

## 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 ( $\mu\text{l CO}_2 \text{ hr}^{-1}$ , hereafter,  $\dot{V}\text{CO}_2$ ) using equipment from Sable Systems International (SSI, Las Vegas, Nevada). Before all trials, we zeroed a CA-10 CO<sub>2</sub> analyzer (accuracy of 1%, resolution of 0.00001%) using N<sub>2</sub> gas and then spanned the analyzer with a gas of known CO<sub>2</sub> concentration (1,200 ppm CO<sub>2</sub> in N<sub>2</sub> ± 1%). For each trial, we placed individual workers in chambers attached to an RM8 multiplexer; 6 contained single ants, and 1 remained empty as a control. Hourly CO<sub>2</sub> values from the empty chamber were subtracted from all experimental chambers to correct for extrinsic CO<sub>2</sub>. 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 CO<sub>2</sub> through tubing affixed to the eighth position on the multiplexer between each experimental trial.

We first scrubbed incurrent air of H<sub>2</sub>O and CO<sub>2</sub> using a drierite/ascarite/drierite column (Lighton, 2008) at a flow rate of 50 ml min<sup>-1</sup>. Flow was generated with an SS-3 subsampler pump and regulated by a 200 ml min<sup>-1</sup> Sierra valve connected to an MFC unit. We then scrubbed this air by a second drierite/ascarite/drierite column and sent it to the multiplexer, which was programmed to switch between chambers using SSI Expedata software. Ambient temperature was continuously recorded adjacent to respirometry chambers using a Thermistor cable. All equipment was interfaced with a computer using a SSI UI-2 control module.

We placed ants individually in chambers containing air scrubbed free of CO<sub>2</sub> as described previously. After 1 hr, we flushed the air out of chambers for 200 sec at 50 ml min<sup>-1</sup>, passed it through a 10-cm<sup>3</sup> column of magnesium perchlorate (Cl<sub>2</sub>MgO<sub>8</sub>) to remove any remaining moisture, and then sent it to the CO<sub>2</sub> analyzer. We ran trials for 6 hr, yielding 6 measurements per chamber. The first hour of data included extrinsic CO<sub>2</sub> and was never used. Thereafter, ants were generally inactive, and CO<sub>2</sub> readings stabilized at a lower value (Lighton, 2008). Each data point was the mean of 5 hourly respiration measurements of a single ant taken during this time.

Following the worker measurements, we removed gasters with a scalpel and sealed the attachment point to the postpetiole with a small drop of nail polish. We then froze the rest of the worker. *Cephalotes atratus* gasters have spiracles, and preliminary trials indicated that the gasters continued to respire normally during subsequent measurements. We returned these gasters to the respirometry chambers and recorded a second set of measurements as before. After these trials, we froze all ant tissue, dried it at 60 C for 24 hr, and weighed it to the nearest 0.1 mg. Because the parasite dramatically increases the mass of infected workers while decreasing overall body size (Yanoviak et al., 2008) (Table I), we also estimated the size-specific energy demands of ants using worker head width (HW) as a body size metric. We measured HW behind the eyes, between the vertex spines (see Yanoviak et al., 2008) using a stereoscope equipped with an ocular micrometer. *Cephalotes atratus* HW is strongly correlated to other body size metrics that have lower repeatability, e.g., appendage lengths, mesosoma length, and gaster dimensions (S. Yanoviak, unpubl. obs.). Because of the counterintuitive effects of the parasite on host body size and mass, comparison of metabolic rates based on HW enabled us to separate the metabolic cost of the parasite from that of its host.

We used SSI ExpeData software to subtract the empty chamber CO<sub>2</sub> from each experimental measurement and correct for small variations in flow rate ( $\pm 0.1 \text{ ml min}^{-1}$ ). We then used this software to generate the variable  $\dot{V}\text{CO}_2$  by transforming CO<sub>2</sub> measurements from ppm to  $\mu\text{l hr}^{-1}$  and integrating these

TABLE I. Average ( $\pm$ SE) size (wet mass and HW) and metabolic rate of infected ( $n = 6$ ) and uninfected ( $n = 8$ ) *C. atratus* used in the study. HW = head width behind the eyes,  $MR_{\text{mass}}$  = mass-specific metabolic rate,  $MR_{\text{HW}}$  = size-specific metabolic rate,  $Z$  = Wilcoxon score.

	Infected	Uninfected	Z	P
Whole ant				
Mass (mg)	35.8 (3.63)	21.9 (3.14)	2.78	0.006
HW (mm)	2.2 (0.13)	2.5 (0.12)	2.07	0.038
$MR_{\text{mass}}$ ( $\dot{V}CO_2 \cdot \text{mg}^{-1}$ )	0.50 (0.102)	1.04 (0.088)	2.90	0.004
$MR_{\text{HW}}$ ( $\dot{V}CO_2 \cdot \text{mm}^{-1}$ )	4.82 (0.513)	6.67 (0.444)	2.13	0.033
Gaster only				
Mass (mg)	13.5 (1.37)	4.8 (1.19)	3.04	0.002
$MR_{\text{mass}}$ ( $\dot{V}CO_2 \cdot \text{mg}^{-1}$ )	0.28 (0.128)	0.80 (0.111)	2.78	0.006
$MR_{\text{HW}}$ ( $\dot{V}CO_2 \cdot \text{mm}^{-1}$ )	1.73 (0.204)	1.29 (0.177)	1.23	0.220

values for trial intervals.  $\dot{V}CO_2$  measurements were standardized to 25°C assuming  $Q_{10} = 2$  (Lighton, 2008). However, temperature corrections were minimal; the average ( $\pm$ 1SD) of 30 hourly temperature measurements was  $21.70 \pm 0.56$ °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 energy demands. 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. neotropicum* 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. neotropicum* 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 measurable ways. Infected workers have slower tempos than uninfected nestmates (Yanoviak et al., 2008), but our results suggest that worker behavior is not driving energetic differences. Specifically, recordings from excised (immobile) gasters indicate that differences in respiration rates between infected and uninfected ants are due to effects of the parasite on ant mass and body size.

We used worker mass and HW to quantify parasite effects on ant size because these variables are easily measured and tend to have high repeatability. However, future investigations may provide more precise measures of metabolic costs (or the lack thereof) by dissecting the parasite mass out of the host and weighing each independently. Similarly, quantification of respiration rates across all stages of nematode and ant development would be an informative extension of this work.

The physiological mechanisms employed by parasites to extract metabolic fuel from their hosts remain largely unknown, in part because they are difficult to isolate and measure (Thompson, 1986; Lettini and Sukhdeo, 2010). A complete understanding of the mechanisms generating the observations in this study will require more detailed investigation, perhaps using parasitized ant colonies maintained in the laboratory. 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.

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