Preliminary Assessment of Metabolic Costs of the Nematode Myrmeconema neotropicum on its Host, the Tropical Ant Cephalotes atratus

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ABSTRACT: The parasitic nematode Myrmeconema neotropicum infects workers of the neotropical arboreal ant Cephalotes atratus. Infected ants exhibit altered behavior, e.g., reduced aggression and slower tempo, as well as physical traits, e.g., gaster changes from shiny black to bright red. These changes are thought to induce fruit mimicry and attract frugivorous birds, which are the presumed paratenic hosts for the nematodes. We used respirometry to measure the energetic costs of nematode infection, testing the prediction of higher metabolic rates for infected workers maintaining both ant and nematode biomass. Contrary to this prediction, infected workers had lower mass-specific metabolic rates than uninfected workers. Parasites are limited to the gasters (abdomens) of adult ants, and infected gasters had 57% more mass, but 37% lower metabolic rates, compared to uninfected gasters. These results use a metabolic currency to measure, in vivo, the energetic costs of parasitism, and they shed light on the complex co-evolutionary relationship between host and parasite.

Social insects are especially vulnerable to parasites due to the intricacies of colony life (Schmid-Hempel, 1998; Moret and Schmid-Hempel, 2000; Hughes, 2002; Cremer et al., 2007). A striking social insect parasite is the nematode Myrmeconema neotropicum, which parasitizes the tropical arboreal ant Cephalotes atratus. The nematode manipulates ant behavior and physiology, presumably to transform ants into fruit mimics and attract frugivorous birds that are the likely paratenic hosts for the nematodes (Poinar and Yanoviak, 2008; Yanoviak et al., 2008). We used this model system to examine the energetic costs of parasitism. Specifically, we quantified the metabolic demands of nematode parasites developing within C. atratus workers.

The eggs of M. neotropicum enter C. atratus nests via infected bird feces that foraging workers feed to developing ant larvae. Infected larvae have reduced growth, and, by the time they pupate, they are laden with reproductively mature nematodes living in their gasters (bulbous posterior portion of the abdomen), where they mate. After infected ants eclose, female nematodes begin egg production and gradually reorganize the ants’ gasters, changing the color from shiny black to bright red, weakening the attachment to the postpetiole, and causing some internal organs, e.g., the ventral nerve cord, to atrophy, while leaving others intact, e.g., the alimentary canal (Poinar and Yanoviak, 2008; Yanoviak et al., 2008). The nematodes simultaneously change the behavior of their hosts, reducing ant aggression and tempo, causing them to maintain their gasters in a relatively elevated position, i.e., gaster flagging, and limiting the production of defensive pheromones (Yanoviak et al., 2008). As infected workers age, they become foragers outside the nest (Corn, 1980), and their gasters, filled with hundreds of nematode eggs, become redder, coordinating peak redness and infectiveness with potential exposure to avian frugivores (S. P. Yanoviak, pers. obs.).

The principal goal of the present study was to provide a preliminary estimate of the metabolic cost of this symbiosis to the host. To do so, we measured the contribution of parasites to worker mass, and then tested the prediction that infection stimulates higher metabolic rates, assuming the living biomass added by the parasite increases the energy demands of the host. We first compared the metabolic rates of infected and healthy workers. We then controlled for potential behavioral effects by comparing the energy demands of excised nematode-laden and healthy gasters that did not move but that continued to respire.

We collected infected and uninfected C. atratus workers in June 2010 on Barro Colorado Island, Panama (9°09’ N, 79°51’ W), a lowland tropical forest managed by the Smithsonian Tropical Research Institute (Leigh et al., 1996). We harvested both types of workers from a single large infected colony and maintained them on sugar water until respirometry experiments were performed (within a week of capture).

We conducted constant volume respirometry and recorded metabolic rate (μl CO2 hr−1, hereafter, VCO2) using equipment from Sable Systems International (SSI, Las Vegas, Nevada). Before all trials, we zeroed a CA-10 CO2 analyzer (accuracy of 1%, resolution of 0.00001%) using N2 gas and then spanned the analyzer with a gas of known CO2 concentration (1,200 ppm CO2 in N2 ±1%). For each trial, we placed individual workers in chambers attached to an RMs multiplexer; 6 contained single ants, and 1 remained empty as a control. Hourly CO2 values from the empty chamber were subtracted from all experimental chambers to correct for extrinsic CO2. Respirometry chambers for individual ants were 10-ml syringe barrels fitted with rubber stoppers, which were cleaned with 95% EtOH between trials. To establish baseline measures of ant respiration, we passed air scrubbed of CO2 through tubing affixed to the eighth position on the multiplexer between each experimental trial.

We first placed an empty colony and maintained them on sugar water until respirometry experiments were performed (within a week of capture). After 1 hr, we flushed the air out of chambers attached to the multiplexer, and then used the empty chamber to establish baseline measures of ant respiration. We placed ants individually in chambers containing air scrubbed free of CO2, as described previously. After 1 hr, we flushed the air out of chambers for 200 sec at 50 ml min−1, passed it through a 10-cm3 column of magnesium perchlorate (Cl2MgO4) to remove any remaining moisture, and then sent it to the CO2 analyzer. We ran trials for 6 hr, yielding 6 measurements per chamber. The first hour of data included extrinsic CO2, and we never used the first hour of data for analyses.

We used SSI ExpeData software to subtract the empty chamber CO2 from each experimental measurement and correct for small variations in flow rate (±0.1 ml min−1). We then used this software to generate the variable VCO2 by transforming CO2 measurements from ppm to μl hr−1 and integrating these values over the duration of each trial, resulting in a single number representing the metabolic rate of an ant colony.
measurements were standardized to 25°C assuming Q_10 = 2 (Lighton, 2008). However, temperature corrections were minimal; the average (±SD) of 30 hourly temperature measurements was 21.70 ± 0.56°C, with a range of 20.70 to 23.19°C. We used nonparametric tests for all analyses due to small sample sizes.

Infected workers were smaller than healthy workers based on HW, but they were heavier due to the parasite mass in their gasters (Table I; cf. Yanoviak et al., 2008). Contrary to our prediction, infected workers had lower mass- and size-specific metabolic rates than uninfected workers (Table I). Both mass- and size-specific results for isolated gasters further indicated that the metabolic demands of parasite eggs were minimal at this stage of their development (Table I).

In combination, our mass- and size-specific results show that gravid nematodes, laden with hundreds of eggs, added substantial mass to the gasters of their infected hosts without significantly increasing host body mass. These results corroborate and clarify aspects of the nematode life cycle described elsewhere (Poinar and Yanoviak, 2008; Yanoviak et al., 2008). Specifically, our results suggest that the majority of M. neotropicae development occurs in larval C. atratus workers, where they feed on hemolymph and other tissues, compromising ant growth and likely exacting metabolic costs. However, as the nematode eggs reach peak infectiveness in adult ants (coinciding with maximum gaster redness), they become quiescent and use little energy. Size-specific gaster results suggest that the slight (but non-significant) increase in metabolic rate attributed to the parasite could be measured given a larger sample size.

Parasites impart energetic costs on their hosts due to direct consumption of tissues and altered physiology (Connors and Nickol, 1991; Booth et al., 1993; Lettini and Sukhdeo, 2010). However, the costs associated with each of these mechanisms are difficult to decouple in vivo (Thompson, 1986). Parasites are known to reduce worker size or induce color changes in other ant species (Lee, 1957; Passera, 1976; Stuart and Alloway, 1988; Trabalon et al., 2000). However, M. neotropicae provides a unique opportunity to contrast the direct and indirect energetic costs of parasitism because it alters both the behavior and morphology of its host in measureable ways. Infected workers have slower tempos than uninfected nestmates (Yanoviak et al., 2008), but infected workers are also heftier due to the parasite mass in their gasters (Yanoviak et al., 2008). Contrary to our prediction, infected workers had lower mass- and size-specific metabolic rates than uninfected workers (Table I). Both mass- and size-specific results for isolated gasters further indicated that the metabolic demands of parasite eggs were minimal at this stage of their development (Table I).

Regardless of the underlying mechanisms, our results illustrate the way in which a metabolic approach can improve our understanding of the complex relationships that exist between parasites and their hosts.

We thank Oris Acevedo, Belkys Jimenez, and the staff of the Smithsonian Tropical Research Institute for logistical support. This project was funded by the National Science Foundation under the grants DEB-0842038 to M.K., and IOS 0843120 to S.P.Y.

<table>
<thead>
<tr>
<th>Whole ant</th>
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<tbody>
<tr>
<td>Mass (mg)</td>
<td>35.8 (3.63)</td>
<td>21.9 (3.14)</td>
<td>2.78</td>
</tr>
<tr>
<td>HW (mm)</td>
<td>2.2 (0.13)</td>
<td>2.5 (0.12)</td>
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<tr>
<td>MRmass (VCO₂·mg⁻¹)</td>
<td>0.50 (0.102)</td>
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<td>MRHW (VCO₂·mm⁻¹)</td>
<td>4.82 (0.513)</td>
<td>6.67 (0.444)</td>
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<tr>
<td>Gaster only</td>
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<td></td>
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</tr>
<tr>
<td>Mass (mg)</td>
<td>13.5 (1.37)</td>
<td>4.8 (1.19)</td>
<td>3.04</td>
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<td>MRmass (VCO₂·mg⁻¹)</td>
<td>0.28 (0.128)</td>
<td>0.80 (0.111)</td>
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<td>MRHW (VCO₂·mm⁻¹)</td>
<td>1.73 (0.204)</td>
<td>1.29 (0.177)</td>
<td>1.23</td>
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</tbody>
</table>

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**LITERATURE CITED**


