Defending your parasite: the effect of hairworm infection on male cricket behavior

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Abstract: Male house crickets (*Acheta domesticus*) exhibit aggressive territorial behaviors, especially in the presence of a female. Currently, there is limited information available concerning the infection of crickets with hairworms. We observed the variation of aggression shown by *A. domesticus* once infected with hairworms. The males were studied in an arena where various aggressive behaviors could be recorded. We found that there was a general increase in aggression by non-infected crickets as compared to infected crickets. However, this discrepancy was not significant. These results suggest that the energy of the infected cricket is being diverted elsewhere as a result of the hairworm infection.

Keywords: Hairworm, Aggression, *Paragordius various*, *Acheta domesticus*, Behavior

Introduction

Males often display signs of aggression toward other male members of a species when selecting mates or defending territory (Alexander 1961). Specifically, male house crickets (*Acheta domesticus*), are known to exhibit these aggressive behaviors (Montroy et al., 2016). They readily engage in distinct acts such as stridulation, shuddering, mandible flaring, and antennation when they feel their territory is being threatened (Alexander 1961). Level of aggression is influenced by male hormone levels, and aggression plays a significant role in cricket fitness. Crickets that exert their dominance through forms of aggressive behavior are more likely to survive and reproduce (Montroy et al., 2016). Few studies have been conducted to examine aggression in crickets infected with macroparasites. The phylum Nematomorpha are parasites of terrestrial arthropods that have a complex life cycle, using hosts in both aquatic and terrestrial systems (Bolek et al., 2015). These parasites are able to manipulate the behavior of the cricket to seek water, the final habitat of the free-living adult worm (Biron et al., 2005). This leads us to hypothesize that male crickets infected with the hairworm, *P. various,* do not display signs of typical male aggression because hair worm infection alters cricket behavior.

Methods

To begin testing the effect of hairworm infection on male house crickets, Physa sp. snails were exposed to *P. varius* larvae. Approximately 250 hairworm larvae were pipetted into each well of Corning® 24-well plates. Each well plate was filled halfway with water, then one snail was added to each well. The snails sat for two days to allow for ingestion of the larvae. Half-grown *Acheta domesticus* house crickets (approximately four weeks old) were obtained from Armstrong Cricket Farm. They were starved for two days so

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Table 1 - Average number of aggressive behaviors by resident males (control and infected).

	Female present	Female absent
Resident - control	3.2	6.9
Resident - infected	2.9	3.9

they would eat the infected snail tissue. The crickets were fed the infected snail tissue, then were housed individually in incubators with access to food and water. Control crickets were set up in the same manner, except they were not fed infected tissue. The incubators were kept at 27 degrees Celsius, and food/water was changed as needed (every 3-4 days). The crickets remained incubated for 2-3 weeks until reaching the point of maturity. Each cricket was labeled as either infected or control.

To test the infection on male cricket behavior, an arena was set up with a video camera to record the observed aggressive behaviors. One non-infected cricket was added to an empty clear aquarium and allowed to acclimate for 5-10 minutes. This cricket serves as the 'resident' male. Then, another non-infected male cricket, labeled the 'intruder male' was added and the males' respective behaviors were observed and recorded for a 15-minute period. This trial served as the control group. Next, a female cricket was introduced to the two males for an additional 15 minutes, and their behaviors were again observed and recorded. This control trial was repeated three times.

This same process was then repeated with an infected male cricket as the 'resident' male and a non-infected cricket introduced as the 'intruder'. The number of behaviors observed were compared both with a female present and a female absent. This trial was also repeated three times. Lastly, the process was again repeated with infected crickets as both the resident and intruder. Their behaviors were observed with both a female present and a female absent, and this trial was repeated three times.

The data was assumed to be normal, and a t-test was conducted using Excel®. The t-test was used to determine the probability values for the collected data. These p-values were analyzed to ascertain whether our results were significant or not.

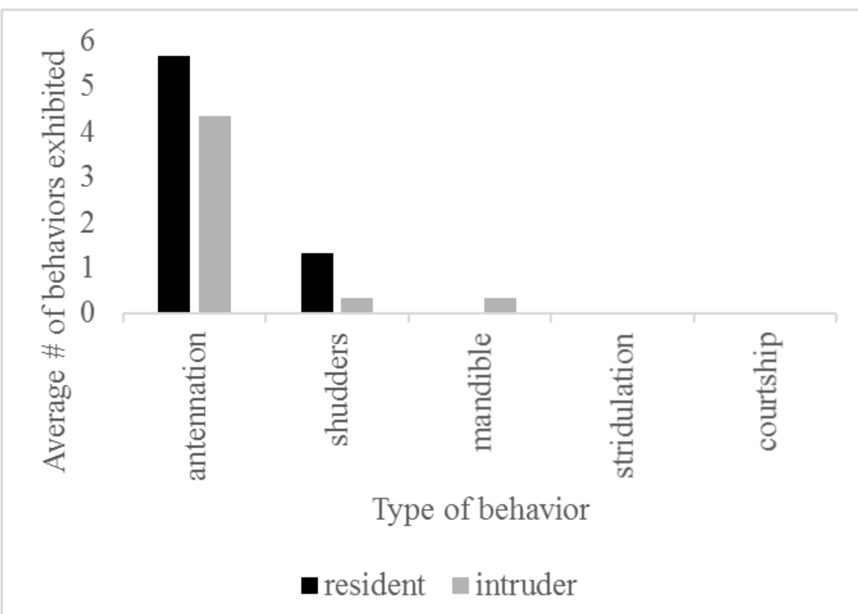

Figure 1 - The average number of aggressive behaviors exhibited between a control male cricket versus a control male cricket without a female present.

Results

A trend occurred between the residents versus intruders and in the presence of females in both control and infected male

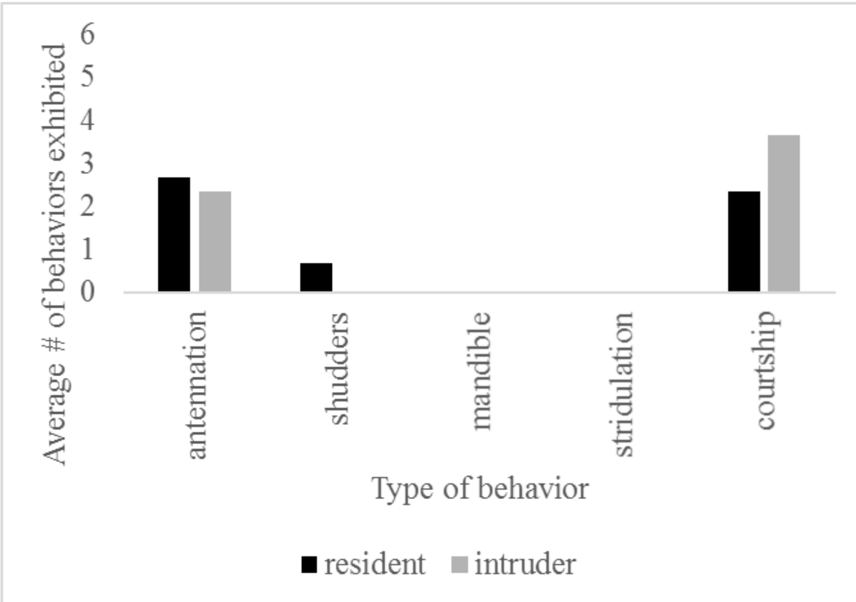

Figure 2 - The average number of aggressive behaviors exhibited between a control male cricket versus a control male cricket with a female present.

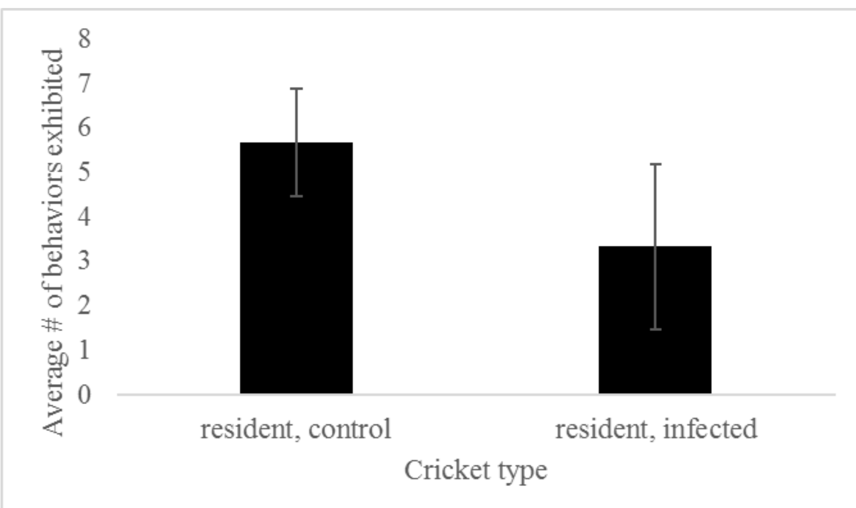

Figure 3 - The average number of aggressive behaviors exhibited by resident control male crickets and resident infected male crickets with and without a female present (t_3=1.055, p=0.175). The error bars represent standard error of the mean.

crickets (Table 1). Additionally, the presence of females decreased aggressive behaviors but increased courtship behaviors (Figures 1, 2, 3). When a control resident cricket was in the arena with another control and no female, the resident showed an average of 6.9 aggressive behaviors while the intruder showed an average of 4.9 (Figure 1). When a control resident cricket was in the arena with another control and a female, the resident showed an average of 3.2 aggressive behaviors and 2.3 courtship behaviors while the intruder showed an average of 2.3 aggressive behaviors and 3.6 courtship behaviors (Figure 2). When an infected resident cricket was in the arena with another infected cricket and no female, the resident showed an average of 3.9 aggressive behaviors and no courtship behaviors. When an infected resident cricket was in the arena with another infected cricket and a female, the resident showed an average of 2.9 aggressive behaviors and 0.3 courtship behaviors (Figure 3). Overall, the resident control crickets showed an average of 5.6 aggressive behaviors and the resident infected crickets showed an average of 3.3 aggressive behaviors (t_3=1.055, p=0.175; Figure 4). However, these results were not significant.

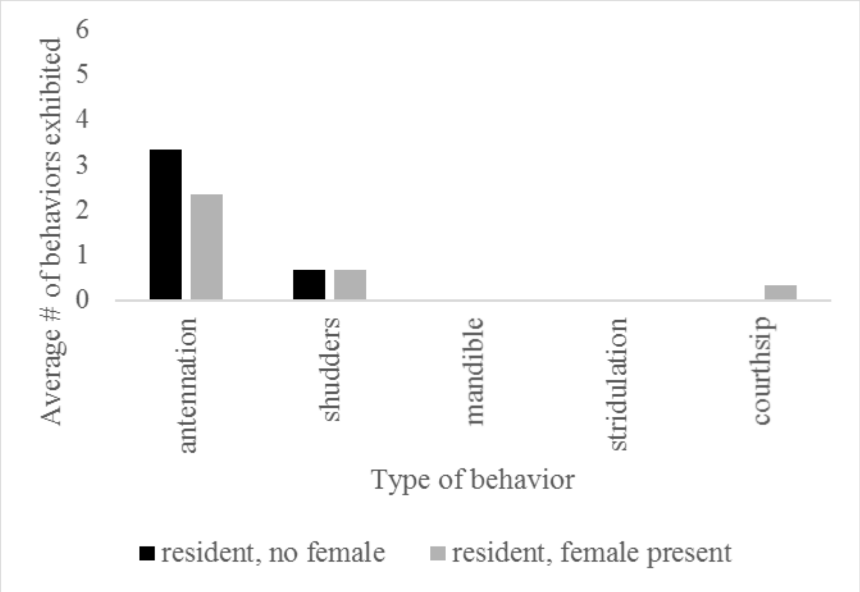

Figure 4 - The average number of aggressive behaviors exhibited by resident infected male crickets with and without a female present.

Discussion

This was a novel study to determine whether male crickets infected with hairworms had an effect on their aggressive behaviors. Based on our results, the overall number of aggressive behaviors exhibited by the infected crickets was lower when compared to control crickets. However, these results were not significant. Additionally, when females were added to the arena, the infected males were less likely to exhibit courtship behaviors in the presence of a female when compared to control males. The data suggests that the infection of hairworms in male crickets results in a decline of aggressive behaviors. The data that we found supported our original hypothesis that the infection of crickets with hairworms alters cricket behavior. The decreased aggressive behavior of the infected males could be due to the hairworms castrating their hosts (Biron et al., 2005). Hormones found in the gonads of crickets are linked to aggression (Dixon et al, 1986). Previous studies have observed that castration of crickets by parasites could be due to the testes containing substances that are harmful to the parasite's development (Adamo et al., 1995). Decreased fighting behaviors could also be linked to the decrease in body fat and weakening of muscles due to the development of the parasite inside the cricket (Adamo et al., 1995). Another explanation for the decreased aggression in infected males is the possibility that some crickets were sick and some were not. It is not clear what causes the sickness, but a higher than normal number of crickets died.

In the future, this experiment could be expanded by conducting the same tests, but collecting the data after the hairworms come out of the host crickets. This could give us a better understanding of how the infection affects the host's behavior. To future our understanding of how cricket behavior is changed due to hairworm infection, we could observe if males are able to successfully reproduce once infected. This research introduces more data and ideas involving the infection of crickets with parasites and the effects that the infection has on their behavior. These findings can help the scientific community understand the effects of parasites not only on crickets but humans alike.

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