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Effect of a Parasitic Nematode, *Chondronema passali* Leidy (*incertae sedis*), on the Size and Strength of the Horned Passalus, *Odontotaenius disjunctus* Illiger (Coleoptera: Passalidae)

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Abstract

The horned passalus, *Odontotaenius disjunctus* (Illiger), is host to a variety of parasites, including a little-studied nematode, *Chondronema passali* (Leidy), that can number in the thousands in a single beetle. We attempted to determine the effects of this parasite on two measures of host fitness, physical strength and body size of adult beetles collected from hardwood logs at two sites in Georgia, USA. For a subset of the beetles, we measured their individual pulling strength using a dynamometer and data-logging apparatus. We also recorded the live weight of all beetles and from another subset a composite measure of body size based on digital images. Beetles were dissected and nematode infection status was assessed for all beetles. Of all beetles examined in this study (49 females, 44 males), we detected *C. passali* in 63 (67.7%) individuals. Prevalence in males did not differ from females. Infections ranged from 10 to over 1,000 individual nematode larvae per beetle. There was no significant effect of infection on maximum pulling force after accounting for body size and gender. Beetles with nematode infections weighed significantly more than those without nematodes and were significantly larger in body size. These results suggest the effects of this parasite are minimal to the host, and infections may even confer an advantage via the increase in size. Based on a review of the relevant literature, this appears to be a rare phenomenon among the many insect-nematode parasite relationships.

Key Words: bess beetle, host-parasite relationship, body weight, prevalence

Beetles and other insects are hosts to a large number of parasitic nematodes (Welch 1965; Poinar 1972; Benham 1974). In most cases, nematode infections cause mortality (*e.g*., Poinar and Thomas 1985) or a range of sublethal effects including reductions in larval weight (Milstead 1980), delayed metamorphosis (Ashraf and Berryman 1970), reduced lifespan and fecundity (Togashi and Sekizuka 1982) and reductions in gallery construction of wood-inhabiting species (Macguidwin and Smart 1979). Those that have clear negative effects are often used as biological control agents in the agricultural industry (Webster 1980; McGraw and Koppenhofer 2008; Morton and del Pino 2008). The horned passalus, *Odontotaenius disjunctus* Illiger (Fig. 1A), a beetle that lives in rotting hardwood logs throughout much of eastern North America, is host to two species of nematodes (Pearse *et al*. 1936, Reinert 1973). One of these nematodes, *Hystrignathus rigidus* Leidy, is found in the beetle gut in low numbers (5–8) throughout the year, while the other, *Chondronema passali* Leidy (Fig. 1B), lives in the body cavity in its larval form and can number in the thousands within individual beetles (Pearse *et al*. 1936). This high within-host abundance suggests that the effects of this parasite, if any, could be especially pronounced.

Despite the fact that *C. passali* was discovered over 160 years ago (Leidy 1852), little is known of its biology. What is known is that it is widespread within *O. disjunctus* populations; infection prevalence varies from 68% in South Carolina (Reinert 1973) to nearly 100% in Maryland (Nickle and Pilitt 1969). In the only exclusive study of the biology of this parasite, Christie and Chitwood (1931) described its morphology and mode of transmission. The entire life cycle of *C. passali* takes place in galleries of *O. disjunctus*. Adult nematodes occur in the frass inside the beetle tunnels. Christie and Chitwood (1931) speculated that the mother nematode with her entire progeny is eaten by the beetle for a *per os* infection. The taxonomic status of the nematode is currently uncertain. It had been placed in the order Tylenchida, although Nickle and Pilitt (1969) could not confirm this because of ambiguities in shared characters with other groups of parasitic tylenchids. They indicated it is a primitive nematode with no close relatives.

No prior studies have examined the effect of this nematode on the welfare of its host, but given its high within-host abundance, any effects may be considerable. We undertook the current study to elucidate the effects of *C. passali* on body size (mass and morphology) of *O. disjunctus* from field collections, and the effect of infection on the strength

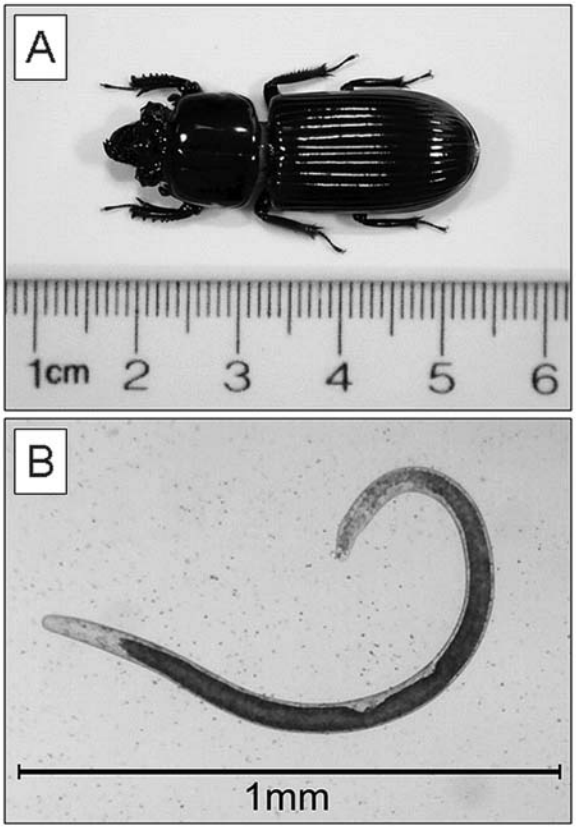

Fig. 1. The horned passalus, *Odontotaenius disjunctus* (A), and a larval *Chondronema passali* (B) taken from the body cavity of a beetle.

of beetles using a device that measures how much force they can pull.

Material and Methods

Beetle Sources. From September through November 2012, we collected adult horned passalus from hardwood logs in two sites near Athens, Georgia (n = 23, 17), and from one site near Savannah, Georgia (approximately 300 km from Athens, n = 52). Initial comparisons indicated there was no difference in live weight of the two Athens collections (Student's T-test, df = 39, t = 0.316, p = 0.753), so these sites were pooled for most analyses. All sites were hardwood forests with minimal anthropogenic disturbance, so that fallen logs were numerous on the forest floor. All beetles were returned to the laboratory at the University of Georgia (Athens) and housed in plastic containers (filled with rotten wood collected from the same site) until strength and parasite assessments (below) one month later. The individuals that were to be used for strength measurements were housed individually in 1.9-L plastic containers, since prior work in our laboratory showed that crowding leads to reductions in strength (Davis *et al*. In Press). All other individuals were housed in groups of 5–7 in 8-L plastic containers.

Measuring Beetle Strength. For a subset of the beetles (those from one of the Athens sites, n = 23), we measured their individual pulling strength using a method described by Davis *et al*. (In Press), and which was adapted from an early study of the strength of this species (Hinds 1901). Briefly, a nylon thread was tied around the beetle (at the junction between the pronotum and the abdomen) and the other end of the thread was attached to a dynamometer (precision = 0.03 Newtons). The beetle was allowed to enter an artificial wooden tunnel (20 mm wide, 15 mm tall) and to pull against the thread. A datalogger recorded the real-time pulling force of the beetle (Fig. 2). We allowed each beetle to pull for a 10-minute period, and from this we obtained the maximum pulling force for each beetle. Prior analyses showed this measure was strongly correlated with mean pulling force over the 10-minute period (Davis *et al*. In Press). After the strength trial, each beetle was weighed with an electronic balance (precision = 0.01 g) then assessed for nematode parasites.

Measuring Beetle Size. For a second subset of the beetles (n = 36, from the Savannah site), we photographed them individually (see Fig. 1A) and used image analysis software to measure certain body

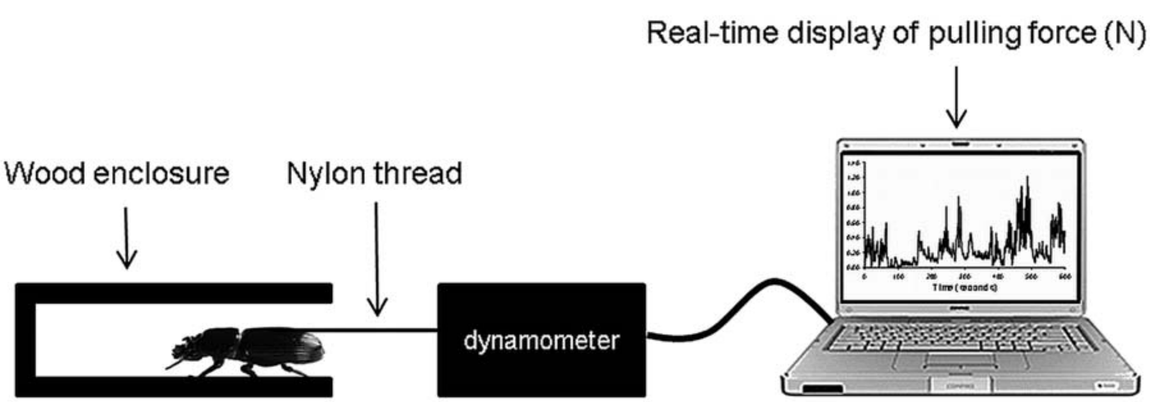

Fig. 2. Schematic of the apparatus used to measure pulling strength of *Odontotaenius disjunctus* in this study.

features that index body size (Davis *et al*. In Press). Specifically, we measured the elytral length, pronotal length, and pronotal width. From these values, we calculated a composite measure of body 'size' with the following equation: size = (pronotal length + elytral length)∗(pronotal width), which is expressed in square millimeters (Davis *et al*. In Press).

Nematode Assessment. All beetles were weighed and killed using chloroform in a killing jar prior to dissections. Their abdomens were dissected under a low power dissection scope. If present, *C. passali* is readily seen in the abdominal body cavity, especially if the insect is freshly killed, since the worms are still moving. We recorded the presence or absence of nematodes in each beetle, along with the beetle gender based on the presence of the male aedeagus (Schuster 1975). For a subset of the beetles (those that were strength-tested, *n* = 23), we also counted the actual number of nematodes in the body cavity. For this, we placed the beetle (after the ventral abdominal plate had been removed) in a 50-ml Eppendorf tube containing 20 ml of water. The tube was sealed and shaken with a vortexer for 20 seconds. The liquid was poured into a gridded Petri dish and the worms were counted under a low-power dissecting scope. This method appeared to remove all worms from the body cavity (searches of the cavity afterward showed no remaining worms) and facilitated their counting.

Data Analyses. Using the subset of beetles in which both pulling strength was measured and nematode counts were made (*n* = 23), we performed two tests to determine the effect of infection on strength (maximum pulling force, N). We first determined that the max force data were normally-distributed, based on visual inspection of their frequency distribution. Next we used ANCOVA to determine the effect of infection status (nematodes present or absent) and gender on max pulling force (response variable), and with body mass as a covariate. An infection by gender interaction term was also included in this model. We also addressed this question using the counts of nematodes (log-transformed); in another ANCOVA model, max pulling force was the response variable, gender was a predictor, and body mass along with nematode count were covariates.

To address the effect of infection on host body size, we used two approaches. First, using all of the beetle collections, a general linear model was used to examine the effect of nematode infection (infected or not) on body mass of the beetles. Body mass was the response variable, and nematode infection, sex, and collection site (Athens or Savannah) were independent variables. All two-way interaction terms were used in this model. Second, we used the body size measurements from the subset of the Savannah beetles (based on elytral length, pronotal length, and pronotal width) in an ANOVA model where nematode infection (infected or not) and sex were predictor variables. The sex∗infection interaction term was also included in this model. All tests in this study were performed using the Statistica 6.1 software package (Statistica 2003).

Results

Infection Prevalence and Intensity. Of the 93 beetles examined in this study (49 females, 44 males), we detected *C. passali* in 63 (67.7%) individuals. Infection prevalence did not differ between male and female beetles; 65% of females were infected while 70% of males were infected. A Chi-square test revealed no difference in the frequency of infected and uninfected beetles in either sex ($\chi^2 = 0.281$, df = 1, $P = 0.596$). Of the beetles from which we counted the number of nematodes, the counts ranged from 10 to 1,145, with a mean of 210 (±306 SD). The frequency-distribution of these counts was left-skewed, with most individuals having fewer than 500 nematodes, while few beetles had greater nematodes burdens.

Beetle Strength. The initial ANCOVA model (where nematode infection was included as a categorical predictor) indicated there was no significant effect of infection on maximum pulling force ($P = 0.2694$, Table 1A). There was also no effect of beetle gender on pulling strength ($P = 0.1558$), nor was the infection∗sex interaction significant ($P = 0.2259$). The only significant predictor of pulling strength was beetle size ($P = 0.0021$, Table 1A); pairwise correlation of body mass and maximum force indicated a positive relationship (r = 0.68, $P = 0.0004$). When the same question was addressed using the nematode counts as a covariate in an ANCOVA, there was still no effect of infection on pulling strength ($P = 0.7287$, Table 1B). Gender was also not significant in this model ($P = 0.4307$), and body mass was again significant ($P = 0.0011$).

Beetle Size. Infections with *C. passali* did have an effect on beetle body mass ($P = 0.0001$, Table 2A), though it was counter to our initial expectations; infected beetles were larger than uninfected beetles, and this effect was similar in both males and females (Fig. 3). Uninfected male beetles weighed an average of 1.52 g (±0.19 SD), but infected male beetles weighed 1.73 g (±0.16 SD), a 14% difference in weight. Meanwhile, infected females weighed 1.83 g (±0.18 SD) on average, compared to 1.67 g (±0.29 SD) for uninfected females, or a 10% difference. The GLM also showed a significant main effect of sex on body mass ($P = 0.0293$, Table 2A), and an interesting site∗sex interaction effect (P = 0.0210). Collectively, these last two effects indicate that female beetles tend to weigh more than male beetles, but the magnitude of this effect differed

Table 1. Summary of two ANCOVA models examining the effect of *Chondronema passali* on maximum pulling strength (response variable) of *Odontotaenius disjunctus*. The first model (**A**) considers nematode infection as a categorical predictor variable; in the second model (**B**), the actual count of nematodes was a continuous covariate. Beetles ($n = 23$) for these analyses were collected from Athens, Georgia, USA.

A.

Independent variable	df	MS	*F*	*P*
Nematode Infection (yes/no)	1	0.14	1.30	0.2694
Sex	1	0.24	2.19	0.1558
Infection*Sex	1	0.17	1.57	0.2259
Body Mass	1	1.39	12.94	0.0021
Error	18	0.11		
Total	22			

B.

Independent variable	df	MS	*F*	*P*
Sex	1	0.07	0.65	0.4307
Body Mass	1	1.67	14.67	0.0011
Nematode Count (log)	1	0.01	0.12	0.7287
Error	19	0.11		
Total	22			

by collection site. Specifically, the sex-based difference in body mass was larger within the Savannah collection than the Athens collection (Fig. 4). Finally, the effect of nematode infection on the composite measure of body size was significant ($P = 0.0229$, Table 2B), with infected individuals being larger (347 mm^2 ±33.2 SD) than uninfected ones (323.1 mm^2 ±38.8 SD) by approximately 7%. Gender was also significant in this analysis ($P = 0.0018$, Table 2B).

Discussion

Parasites, by definition, subsist on resources from their host. Because larval *C. passali* grow and develop entirely within the body cavity of *O. disjunctus*, they must obtain all of their nutrition from the host. We hypothesized that this would reduce host fitness, if not from the draining of host nourishment or tissue, then from the chronic expenditure of an immune response to combat the parasite. Because

Table 2. **A.** General linear model examining the effect of infection with *Chondronema passali* (infected or not), gender, and collection site (Athens or Savannah, Georgia USA, $n = 93$) on body mass of *Odontotaenius disjunctus*. **B.** Results of ANOVA model examining effects of nematode infection on a composite measure of beetle body size based on morphological measurements. A subset of beetles from the Savannah site ($n = 36$) were used in this analysis.

A.

Independent variable	df	MS	*F*	*P*
Collection Site	1	0.059	1.49	0.2251
Nematode infection	1	0.676	17.15	0.0001
Sex	1	0.193	4.91	0.0293
Site*Infection	1	0.002	0.05	0.8233
Site*Sex	1	0.218	5.53	0.0210
Infection*Sex	1	0.005	0.13	0.7177
Error	86	0.039		
Total	92			

B.

Independent variable	df	MS	*F*	*P*
Sex	1	11456.26	11.54	0.0018
Nematode Infection	1	5672.16	5.71	0.0229
Infection*Sex	1	443.19	0.45	0.5089
Error	32	992.87		
Total	35			

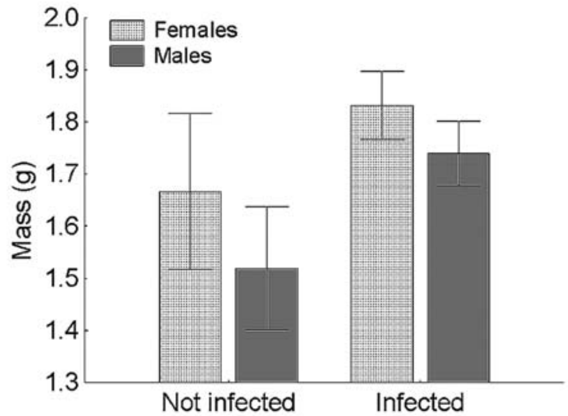

Fig. 3. Average body mass of male and female *Odontotaenius disjunctus* with and without infections of *Chondronema passali*. Error bars represent 95% confidence intervals. Beetles were collected in Athens ($n = 40$) and Savannah ($n = 52$), Georgia, USA.

of this, it was therefore surprising to find 1) no effect of nematode infection on physical strength of the beetles, and 2) an *increase* in body size with infection, rather than a decrease. Combined, these results suggest this parasite has no measurable detrimental effects on the host, and we could even say it confers an advantage to the horned passalus via the increase in size. There are many advantages to being larger for beetles and other insects; larger beetles are physically stronger (Block 1959, this study), they tend to win fights against smaller opponents (Otronen 1988), and they are more fecund (Juliano 1985; Fox *et al*. 1995). While we did not attempt to measure fecundity in infected and uninfected beetles in this study, this would certainly be a direction for future research.

The mechanism for the increase in body size with infection is not clear. It may be that infected

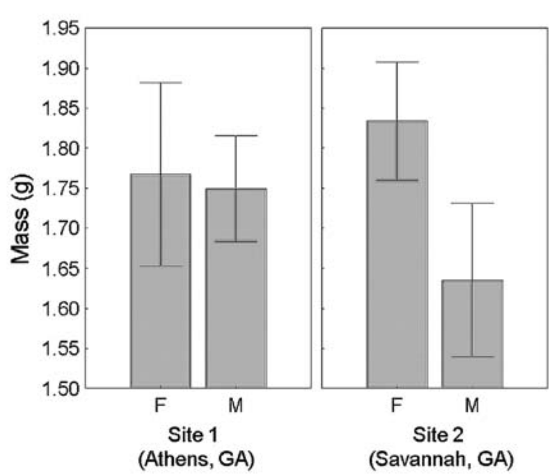

Fig. 4. Average body mass of male (M) and female (F) *Odontotaenius disjunctus* from Athens and Savannah, Georgia, USA. Error bars represent 95% confidence intervals.

beetle larvae increase feeding to compensate for the effects of the infection. There is also the possibility that the size patterns we observed in the adult beetles is the result of selective mortality of infected beetles; those that could not tolerate the infection (perhaps the smallest individuals) died early in life and were removed from the adult population that we sampled, leaving only the large, infected individuals. To determine if this is the case would require additional investigation of nematode infections within the larval beetles to see if infected larvae differ in size from uninfected ones. However, even if selective mortality was the reason, one could argue that the overall result of the nematode is positive on the population as a whole (since it ultimately leads to larger adult beetles).

The majority of the literature regarding insect-nematode (and other parasite) interactions indicates infections lead to reductions in growth and/or body size (see reviews by Welch (1965) and Thompson (1983)). However, there are some rare cases where insects infected with nematodes were found to be larger than uninfected individuals, and one of these was noted over 100 years ago. Strickland (1911) observed that larval black flies infected with a nematode species were larger than uninfected ones, but it was also indicated that the infected individuals were 'more sluggish'. In addition, alfalfa weevils, *Hypera postica* (Gyllenhal), infected with the nematode *Hexamermis arvalis* (Poinar and Gyrisco) were larger than healthy ones (Poinar and Gyrisco 1962). We also point out that there are other parasites of vertebrates that are known to increase host growth rates (reviewed in Thompson 1983), and/or where infected individuals are larger than uninfected ones (*e.g.* Davis *et al*. 2009). Thus, the results of the current study are not without precedent, but this does appear to be a rare phenomenon.

Finally, there are many questions that remain regarding this interesting and little-studied host-parasite system. For example, the extremely large number of *C. passali* within this host is interesting in itself. Why would so many of them be necessary for transmission to the next host, especially if the adult beetles typically have only 2–3 progeny in their wood gallery? Is their mode of transmission inefficient, or do some of them not escape the immune response of their subsequent host? Indeed, future directions for research into this host-parasite system could involve examining the host immune response (encapsulation and melanization), if there is any, to infection (Wang *et al*. 1994; Castillo *et al*. 2011; Ebrahimi *et al*. 2011). It would also be important to examine worm burdens in larval beetles to look for evidence of selective mortality in immature hosts, and to compare the effects of infection on beetle fecundity.

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