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Infection increases the value of nuptial gifts, and hence male reproductive success, in the *Hymenolepis diminuta–Tenebrio molitor* association

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During copulation, male insects pass accessory gland components to the female with the spermatophore. These gifts can affect female reproductive behaviour, ovulation and oviposition. Here, we show that female mealworm beetles, *Tenebrio molitor*, mated with males infected with metacestodes of the rat tapeworm, *Hymenolepis diminuta*, produced significantly more offspring than those mated with uninfected males. There is a significant positive relationship between parasite intensity in the male and reproductive output in the female. Infection results in a significant increase in bean-shaped accessory gland (BAG) size. We suggest that infected males pass superior nuptial gifts to females and discuss the confounding effects of infection in male and female beetles upon overall fitness costs of infection for the host and the likelihood that the parasite is manipulating host investment in reproduction.

Keywords: *Hymenolepis diminuta* metacestodes; *Tenebrio molitor*; nuptial gifts; male reproductive fitness

1. INTRODUCTION

In common with many parasitized insects, female *Tenebrio molitor* experience some loss of reproductive fitness when infected with metacestodes of the rat tapeworm, *Hymenolepis diminuta*. This loss is associated with an impairment of the process of vitellogenesis resulting in a reduction in the yolk protein content of eggs and in fertility (Webb & Hurd 1999).

Although we have less information concerning the effects of infection in male *T. molitor*, it appears that they may be very different from those in the female. Male *T. molitor* attain sexual maturity one week post-emergence (Happ 1987). During this week, the bean-shaped accessory glands (BAGs) increase in volume and begin to synthesize proteins that will contribute to seminal fluids, spermatophores and copulatory plugs. Size, and wet and dry weight rise to a plateau on day 6 post-emergence in uninfected beetles but continue to increase up to day 10 post-emergence in infected males (Carver & Hurd 1998). BAG total protein content and trehalase activity is significantly increased and spermatophores produced by infected males contain significantly elevated protein content and trehalase activity (Carver *et al.* 1999).

Components in the male ejaculate may affect the behaviour, fecundity, ovulation and oviposition of female insects (Gillott 2003). Our findings concerning the effect of infection on *T. molitor* BAGs suggest that metacestode-infected males may be providing superior nuptial gifts to the female in comparison with uninfected males. This has led to the hypothesis that uninfected females mated with infected males may be more reproductively successful. Here, we report the findings of an experiment designed to test this hypothesis.

2. MATERIAL AND METHODS

Beetles were maintained and males infected as reported previously (Carver & Hurd 1998). Because a trend for larger female *T. molitor* to produce more offspring has been reported (Worden *et al.* 2000), newly emerged females were weighed within the first 24 h and egg production was expressed in terms of female mass (adjusted reproductive success).

Parasite infections were allowed to develop in the males for 6 days, during which time the male sexual accessory glands mature (Happ 1987) and male responsiveness to females reaches a peak (Happ 1970). Individual males (9 days old) were then placed with a 9-day-old female in a Petri dish and observed for 10 min. Male beetles were marked with a spot of Liquid Paper correction fluid on the elytra, which has been shown to have no effect on mating potential (Carver 1997). Any males not initiating courtship, mounting the female and extending the genitalia to be in contact with the female (Hurd & Parry 1991) were removed from the study. Control, uninfected males were treated in the same way. Following the 10 min observation period, pairs were kept together for 24 h then females were removed and placed in fresh bran, supplemented every third day with an apple slice.

Following mating, males were removed for dissection by a dorsal incision of the abdomen and the haemocoel was flushed out with *Tenebrio* saline (76 mM NaCl, 36 mM KCl). All metacestodes from infected males were counted and their developmental stage noted. The width and height of the BAGs from infected and non-infected males were recorded (Carver & Hurd 1998).

Females were left for a further 6 days then the total number of eggs laid was counted on this and every third day for a further 15 days. At each egg count, females were removed and placed in a fresh dish to decrease the chance of cannibalism. Dishes containing eggs were maintained at 26–27 °C for 14 days, by which time all viable eggs had hatched (Cotton & St George 1929). Larvae per dish were counted and egg viability assessed as the percentage of eggs hatched. In total, 30 female/infected male pairs and 30 female/control male pairs were observed.

3. RESULTS

All males exposed to infection harboured metacestodes at various stages of development, the majority at early stage four, and there were only three beetles containing any fully mature parasites (Hurd & Burns 1994). Parasite density ranged from 11 to 132 with a mean value of 57.43 ± 5.54 (s.e.) metacestodes per beetle. Comparison of BAGs from 9-day-old males confirmed our earlier finding that infection significantly increased the width of these glands (two-tailed *t*-test; d.f. = 58, $p < 0.001$; figure 1). However, the increase in height was not significant at this age (d.f. = 58, $p = 0.717$). There was no relationship between BAG width and density of parasite infection (figure 2) and no correlation was found between BAG width and the number of eggs produced by infected or uninfected females (Pearson's correlation: 0.047, $p = 0.806$ and 0.218, $p = 0.246$, respectively).

Females mated with infected males laid significantly more eggs than those mated with uninfected males during 21 days post-mating (mean ± s.e. = 34.67 ± 3.46 and 25.70 ± 2.19,

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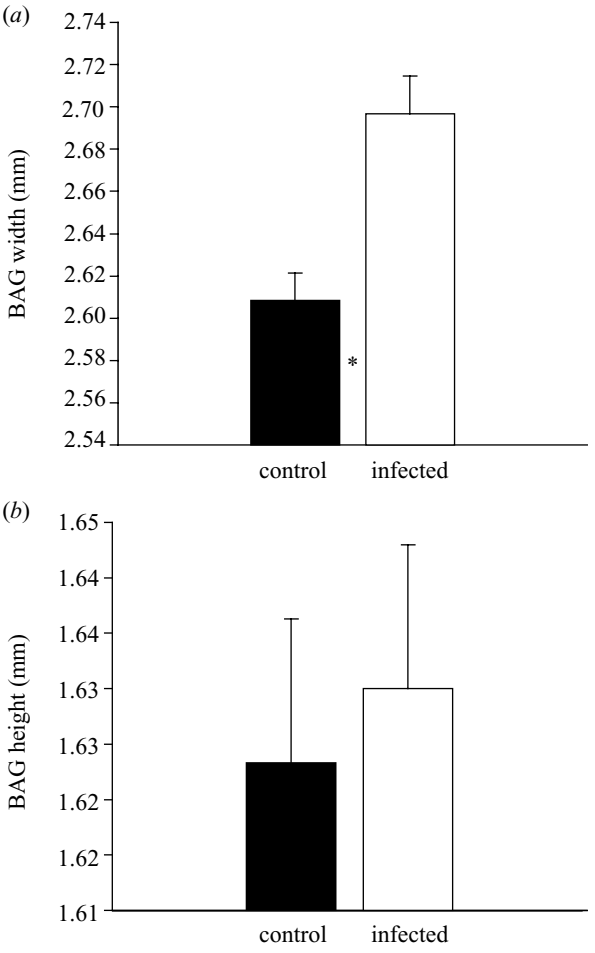

Figure 1. The effect of *Hymenolepis diminuta* infection on (*a*) the width and (*b*) the height of the BAGs of *Tenebrio molitor*, measured on day 7 post-infection. Error bars, s.e.m., $n = 30$ infected and 30 uninfected males; *significantly different ($p < 0.001$; two-tailed *t*-test).

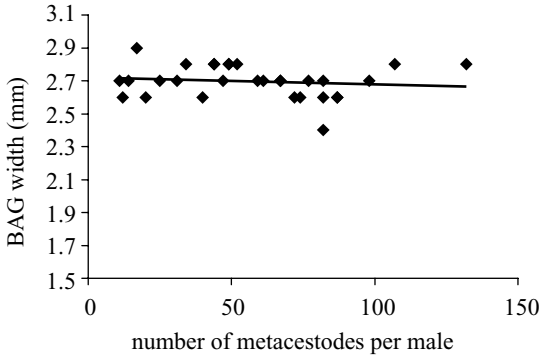

Figure 2. The relationship between the density of *Hymenolepis diminuta* infections in the male and the width of the BAG on day 7 post-infection. Nine-day-old males were mated with 9-day-old females and oviposition monitored on day 6 post-mating and every third day thereafter for a further 15 days. $n = 30$ males; *Y* (BAG width) = 0.2722–0.00041 × (parasites); $t = 0.714$, significance $p = 0.481$, d.f. = 28, adjusted $r^2 = -0.077$.

respectively). Mass-adjusted female egg production was significantly increased by infection (figure 3). The percentage of eggs that hatched was not affected (infected = 94.2%, uninfected = 95.3%), therefore the production

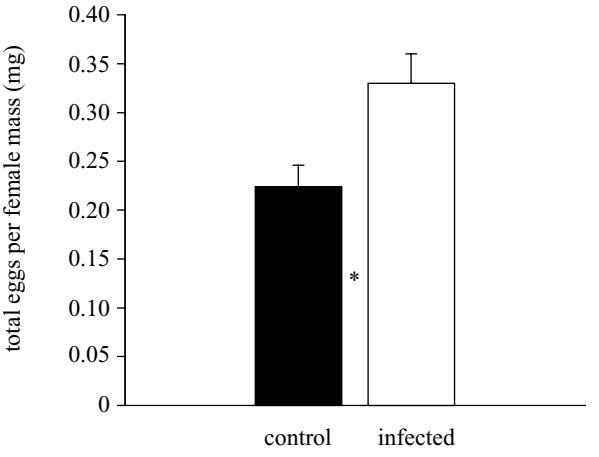

Figure 3. Comparison of egg production by female beetles mated to *Hymenolepis diminuta*-infected or uninfected males. Egg production was monitored for 21 days post-mating and expressed in relationship to female mass at the time of emergence. Error bars, s.e.m.; $n = 30$ females; *significantly different ($p = 0.029$; two-tailed *t*-test).

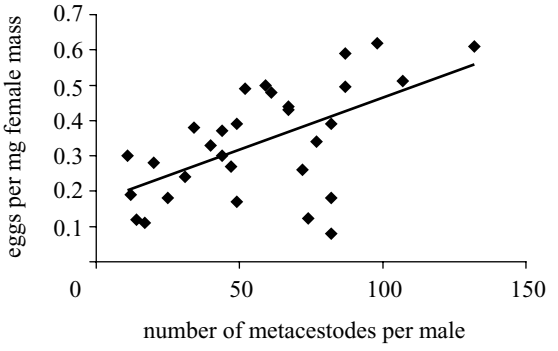

Figure 4. Relationship between the density of *Hymenolepis diminuta* infections in the male and the number of eggs laid by their non-infected mates during a 21-day period. Nine-day-old males and females were mated and oviposition monitored on day 6 post-mating and every third day thereafter for a further 15 days. $n = 30$ females, each housed individually. Egg production is expressed in relation to female wet weight at the time of emergence. *Y* (adjusted female egg production) = 0.136 + 0.003408 × (parasites); $t = 4.196$, significance $p < 0.001$, d.f. = 28, adjusted $r^2 = 0.364$.

of live larvae was increased by infection(d.f. = 58, $p = 0.030$). Furthermore, egg and larvae production was found to be significantly related to the intensity of infection in the male (figure 4).

4. DISCUSSION

Our finding that mating with *H. diminuta*-infected males significantly increases egg laying in uninfected females led us to conclude that the ejaculates from infected males provide superior nuptial gifts to their mates that result in increased reproductive fitness for both partners that is dependent on parasite density.

Lewis & Austad (1994) suggest that nutrient transfer to female *Tribolium castaneum* (another Tenebrionidae) via the male ejaculate may affect female fertility. The total protein content of spermatophores from *T. molitor* is

significantly elevated by *H. diminuta* infection (Carver *et al.* 1999). However, no functional role has been assigned to *T. molitor* spermatophore proteins and sex peptides have not yet, to our knowledge, been identified (Happ 1987). To date, we do not know whether secretions from the enlarged BAGs are responsible for the observed increase in reproductive fitness or whether other components present in the ejaculate are involved, but the lack of relationship between BAG width and parasite density or egg production suggests the latter.

Male-produced juvenile hormone (JH), passed to the female during mating, enhances vitellogenesis and stimulates endogenous JH synthesis in several insects (Gillott 2003). Mating increases JH titre in *T. molitor* females but this is not enhanced by mating with infected males (Cole *et al.* 2003), thus JH is unlikely to be the causal component in the ejaculate.

Our findings contrast with Worden *et al.* (2000) who reported a negative relationship between male infection and female fertility in this association. However, Worden *et al.* did not infect males until 13 days post-emergence, when BAGs were fully developed (Happ 1987) and unlikely to be affected by the parasites. In addition, enhanced fecundity was only observed when females mated with males that had infection intensities of greater than 275 parasites and in two out of six beetles densities approached one thousand, far in excess of those reported in natural infections in wild *T. molitor* (Rau 1979; fewer than 83 parasites per beetle).

Our study was designed to investigate changes in male reproductive fitness that only operate via the effect of nuptial gifts, thus no mate choice was allowed and only copulating males were investigated. Male reproductive fitness is, however, dependent upon sequential events including mate attraction, courtship, copulation and insemination. *T. molitor* sperm number and viability are not affected by infection (Carver 1997) but the response of males to female copulatory release hormone is depressed by 50% when immature parasites are present (Hurd & Parry 1991). In conditions where males were competing for females, infected males may not be so successful.

Polak & Starmer (1998) reported that parasite-induced risk of mortality elevates reproductive success in male *Drosophila*. However, cases of increased host reproductive effort are rare. The phenomenon of fecundity compensation has been demonstrated where, early in infection, host reproductive output is increased, only to be depressed or cease later in the infection (Thornhill *et al.* 1986). This also occurs in *H. diminuta*-infected female *T. molitor* between day 3 and day 6 post-infection (Cole *et al.* 2003), although we do not know whether the viability of these eggs was decreased.

Life-history costs of infection are clearly complex in this association. A male beetle gains from mating with an uninfected beetle since her eggs will be more fertile than an infected female's and, if parasite resistance is heritable, he may produce offspring that are more resistant to infection. Infected females produce less sex pheromone (Hurd & Parry 1991), so sexual selection could be based on honest indicators (Grafen 1990) making it more likely that the male will mate with an uninfected beetle if he has a choice. Female beetles gain a direct benefit, in the form of superior nuptial gifts, by mating with an infected male. However, they may then produce offspring that are more susceptible to infection. Do female beetles choose their mates and, if so, do they choose infected or uninfected males?

Selection pressures will also be operating on the parasite and may result in manipulation of the host to enhance parasite fitness. It is possible that metacestodes are able to manipulate the production of nuptial gifts and thereby enhance the inheritance of susceptibility genes and that the host is either unable to counter this strategy or the trade-off in so doing is not sufficiently beneficial to have evolved (Hurd 1998).

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