis diminuta*) infection in flour beetles (*Tribolium confusum*): does it cause a trade-off between host fecundity and egg size?

Allen W. Shostak

Department of Biological Sciences, University of Alberta

Edmonton, Alberta, Canada T6G 2E9

al.shostak@ualberta.ca

Corresponding author:

Allen W. Shostak

Department of Biological Sciences, University of Alberta

Edmonton, Alberta, Canada T6G 2E9

Phone: 780-492-1293

Fax: 780-492-9234

al.shostak@ualberta.ca

Author: Allen W. Shostak

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Abstract: Inter- and intra-specific comparisons commonly reveal an inverse relationship between fecundity and offspring size. Many animals also vary egg size in response to environmental conditions. Infection of flour beetles *Tribolium confusum* Jaquelin Du Val, 1868 with the rat tapeworm *Hymenolepis diminuta* (Rudolphi, 1819) Weinland, 1858 causes a major reduction in host fecundity. This study tested if this fecundity reduction was associated with changes in host egg size. Age-matched beetles were either fasted and then exposed to parasites, fasted only, or neither, and egg production and egg length determined for 5 weeks post-exposure. Control beetles that were neither fasted nor exposed to parasites had steady egg production, but produced smaller eggs as they aged. Beetles that were fasted only, produced fewer but larger eggs for 1-2 weeks after the fast ended. Then, fecundity and egg size returned to control levels. Infected beetles also produced fewer, larger eggs for 1-2 weeks, but at levels indistinguishable from beetles that had been fasted only. After 2 weeks, while fecundity of infected beetles remained low, egg size became similar to non-infected hosts. Beetles appeared to trade-off fecundity and egg size in response to reduced feeding, but not to the presumed nutritional stress of parasitic infection.

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Introduction

Offspring size is a fundamental trait of organisms and reflects selection pressures on the species and environmental influences on the parent. The most common approach to studying offspring size has used inter-specific comparisons, but high levels of variation in offspring size within species also occur. Variation among populations is associated with habitat quality or latitudinal clines, and variation within populations results from body size, stressors or age of parent (Marshall and Keough 2008). An early model (Smith and Fretwell 1974) proposed that limited resources may necessitate a trade-off between the size and number of offspring, as long as offspring size does not decrease beyond the minimum size required for viability. Many studies have since documented that offspring size–fecundity relationships exist for diverse groups of animals at many scales of observation, but also that the relationship is not universal and that factors other than resources limitations may be involved (Bernardo 1996; Fox and Czesak 2000; Marshall and Keough 2008).

Parasitism is a source of stress that reduces fecundity of hosts as varied as molluscs (Kube et al. 2006), crustaceans (Decaestecker et al. 2005), insects (Guinnee and Moore 2004), fish (Heins and Baker 2003), and mammals (Newey and Thirgood 2004). With fecundity–egg size relationships common in other animals, and with parasitism a common cause of reduced fecundity, it is surprising that few studies have addressed the effects of parasitism itself on host egg size. Stickleback *Gasterosteus aculeatus* L., 1758 infected with the tapeworm *Schistocephalus solidus* (Mueller, 1776) Creplin, 1829 are often castrated, but if not they produce smaller eggs when parasite mass is large relative to host mass (Heins and Baker 2003). Some populations of marine snails with a high prevalence of trematode infection produce larger

eggs than less commonly infected populations (Fredensborg and Poulin 2006). Infection by the rat tapeworm *Hymenolepis diminuta* (Rudolphi, 1819) Weinland, 1858 reduces fecundity of the tenebrionid beetle *Tenebrio molitor* L., 1758 in conditions of extreme host crowding, and also the volume of retained eggs in virgin females, although not the volume of eggs released by mated females (Hurd and Arme 1986a). These limited observations would suggest that infected hosts do not compensate for reduced fecundity by investing more resources in the eggs that they are able to produce. Rather, the host may produce not only fewer, but also smaller, eggs.

Tribolium confusum Jaquelin Du Val, 1868, although only about 2% the body size of *T. molitor* (Holloway et al. 1987), has also been a common model system to study effects of infection with *H. diminuta* (Shostak et al. 2008). Infection causes a rapid, persistent and intensity-dependent reduction in fecundity (Keymer 1980; Maema 1986). Infection likely results in nutritional stress on the host, mimicking the effects of host starvation (Shostak et al. 2008). At 28 ºC the parasite grows exponentially and reaches its maximum growth rate 7 days post-exposure (PE), then grows at a slower rate for the next 7 days while it completes differentiation; after 14 days PE parasite growth ceases (Shostak et al. 2008). The effect of infection on egg size that was noted for *T. molitor* (Hurd and Arme 1986a) has not been examined for *T. confusum*. However, egg size varies among individuals, strains and species of *Tribolium* with coefficients of variation in lengths or mass reported to be 3-16% (Arnaud et al. 2005; Holloway et al. 1987; Sokoloff 1972), providing background variation on which parasitic infection might act. An egg size–fecundity relationship is strong among iteroparous species of insects such as *T. confusum* (Holloway et al. 1987) and egg size is known to be plastic in other species of beetles (Fox and Czesak 2000; Fox et al. 1997). *Tribolium confusum* might reveal more detailed and consistent effects of parasitism on host fecundity and egg size than would *T. molitor*, assuming that the

same parasite growing in a much smaller host might be expected to have exaggerated effects on the host.

The present study examined whether the previously-documented reduction in fecundity of *T. confusum* following infection by *H. diminuta* is also accompanied by alteration of host egg size. Three alternative hypotheses were considered: (i) fecundity reduction following infection is not associated with changes in egg size; (ii) fecundity reduction is also accompanied by a decrease in egg size, or (iii) fecundity reduction is accompanied by an increase in egg size.

Materials and methods

Beetles

A colony of *T. confusum* of unknown strain originally purchased from a biological supply company has been maintained since the mid 1960s on a medium comprising unbleached flour supplemented with 5% brewer's yeast. Beetles were stored under unmonitored ambient conditions (~20–22 ºC) until 1999, and thereafter in an incubator in the dark at constant 28 ºC but at uncontrolled ambient humidity that varies seasonally within the incubator (10–40%). The source colony was maintained in 2–4 dishes containing 500 g medium, and the medium was replaced 2–3 times per year and all live beetles recovered from the old medium are mixed and redistributed among dishes of fresh medium. No attempt was made to control beetle density.

Parasites

Hymenolepis diminuta was maintained in male Sprague-Dawley rats, infected at 200–250 g with 10 cysticercoids each. Eggs were collected by macerating fresh rat feces in tap water, passed through a series of sieves to retain the 45–80 μm fraction, cleaned by centrifugation (10 min at 1000 rpm) over 1 M sucrose, and washed 2× in distilled water. Eggs for control infections were treated similarly but were heat killed (60 ºC for 1 hour) before use. Procedures

involving rats were done in accordance with current guidelines of the Canadian Council on Animal Care, and the protocol was approved by the Animal Care and Use Committee for Biosciences for the University of Alberta.

Experimental design

Except for handling, parasite exposure and census, which were done under ambient conditions, beetles were stored in the incubator. Beetle pupae were collected and sexed, and sexes stored separately. Adults emerging over a 4-day span were randomly paired (1 male and 1 female per vial containing 2 ml fresh medium). At week -4 of the experiment (4 weeks prior to exposure to parasites), when the adults were 9–12 days old and all pairs were producing eggs, a census of eggs in each vial was conducted once each week until week 5 (5 weeks post exposure to parasites). Medium was passed through a 250 μm sieve to determine adult survival and egg production, and the adults were placed on 2 ml fresh medium. Vials containing any dead adults were removed from the experiment. Commencing week -1, eggs recovered from each beetle pair at each weekly census were photographed digitally (1200 × 1600 pixels) over a background grid for later measurement.

At week -1 vials were randomly allocated to one of three groups. A treatment group ("T") was fasted in empty vials for 6 days at 28 ºC (a period of fasting promotes infection (Dunkley and Mettrick 1971)) and then, 1 day prior to week 0, were exposed to live parasite eggs. Each pair of beetles was placed in an exposure arena (Shostak et al. 2008) containing 1 oatmeal flake on which had been placed 20 μl distilled water containing a total of 2000 freshly collected parasite eggs. After 24 hours the beetles were returned to vials containing 2 ml medium. A fasting control ("C2") was fasted for 6 days, but then exposed only to heat-killed parasite eggs. Group C2 beetles were thus maintained on a similar feeding regime to Group T

except for the presence of an infection. Since, for logistical reasons, the infection procedure was carried out on the bench top under ambient conditions, an environmental control ("C1") was added to control for effects of these conditions. "C1" beetles remained on food while the other groups were being fasted, but throughout the experiment were moved in and out of the incubator to match the location and environmental conditions of groups "T" and "C2".

Necropsy of beetles was done following the week 5 census. Each beetle was weighed (nearest 0.1 mg), sexed, killed and dissected. The number of parasite cysticercoids present was recorded.

Egg measurement

Eggs of *T. confusum* are normally covered by a sticky exochorion to which flour particles readily adhere during laying (Sokoloff 1972). Photographed eggs were selected for measurement only if the poles of the egg were free enough of flour to be discerned. Maximum length in pixels was measured on all such eggs. Some eggs were nearly free of attached flour; on these maximum width in pixels was also measured to determine how egg shape varies with egg length. Measurement of the background grid on each photograph was used to convert pixels to mm. All eggs that could be measured were measured, to eliminate bias in the selection of eggs; this was actually quicker than applying formal procedures to select and measure only a random subset of eggs.

The accuracy and repeatability of egg measurements was assessed in two ways. First, a sample of $N = 30$ eggs was measured using an ocular micrometer, then photographed and measured blind from the photographs as described above. Second, because measurement on the large number of eggs required several months, photographs from 30 beetles were chosen at random from among those whose eggs were photographed at week -1, and $N = 5$ eggs from each

beetle were randomly selected and re-measured, blind, approximately 12 months after the original measurements.

Data analysis

Fecundity was estimated as the number of eggs present in each vial at the weekly census. Egg cannibalism by adult beetles was assumed to be negligible because of the low beetle density employed (Yan and Stevens 1995). Egg cannibalism by larvae was assumed to be negligible because larvae were few (mean of 6/culture at time of census) and small (<2 days old based on preliminary observations that egg hatching in our culture conditions normally commences 5-6 days after laying), and that larvae of *T. confusum* have a much lower tendency than adults to cannibalize eggs (Alabi et al. 2008). The mean of week -2 and week -1 fecundity determinations was used as the base, or pre-exposure, fecundity for each female. Egg length unless indicated otherwise was the mean length of all measurable eggs from each female for each census period. Mean egg length at week -1 was used as base egg length for each female. For some analyses, group "T" beetles were subdivided to produce different ranges of infection intensities: "T1" was 1–7 parasites per host, likely with host resources in excess of parasite needs (Shostak et al. 2008); "T2" was 8–12 and "T3" was >12 (the division was chosen arbitrarily to maintain equal sample sizes in "T2"and "T3".

Statistical analyses were performed using procedures in SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Simple regressions used PROC REG and included visual examination of residual plots to confirm linearity of plots and normal distribution of residuals. Changes in fecundity and mean egg length of post-infection beetles among treatment groups were tested using PROC MIXED in a repeated measures analysis of variance design, with female weight and either base fecundity or base egg length as covariates, and using Kenward-Roger

adjustment of degrees of freedom. Prior to analysis, post-exposure fecundities and egg lengths for each female were converted to proportions of their base values. Choice of covariance structure was based on a combination of graphical tools and information criteria (Littell et al. 2006) and resulted in the use of a Toeplitz model for fecundity data and an autoregressive plus random effects model for egg lengths. Tests of differences among treatments each week post exposure used the SLICE option. Variance components for egg length (egg lengths within individual beetles vs. among beetles) were estimated using PROC MIXED by the residual maximum likelihood method. Statistical significance was determined using $\alpha = 0.05$. Coefficients of variation (CV) were calculated as 100 × SD/mean. Data are presented as mean ± SD unless indicated otherwise.

Results

The experiment was initiated with 150 pairs of beetle pupae. There were 114 pairs of adults alive by the time of parasite exposure at week 0, and only 104 pairs survived to week 5 PE. Eggs measured from photographs were 0.69 ± 0.043 mm long, slightly (0.01 ± 0.015 mm) longer than measurements of the same eggs made using an ocular micrometer (paired *t* tests: lengths, $t_{29} = 3.82$, $p < 0.001$). Re-measurement of photographed eggs ca. 12 months apart produced variance components for egg length of 0.00035 ± 0.00016 SE (22% of total variance) among beetles, 0.00119 ± 0.00016 (74%) among eggs within beetles, and only 0.00007 ± 8.1×10^{-6} (4%) among replicate measurements of each egg.

Fecundity increased rapidly during the first few weeks of the experiment and then began to plateau (Fig. 1) at a mean of 50 eggs/female/week. At week -1, most females produced 30–70 eggs/week, although one individual produced <10. Because that individual had consistently produced 3–7 eggs during each week prior to exposure, and lived until week 5 PE, it was

assumed to be healthy and its data were retained. Interestingly, this individual was assigned to the "T" group and also happened to acquire the largest infection, 27 cysticercoids. Overall, females in the experiment (N = 104) produced 7.0 ± 1.45 eggs per day (CV = 20.7%) during the 2 weeks prior to infection. Mean egg length per beetle at week -1 varied from 0.64–0.74 mm, while individual egg lengths were more variable (0.55–0.85 mm).

At necropsy, all beetle pairs were confirmed to comprise 1 male and 1 female. No control beetles were infected. All parasite-exposed beetles were infected and had 1–27 parasites (mean = 9.5 ± 7.0). The final number of pairs was 33 in group "C1", 29 in "C2" and 42 in "T". The intensity subdivisions in group "T" comprised N = 20 pairs in "T1", 11 in "T2" and 11 in "T3".

There was no relationship between female mass (as determined at the end of the experiment) and either fecundity (Fig. 2a) or mean egg length (Fig. 2b) (as determined at week -1, prior to allocation of experimental treatments). Although there was a significant difference in mean final mass of females allocated at random to treatments (ANOVA, $F_{2,100} = 3.25$, p = 0.043), the differences among groups were small ("C1": 2.75 ± 0.047 SE mg; "C2": 2.59 ± 0.050 mg; "T": 2.64 ± 0.041 mg) compared to the overall range in mass of individual beetles (Fig. 2). Overall, females weighed at the end of the experiment were 2.66 ± 0.27 mg (N = 104, CV = 10.2%). Females that produced more eggs at week -1 tended to produce significantly smaller eggs (Fig. 2c) but this relationship accounted for only a small portion of the variability in mean egg length as measured by R^2.

Following exposure to parasites, changes in fecundity over time differed among treatments, as revealed by a highly significant interaction term (repeated measures ANOVA: $F_{16, 294} = 9.59$, $p < 0.001$). Fecundity of environmental control "C1" beetles remained near pre-

exposure levels from week 1 through week 5 (Figure 3). Fecundity of fasted control beetles "C2" dropped to about 50% of pre-exposure levels at week 1 post-exposure, then returned to "C1" levels by week 2 and remained there for the duration of the experiment (Fig. 3). Although fecundity of all infected beetles dropped to about 50% of pre-exposure levels at week 1 post-exposure, subsequent fecundity varied according to number of parasites present. Group "T1" fecundity was at "C1" and "C2" levels by week 2, whereas "T2" fecundity remained significantly lower than control levels for the duration of the experiment and "T3" fecundity was lower than "T2" fecundity until week 5 (Fig. 3). At week 1 the fecundity of all infected beetles was indistinguishable from that of the fasting control (Fig. 3).

A total of 24 856 eggs were recovered during the experiment; 14 225 (57%) of these were measured for length (N = 2 282–2 401 per week), and 2 238 uncoated eggs (9% of all eggs recovered) were also measured for width. Mean egg length per female during the 1 week prior to infection was 0.699 ± 0.018 mm (N = 104, CV = 2.6%) and length of individual eggs was 0.699 ± 0.039 mm (N = 2 412, CV = 5.6%). Following exposure to parasites, changes in mean egg length per beetle over time differed among treatments, as revealed by a highly significant interaction term (repeated measures ANOVA: $F_{16, 319} = 3.55$, $p < 0.001$), but the pattern of change among treatment groups (Fig. 4) differed from the pattern for fecundity (Fig. 3). Egg length of environmental control "C1" beetles gradually decreased from their pre-exposure levels, from about 1% shorter than pre-exposure length at week 1 to about 2% shorter by week 5 (Fig. 4). Fasting control "C2" and all infected beetles "T1"–"T3" produced eggs that were not only significantly longer than "C1" beetles at week 1, but were also 1–2% longer than their pre-exposure lengths (Fig. 4). Egg length in "C2" and "T1"–"T3" also declined as the experiment progressed and egg size in all groups became indistinguishable with few exceptions: "T1" eggs

were shorter than those of "C1", "C2" and "T2" at week 4, and "T2" eggs were shorter than those from "C2" and "T3" at week 5 (Fig. 4).

Overall, egg widths (Y) increased with increases in egg length (X) according to the relationship $\log Y = 0.272 \log X - 0.414$ (ANOVA, $F_{1, 2236} = 152$, $p < 0.01$). Testing the effects of fasting or infection on egg shape was complicated by the small number of beetles producing uncoated eggs, particularly those assigned the infection treatment, and to the reduced and often sporadic egg production by infected beetles. The following qualitative analysis was performed. The slope m of the relationship $\log Y = m \log X + b$ was calculated each week for each beetle that produced at least 5 uncoated eggs that week. In general the slopes were positive, with some significantly greater than 0 but most not differing significantly from 0 (Fig. 5). Given the variation in slopes among "C1" beetles (Fig. 5a), slopes in "C2" beetles (Fig. 5b) appeared similar. Slope estimates of infected "T" beetles (Fig. 5c), which were based on smaller sample sizes due to reduced host fecundity (Fig. 5), were more variable but exhibited only one major departure from the pattern for the control beetles: one beetle at week 2 actually produced eggs that were significantly narrower as they lengthened (Fig. 5c). Nothing unusual was noted about this beetle, which harbored only 9 parasites.

A majority (76–90%) of the random variation in egg length was attributable to variation in egg length within each beetle, with the remainder attributable to variation among beetles (Table 1), and the differences in level of this random variation among treatments or times did not correspond to the pattern of changes in fecundity or egg length that occurred throughout the experiment (Figs. 3, 4). Levels of variation in egg length relative to mean egg length were virtually constant, not only across time but also among treatment groups (Table 1).

Discussion

Basic observations on fecundity and life-history relationships in the present study appear to be fairly typical for this host and parasite. Although lab-reared for several decades, these beetles exhibited levels of variation in mass, fecundity, mean egg length among females, and individual egg lengths that are similar to reports for female fecundity and mass, and egg mass, from other sources of *T. confusum* (Arnaud et al. 2005; Holloway et al. 1987). The present study also confirmed results of a previous study (Arnaud et al. 2005) in which there was a significant relationship between egg number and mass among individuals within species of *Tribolium*, but no relationship of egg number or mass to female mass. Mean fecundity of ca. 7 eggs/day in uninfected females corroborated previous reports (Keymer 1980; Maema 1986). The lack of effect of parasitism on fecundity during the first week post-infection, and the strongly reduced fecundity commencing the second week (but with that effect diminishing over the next several weeks) had also been noted by Maema (1986). The precise handling of control beetles in previous studies (Keymer 1980; Maema 1986) was not described, but clearly those studies only used one control group. The use of two controls in the present study strengthens the conclusion that the period of pre-exposure fasting is the sole cause of the fecundity reduction observed during the first week, that fasting does not have any residual effects after the first week, and therefore that the intensity-dependent reductions in fecundity after the first week are due solely to the effects of parasitism.

The present study initially posed three hypotheses regarding the effect of parasitism on the relation between host fecundity and egg size. Hypothesis I: Egg size does not vary with reduction in fecundity. This might indicate that the infected host adjusts only egg number while continuing to produce normal-sized, and presumably normal quality, eggs. This appears to be the outcome for *T. molitor* infected with *H. diminuta*, because while yolk content in virgin

females may be reduced by infection, eggs actually laid by mated females have normal yolk content (Hurd and Arme 1986a). Hypothesis II: Reduction in fecundity is accompanied by a decrease in egg size. This might indicate an exceptionally severe impairment of host reproductive machinery. This is the outcome reported for *G. aculeatus* infected with *S. solidus* (Heins and Baker 2003). Hypothesis III: Reduction in fecundity is accompanied by an increase in egg size. This might indicate an attempt by infected individuals to compensate for fewer eggs by producing larger, and possibly higher quality, eggs. Although it is well established that snails infected with trematodes may exhibit a temporary increase in egg numbers known as fecundity compensation (Blair and Webster 2006; Minchella 1985), and at the population level may tend to produce larger eggs (Fredensborg and Poulin 2006), there appear to be no prior studies on egg size in parasite-infected hosts that provide evidence at the level of the individual host in support of hypothesis III. Interpretation of egg size in the present study was complicated by the observation that even the uninfected, non-fasted control beetles tended to produce shorter eggs as the beetles aged, so any effects of fasting and infection were taking place on this shifting background. Females clearly produced longer eggs during the two weeks following fasting, even though they only had reduced fecundity during the first week, and therefore response of egg length to fasting alone is consistent with hypothesis III. However, while infected beetles also produced longer eggs, results from the fasting control indicate that this length increase is explained solely by the fasting that infected beetles underwent prior to exposure and not the infection itself. Therefore, the response to parasitism in egg size of *T. confusum* is consistent with hypothesis I and with previous observations on the related *T. molitor* (Hurd and Arme 1986a).

316 The present study demonstrated that individual beetles are capable of adjusting egg size
317 in response to the presumed nutritional stress following a period of fasting. Patterns of parasite
318 growth in *T. confusum* are consistent with depletion of host nutrients in a manner similar to host
319 starvation (Shostak et al. 2008). Since egg size changed as a result of fasting, it was surprising
320 that parasitism produced no clear effect on egg size. (The few cases of significant reduction in
321 egg size of parasitized beetles relative to controls were sporadic, 4-5 weeks post-infection, and
322 without apparent pattern in relation to infection intensity; for the present it is assumed that these
323 reflect the accumulation of subtle differences in the age-related decline in egg size in the
324 different groups of beetles.) It may simply be that there is no effect of parasitism on egg length.
325 The effects of helminth parasitism on host nutrient pathways have been only partially elucidated
326 in tenebrionid beetles (Shostak et al. 2008) and the parasite effect may be through mechanisms
327 that alter egg numbers but play little role in egg size. For example, apart from simple
328 interference competition with the host for hemocoele nutrients, it is known that *H. diminuta*
329 secretes molecules that alter host vitellogenin pathways, at least in *T. molitor* (Warr et al. 2006).
330 On the other hand, the effects of parasitism and the pre-exposure fast may have been
331 compensatory and not additive. Just as there may be a minimum to the size of viable eggs that
332 might be produced by an individual beetle (Smith and Fretwell 1974), the amount an individual
333 beetle might adjust its egg size upwards must also be limited. Other insects can increase egg
334 length or area by only up to about 10% (Fischer et al. 2003; Fox et al. 1997). Fasting alone may
335 have elicited the maximum possible increase in egg size for *T. confusum*, with no further
336 adjustment possible even in infected hosts. Since the bulk of parasite growth occurs within two
337 weeks of infection (Shostak et al. 2008) and the effects of fasting on egg size lasted that long,
338 this might seem a reasonable explanation. Unfortunately, confirmation by isolating or removing

the effect of fasting from infected beetles during the first 2 weeks will prove difficult because eliminating the fast prior to exposure will lower resulting parasite numbers (Dunkley and Mettrick 1971) and with fewer parasites the effect of parasite on host may become increasingly difficult to detect. In the absence of evidence to the contrary, however, the most supportable conclusion based on results of the present study is that parasitism depresses fecundity but does not affect egg size.

Observations on variation in egg size were intriguing. In spite of a considerable range in mean egg length among beetles, most of the variation in length of individual eggs occurred within individual beetles. Given variation within a beetle, there are two ways an increase in mean egg length could be accomplished. First, beetles could narrow the range of egg sizes produced, towards the size of the larger eggs they normally produce. This would produce a decrease in variability in egg size, but would not require the beetle to produce any eggs larger than normal. Second, beetles could proportionately increase the size of all eggs. This would increase mean size but maintain levels of variation. The maintenance of nearly constant CV in egg length and within-host variance components in the present study, across conditions that produced large changes in fecundity and changes in mean egg length, supports the latter scenario. There may be selective pressure to produce variable-sized eggs, or the mechanism producing variable egg sizes with each female may simply be independent of the effect of fasting.

Although parasitism did not affect egg length, fasting of the host clearly did. Larger eggs may enhance fitness to some extent (Fox et al. 1997; Smith and Fretwell 1974) although fitness effects may be context dependent (Fischer et al. 2003; Marshall and Keough 2008). It must also be recognized that some insects may provision eggs of similar size differently (Ferdig et al.

1993; McIntyre and Gooding 2000) and in those systems egg size may not predict egg quality (McIntyre and Gooding 2000). While there is differential provisioning of eggs in *Hymenolepis*-infected *T. molitor* (Hurd and Arme 1986b), it is associated with differences in egg volume (Hurd and Arme 1986a). Within the genus *Tribolium* there are inter-specific correlations between egg mass and various life-history traits, but intra-specific comparisons do not reveal any relationship between egg mass and development time or subsequent adult mass (Arnaud et al. 2005). The populations of *T. confusum* studied by Arnaud et al. (2005) had a CV in egg mass of 14-16%. Since eggs in the present study changed mainly in length, and not width, a 3% increase in egg length (the largest observed differential in length of eggs from fasted beetles relative to controls in the present study) would likely translate into < 5% increase in mass. It therefore seems unlikely that increased egg length following fasting, while statistically significant, would be a biologically relevant trade-off for the drastic reduction in fecundity that occurs in *T. confusum* following fasting or infection with *H. diminuta*.

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Table 1. Measures of variation in length of eggs from environmental control (C1), fasting control (C2) and *Hymenolepis diminuta*-infected (T) *Tribolium confusum*.

Group	Measure	Week -1	Week 1	Week 2	Week 3	Week 4	Week 5
C1	*N* [a]	1536	1938	1684	1856	1724	1602
	Mean [b]	0.702	0.692	0.685	0.690	0.681	0.685
	5% [c]	0.632	0.625	0.618	0.619	0.605	0.613
	95% [d]	0.767	0.753	0.744	0.753	0.743	0.746
	Variance [e]	82%	80%	76%	78%	79%	82%
	CV [f]	6%	6%	6%	6%	6%	6%
C2	*N*	1328	1278	1454	1680	1570	1520
	Mean	0.691	0.708	0.697	0.691	0.683	0.686
	5%	0.623	0.639	0.64	0.635	0.627	0.624
	95%	0.750	0.765	0.747	0.740	0.739	0.747
	Variance	79%	90%	84%	88%	81%	85%
	CV	6%	6%	5%	5%	5%	6%
T	*N*	1910	1550	1440	1642	1560	1588
	Mean	0.701	0.707	0.694	0.692	0.678	0.681
	5%	0.637	0.637	0.636	0.636	0.623	0.625
	95%	0.761	0.762	0.744	0.738	0.732	0.734
	Variance	88%	84%	88%	79%	77%	78%
	CV	6%	6%	5%	5%	5%	5%

[a] Total number of individual eggs measured; [b] Mean egg length (mm); [c] Fifth percentile of egg length (mm); [d] Ninety-fifth percentile of egg length (mm); [e] Variance component for egg length within each beetle expressed as a percentage of total random variation in egg length (within and among beetles); [f] Coefficient of variation for individual egg lengths pooled among beetles within each group and week.

Figure captions

Figure 1. Fecundity of flour beetles *Tribolium confusum* during the four weeks prior to exposure to *Hymenolepis diminuta* at week 0. Values are mean eggs per week per female ± SD, $N = 104$.

Figure 2. Relationships between fecundity, mass and egg length of female flour beetles *Tribolium confusum* determined one week prior to exposure to *Hymenolepis diminuta*. (a) Fecundity vs. mass; (b) Mean egg length per female vs. mass; (c) Mean egg length per female vs. fecundity. Equations are regression formulae and fit statistics against the null hypothesis that the slope = 0.

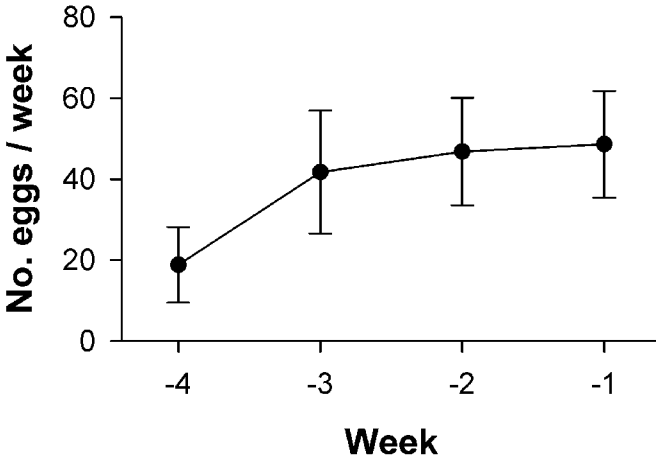
Figure 3. Fecundity of female flour beetles *Tribolium confusum* following exposure to *Hymenolepis diminuta*, expressed as a mean proportion ± SE of same-beetle fecundity determined during the two weeks prior to exposure. "C1", environmental control; "C2", fasting control; "T1", 1–7 parasites present; "T2", 8–12 parasites present; "T3", >12 parasite present. Within each week, letters represent the results of pair-wise contrasts among groups; values with the same letter do not differ significantly ($p > 0.05$).

Figure 4. Mean egg length of female flour beetles *Tribolium confusum* following exposure to *Hymenolepis diminuta*, expressed as a proportion ± SE of same-beetle mean egg length determined during the week prior to exposure. "C1", environmental control; "C2", fasting control; "T1", 1–7 parasites present; "T2", 8–12 parasites present; "T3", >12 parasite present. Within each week, letters represent the results of pair-wise contrasts among groups; values with the same letter do not differ significantly ($p > 0.05$).

Figure 5. The slope m of the relationship $\log Y = m \log X + b$ calculated each week for each environmental control (a), fasting control (b), and *Hymenolepis diminuta*-infected (c) flour beetle *Tribolium confusum* that produced at least 5 measurable eggs that week, where Y = egg width

and X = egg length in mm. Open circles represent slopes that do not differ significantly from 0 ($p > 0.05$); closed circles represent slopes that differ significantly from 0 ($p < 0.05$). No measurements were done for week 0 when beetles were exposed to parasites.

483 Fig 1



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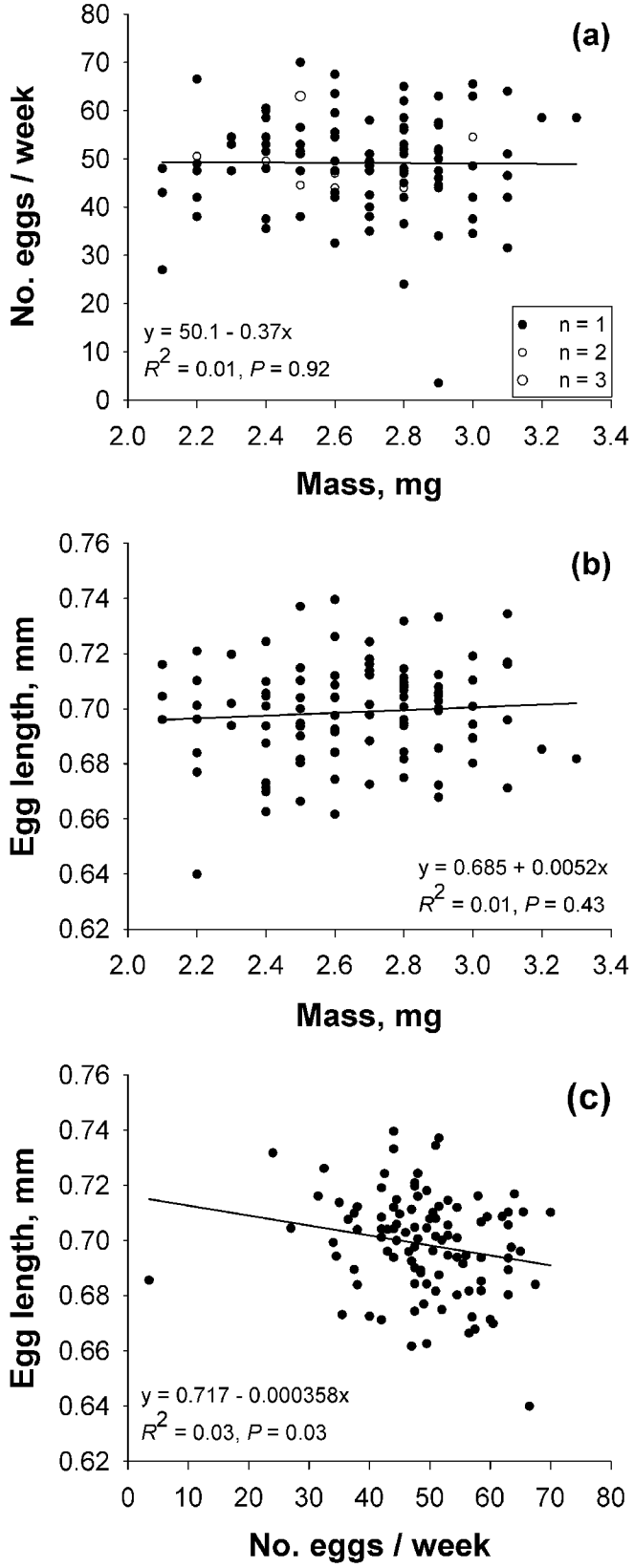
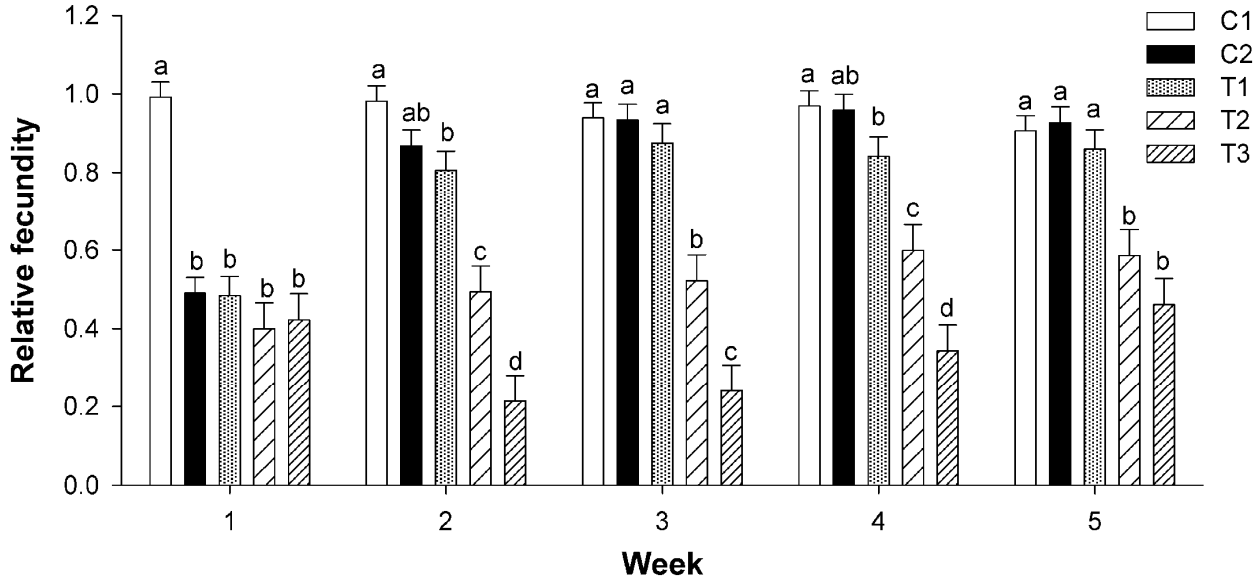
486 Fig 2


(a)
No. eggs / week
y = 50.1 - 0.37x
R^2 = 0.01, P = 0.92
n = 1
n = 2
n = 3
Mass, mg
(b)
Egg length, mm
y = 0.685 + 0.0052x
R^2 = 0.01, P = 0.43
Mass, mg
(c)
Egg length, mm
y = 0.717 - 0.000358x
R^2 = 0.03, P = 0.03
No. eggs / week

487

488 Fig 3



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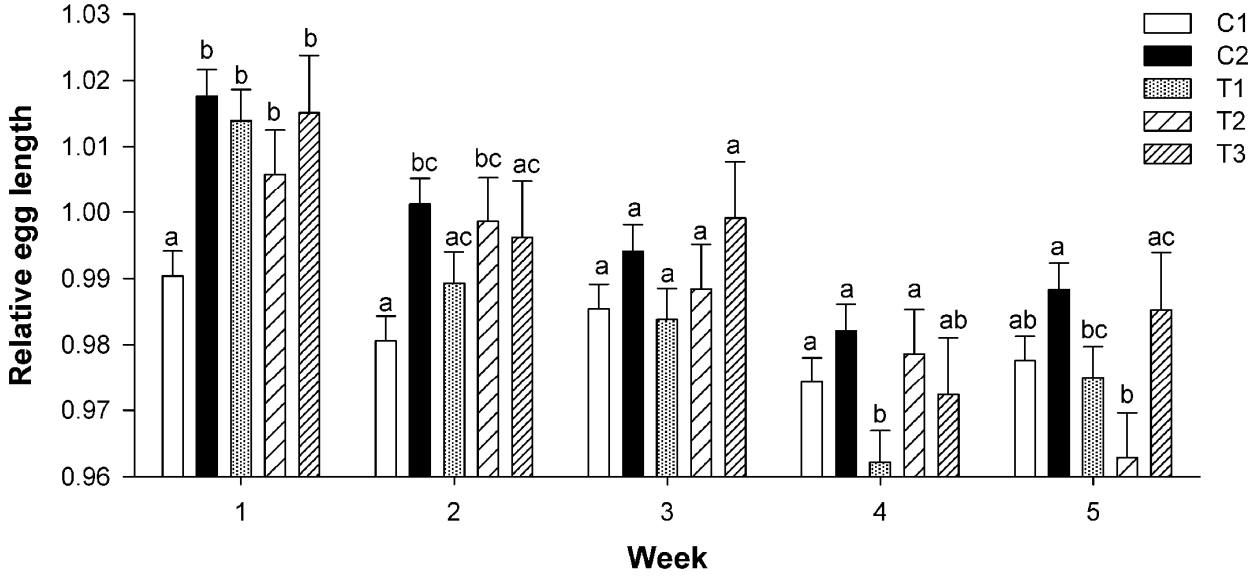
491 Fig 4



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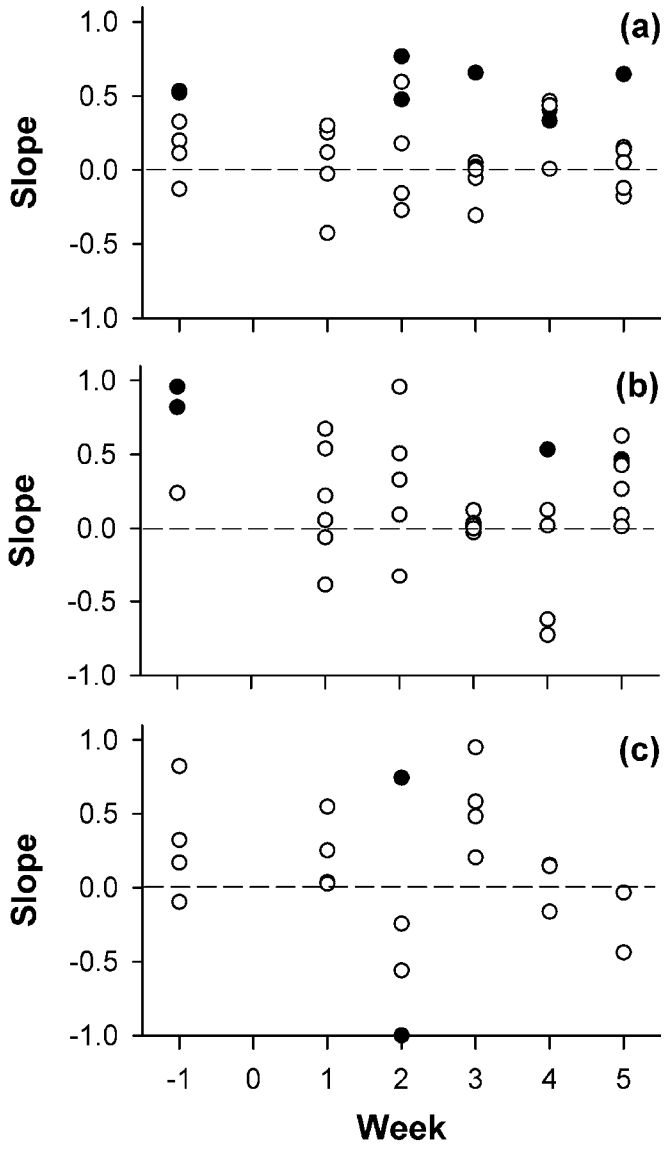
494 Fig 5



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